

**FUNCTIONAL ASPECTS OF THROMBOLYTIC THERAPY**

**BY**

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## ABSTRACT

The benefits of thrombolytic therapy in acute myocardial infarction are established, yet there remain important questions, including the safe interval for effective readministration of the streptokinase-containing thrombolytic agents, and how to expedite the administration of therapy and the restoration of coronary patency. The work in this thesis addresses some aspects of these questions.

The administration of thrombolytic agents by bolus injection has practical attractions. The pharmacokinetic properties of streptokinase (SK) and anistreplase in their standard administration regimens are compared, and demonstrate the significantly earlier, higher plasma concentrations and longer half-life achieved by bolus administration of anistreplase.

The significance of antibodies to the SK-containing thrombolytic agents is largely unknown. Pretreatment SK resistance titre and anti-SK IgG concentrations were measured in 128 patients with acute myocardial infarction treated with SK or anistreplase. A significant minor negative influence of SK resistance titre on coronary patency was observed. The haemodynamic responses to these agents were observed in detail, and blood pressure falls found to be usually short-lived and not require specific intervention. An association was sought between hypotensive episodes and markers of immunological resistance, markers of thrombin activity, and plasma viscosity. No relationship was found, refuting their implication in the hypotensive mechanism.

The time course of the development of changes in SK resistance titre and anti-SK IgG concentration were documented in detail in the same patients over 30 months. Both indices peaked at 2 weeks following therapy, and declined slowly, with 50 & 58% respectively of the population returning to within two standard deviations of pretreatment levels within 2 years. The functional sequelae of these antibody responses were studied in vitro using a pooled clot lysis assay. These data demonstrated near complete inhibition of lysis up to 9 months, with 75% recovery at 30 months.

Two novel administration regimens of alteplase, as 2 intravenous boluses of 35mg, or 3 of 20mg, were assessed by their ability to achieve high plasma concentrations of t-PA and coronary patency. The former regimen achieved coronary patency in 87% at 90 minutes, but while the latter achieved high early patency there was a 30% reocclusion rate, making this regimen unlikely to be suitable for clinical use.

The use of SK-containing thrombolytic agents may be limited by haemodynamic and immunological reactions, and bolus administration of non-immunogenic thrombolytic agents remains attractive.

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## **DECLARATION**

This thesis describes research undertaken in the Department of Medicine and Therapeutics at Stobhill General Hospital, Glasgow, during the period from August 1987 to July 1991, from my position there as Research Fellow and Registrar. I have been fortunate in having the cooperation and collaboration of several colleagues who are formally acknowledged. Otherwise, the work of this thesis has been my own, and in particular, the writing of the substance of the text has been entirely my own undertaking. Some of the work discussed in this thesis has been published in academic journals, and details thereof constitute Appendix III.

## **ACKNOWLEDGEMENTS**

The period of research leading to the submission of this thesis was carried out in Stobhill General Hospital, Glasgow, and during my four years there I was most fortunate in having the benefit of advice and collaboration of many of my colleagues there. I wish to express by profound gratitude to all of them.

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Although the major part of this thesis has been my own work, the database of this thesis is derived from a large patient pool, and the clinical nature of this work, demanding constant availability over the four year period of data acquisition, has involved a team approach by the physicians involved in the thrombolysis research programme conducted in Stobhill. I formally acknowledge and thank the nursing staff of the coronary care unit, the ECG technicians, and my medical colleagues, Dr. W.S. Hillis, Dr. F.G. Dunn, Dr. A.P. Rae, Dr. K.J. Hogg, and Dr. P.D. MacIntyre. To participate in this programme and share the clinical responsibilities with the above has been a privilege and a pleasure.

Last, but by no means least, I thank my wife, Brenda, for her sacrifices and patience, and I dedicate this work to her.

**CHAPTER ONE**

**A HISTORICAL OVERVIEW OF MYOCARDIAL INFARCTION AND CORONARY  
THROMBOSIS**

"The heart .....is a firm, thick mass so richly supplied with fluid that it does not suffer harm or manifest pain."  
Hippocrates (460 - 370 BC)

The heart in itself is not the beginning of life, but it is a vessel made of dense muscle, vivified and nourished by the artery and vein, as are the other muscles."  
Leonardo da Vinci (1452 - 1519)

In early times, the heart was thought to be the origin of life and the seat of the soul. Although the function of this essential organ was not recognised by Hippocrates, he taught the concept that the heart was not affected by disease processes - "cor non aegrotaro potest". It was not until the second century that Galen (130 - 200 AD) recognised the motor power of the heart and first termed the arteries of the heart coronary arteries.

Knowledge of the heart advanced little during the Middle Ages, until the time of the celebrated English physician, William Harvey (1578 - 1657) who first described the circulation of the blood in "de Motu Cordis" in 1628. Harvey also described rupture of the left ventricle coincident with thrombosis in a calcified coronary artery in "Exercitationes II ad Riolanum" in 1660, probably the earliest description of acute myocardial infarction. "Ossification of the coronary arteries" was described in the late seventeenth and early eighteenth centuries by Drelincourt (1633 - 97), Bellini (1643 - 1704) and

Thebesius (1708). Lancini of Rome (1654 - 1720) included calcified coronary arteries as one cause of "aneurysm of the heart", a term used at that time to describe cardiomegaly.

In 1749, Senac again described ossified coronary arteries associated with thin-walled parietal aneurysms of the left ventricle. Morgagni in "De Sedibus et Causis Morborum" (1761) described patients who in life had typical symptoms of angina pectoris and myocardial infarction, and who at autopsy had obstructed coronary arteries, although the connection between these findings was not made.

William Heberden (1710 - 1801) in 1768 and again in 1782 gave perfect graphical accounts of the symptom complex of angina pectoris, although he did not offer an explanation of the pathophysiology of this syndrome. The connection between angina pectoris and the heart and in particular the coronary arteries was first suggested by three men - Fothergill, Parry and Jenner.

John Fothergill (1712 - 80) was a graduate of Edinburgh university who described a case of a man with angina pectoris who proceeded to a sudden death, and attributed his symptoms and demise to the heart. After an autopsy was performed by the famous dissector, John Hunter, on a 63 year old man with angina, Fothergill described the heart as being

of "a ligamentous consistence and in many parts of the left ventricle almost white and hard"...."the two coronary arteries from their origin to many of their ramifications upon the heart were become one piece of bone."

Edward Jenner (1749 - 1823) and Caleb Hillier Parry (1755 - 1822) claimed that myocardial ischaemia due to coronary obstruction was the cause of angina, and Parry in 1799 described "syncope anginosa", although undoubtedly some of his cases of angina had suffered myocardial infarction. Publication of some of these theories was delayed until after the death of the dissector, John Hunter, in 1793. In life he suffered from angina pectoris, and his friends were reluctant to predict his demise due to coronary obstruction, but after his death, as predicted, he indeed had extensively calcified coronary vasculature.

On the experimental front, in 1809 Allan Burns, proved that deprivation of blood flow caused pain by occluding the brachial artery with a ligature. He suggested that ischaemic pain of the heart was the cause of angina pectoris.

Latham (1789 - 1875) was probably the first to differentiate patients with chronic stable angina pectoris from those with a shorter, less effort-related history of greater severity and associated with early death, probably due to myocardial infarction. However, he did not relate these events to the coronary arteries.

The late eighteenth and nineteenth centuries were the era of "physical medicine", led by Laennec (1781 - 1826) and Corvisart (1755 - 1821), when the recognition and demonstration of physical signs was considered all-important. In this atmosphere, because of the absence of physical signs, angina was largely dismissed as either a "rheumatic" or "nervous" complaint. Rokitansky and Virchow both regarded left ventricular aneurysms as inflammatory in origin and the pain of angina due to "nervousness".

It was Richard Quain (1816 - 1889) in 1850 who clearly related diseased coronary arteries to angina and myocardial changes in "On Fatty Diseases of the Heart". He wrote of "local modification of nutrition" and described again ossification of the coronary arteries. He also wrote that, in the absence of anastomoses between the coronary arteries, "obstruction from any cause in one will not admit of the deficiency being compensated for by the supply of the other". He described localised fatty degeneration of the myocardium in the context of alteration of the coronary artery to this part: "I have seen the coronary extensively ossified, going directly to the only part of the heart affected" and "at least, arteries proceeding to the principal seat of the disease, are found more or less obstructed." He understood the basic pathophysiology of ischaemic heart disease, writing that ossification of the

coronary arteries "caused interference with the nutritive function of the artery and thus produced the softening which was attended by pain on effort."

The late nineteenth century German physicians had an understanding of ischaemic heart disease not dissimilar to that of the modern era. Karl Weigert in 1880 wrote "in atheromatous changes of the coronary arteries not infrequently thrombotic or embolic obstructions form in the branches of the arteries. If the obstruction forms slowly, or at least in such wise that collateral channels exist, but not enough to keep up nutrition, a slow atrophy occurs with destruction of the muscle fibres without injury to the connective tissue. The muscle fibres that disappear are replaced by fibrous connective tissue, and the so-called chronic myocarditis is no more than such a process." In 1882, Karl Huber clearly described the process by which atheroma of the artery caused diminution of myocardial blood supply leading to anaemic, atrophic or necrotic lesions which ultimately become areas of fibrosis. Two years later in 1884, Ernst Leyden described differing rates of development of coronary obstruction causing a spectrum of clinical disorders, from angina pectoris through myocardial infarction and sudden death to incipient congestive cardiac failure.

In 1842, Marshall Hall speculated that sudden death was often due to arrest of the coronary circulation and John Erichsen showed that ligation of a major coronary artery in the dog caused syncope. Further experiments in dogs were reported by Connheim in 1881, who showed that ligation of large coronary arteries caused death within two minutes. Although he also showed that ligation of smaller coronary vessels or more gradual obstruction caused much lesser or indeed no ill effects, the medical world concentrated on the sudden death aspects of coronary occlusion and extending the conclusions of these observations to man, decided that coronary occlusion was incompatible with life and therefore not worthy of further major investigation. It was unfortunate that this opinion dominated at the time of the major surge in matters medical led by the innovative work of Koch and the pioneer surgeons, with the effect that cardiological knowledge was not substantially advanced by this wave of medical enthusiasm (from Herrick, 1942).

Herrick in 1912 described in detail two patients with myocardial infarction who survived 52 hours and seven days, the first case having suffered a thrombotic occlusion of the proximal left anterior descending coronary artery (Herrick, 1912). He also reviewed the historical literature, emphasising experiments where the animal survived in the

short term following coronary occlusion, and the occasional description of patients coming to autopsy with evidence of previous coronary occlusions and myocardial infarctions which they had survived for some time.

However, even after it was accepted that it was possible to survive a coronary occlusion and ensuing myocardial infarction, there remained one major argument which raged for at least half a century, namely the question of whether coronary thrombosis was the cause or effect of myocardial infarction. Numerous papers concerning this controversy were published, supporting both sides of the argument.

Early in the discussion, there had always been difficulty in accepting that coronary thrombus was an important initiating event in vivo, as coronary thrombosis had previously been observed as an incidental finding in autopsies, notably in the nineteenth century. At that time, medical teaching instructed that coronary occlusion was incompatible with life and it therefore followed that these findings were an irrelevant post-mortem artifact. However, once it was accepted that man was able to survive coronary occlusion, and indeed that such an event could be asymptomatic, this argument was no longer valid.

There remained, however, other arguments favouring coronary thrombosis as a secondary phenomenon (Roberts & Buja, 1972, Silver et al., 1980, Baroldi et al., 1979, Roberts, 1974, Hellstrom, 1979). Coronary thrombosis had been documented in the absence of myocardial infarction. This can be explained by the presence of sufficient coronary collateralisation (Blumgart et al., 1940) and a slow onset of coronary occlusion.

Post-mortem studies in sudden death failed to demonstrate coronary thrombus (Roberts et al., 1972). This argument is only valid if it is assumed that myocardial infarction is the cause of sudden cardiac death. However, other studies have demonstrated that patients resuscitated from sudden death do not necessarily develop evidence of myocardial infarction (Cobb et al., 1975, Liberthson et al., 1974a, Liberthson et al., 1974b). It was subsequently revealed that sudden death in ischaemic heart disease was due to primary ventricular arrhythmias, often associated with an acute ischaemic event, but not necessarily with major coronary occlusion and myocardial infarction, or else due to primary arrhythmia in chronic ischaemic heart disease in the absence of acute ischaemia.

Estimates of the incidence of coronary thrombosis as judged by post-mortem studies vary enormously. For example, Branwood et al., 1956, reported an incidence of 21%, and at the other extreme, Ridolfi et al., (1977) 93%. Several authors have reported cases of myocardial infarction in the

absence of coronary thrombosis (Ehrlich et al., 1964, Maseri et al., 1978a,b). This finding can be explained in a number of ways. Not all myocardial infarctions are necessarily due to occlusive coronary thrombus. Other causes of coronary obstruction causing myocardial infarction without primary coronary thrombosis include coronary artery spasm (Maseri et al., 1978a, Braunwald, 1978), coronary embolism (Ehrlich et al., 1964), coronary dissection, and rupture of and haemorrhage into an atherosclerotic plaque (Willerson et al., 1980). It is important to differentiate between anatomically discrete, regional myocardial infarction and more diffuse global, subendocardial or laminar myocardial infarctions or multiple micro-infarcts. This distinction is not always clearly made in the literature. Diffuse myocardial infarction is more likely to be due to systemic causes such as severe hypotension, anoxia, anaemia, coronary embolus or conditions of increased myocardial oxygen demand, such as tachycardia or hypertensive crises (Buja et al., 1981), although systemic causes on the background of a critical coronary stenosis may precipitate a discrete myocardial infarction. Obversely, in general, discrete segmental infarcts are more likely to be associated with acute coronary thrombosis, probably superadded to a coronary stenosis, possibly precipitated by atherosclerotic plaque rupture.

This view was supported by Ehrlich and Shinohara in 1964 in a careful pathological study with block dissection of coronary arteries in 38 patients dying after myocardial infarction and 29 controls dying from unrelated causes. Although coronary thrombus was identified in only 50% of all 38 patients with myocardial infarction, the prevalence was 94% (17 of 18) of patients with unicentric myocardial infarction, compared to 10% (2 of 20) of patients with multicentric infarction. Similar post-mortem studies have confirmed these findings (Buja et al., 1981). In addition, post-mortem studies take no account of the possibility of ante-mortem recanalisation (deWood et al., 1980), post-mortem thrombolysis or clot retraction after death.

The incidence of coronary thrombosis found at post-mortem studies may depend on the rigour of the pathological and histological techniques employed (Ehrlich et al., 1964) and also the delay between the onset of myocardial infarction and pathological examination. Although some authors have found higher incidence of thrombus in older myocardial infarctions (Spain and Bradess, 1960), this would not be in keeping with the data of deWood (1980) - vide infra. It is important to note that post-mortem studies are by definition performed on a selected sub-group of patients with myocardial infarction, and it would hardly be surprising if some systematic bias was introduced to these studies.

The pathological studies also made efforts to differentiate post-mortem clots from in vivo thrombus, and although they can be differentiated histologically, they are easily confused on naked eye examination. This differentiation may have added a further confounding factor to the pathologists' studies.

The observation that larger and transmural infarcts, especially if associated with cardiogenic shock, or severe congestive cardiac failure, had a higher incidence of coronary thrombus was also used to suggest that coronary thrombosis was secondary to myocardial infarction. These studies are flawed by the patient selection inherent in these studies and it is not surprising that larger infarcts are associated with larger, more proximal and more persistent coronary thrombus, and therefore more likely to be identified in pathological studies.

Early studies with radio-labelled fibrinogen injected several hours after the onset of myocardial infarction showed that fibrinogen continued to be incorporated into the coronary thrombus, and this was interpreted as meaning that coronary thrombosis was secondary to myocardial infarction (Erhardt et al., 1973 & 1976). However, subsequent studies with radio-labelled fibrinogen have suggested that the label may continue to be incorporated into clot formed prior to the injection of the tracer (Salimi et al., 1977) and that a radio-negative core can be demonstrated in most human thrombi (Fulton et al., 1977). These later findings

illustrate that coronary thrombus occurs before the injection of radio-label and that formed thrombus remains in a dynamic state of equilibrium after its formation in the coronary arteries.

By 1980, there had been a number of post-mortem and clinicopathological studies published which showed that there was a strong association between acute coronary artery occlusion in the infarct related artery and regional myocardial infarcts, and a temporal relationship between the age of the thrombus and the age of the infarct, albeit recognising the limitations of the histological techniques in this regard (Willerson et al., 1980). Thus it was reasonably clear that coronary thrombosis was an associated event, rather than a post-mortem artifact in acute myocardial infarction, and it was thought that the thrombus was a major factor in the causation of myocardial infarction.

In 1980, deWood and his colleagues published a classical paper which to a great extent resolved many of the previous arguments, as well as establishing the safety and feasibility of coronary angiography in the early stages of myocardial infarction. They performed selective coronary angiography on 322 patients with proven acute myocardial infarction within 24 hours of onset of chest pain. It was shown that if the artery was examined within 4 hours of onset of myocardial infarction, coronary occlusion could be

demonstrated in 87% with a subtotal lesion in a further 10%. However if arteriography was delayed to at least 12 hours, the occlusion rate had fallen to 65%, with subtotal lesions in 16%. Patients with cardiogenic shock showed a strong, but not significant trend towards persistent total occlusion (86%) throughout 24 hours.

This important study demonstrated the central role of coronary occlusion due to thrombosis in acute myocardial infarction and the occurrence of spontaneous reperfusion. It also suggested the application of thrombolytic drug by the intracoronary route of administration in myocardial infarction, and stressed the essential elements of pretreatment patency and influence of time on coronary patency in the interpretation of future patency/reperfusion studies of thrombolytic therapy in acute myocardial infarction.

Since this work, coronary angiography has been performed early in the course of acute myocardial infarction in many patients, both for confirmation of diagnosis and administration of therapy. The findings confirm the high incidence of coronary occlusion early in the process of infarction, being 74-86% in the first 12 hours of myocardial infarction (Rentrop et al., 1981, Anderson et al., 1983, Kennedy et al., 1983, TIMI-1, 1985, Simoons et al., 1985, Blanke et al., 1985, Williams et al., 1986).

Once the causative role of coronary thrombosis in myocardial infarction was accepted, the way was open for the logical trial of thrombolytic therapy, and the next chapter discusses the development of treatment with fibrinolytic agents.

**CHAPTER TWO**

**A REVIEW OF THROMBOLYTIC THERAPY IN THE MANAGEMENT  
OF ACUTE MYOCARDIAL INFARCTION**

## **Early experience with streptokinase in acute myocardial infarction**

Streptokinase is derived from streptococci and was first used to dissolve collections of sanguineous, fibrinous, and purulent fluid in man (Tillett and Sherry, 1949) and was subsequently used experimentally to lyse in vivo intravascular blood clots (Johnson and Tillett, 1952). From an early stage in the drug's development it was appreciated that streptokinase exerted its effects by stimulation of the intrinsic lytic cascade by activating plasminogen, which it does with great efficacy (Sherry, 1954).

In 1959, Ruegsegger et al. reported on the experimental use of fibrinolytic therapy with intravenous and intracoronary infusions of thrombin to lyse thrombi, formed in coronary arteries in dogs by the introduction of serum into a restricted segment of coronary artery. They followed events by serial coronary arteriography and demonstrated lysis of the intracoronary clots, after 3 to 7 hours in 4 of 5 dogs. Histological examination of the treated and control animals' myocardium showed less oedema and capillary damage in the lysed group, and perhaps more importantly did not find any evidence of damage induced by the thrombin therapy, and a little surprisingly did not even identify intramyocardial haemorrhage. Although this study was performed in small numbers of surviving animals, and used a drug which could not be widely applied because

of its scarcity, it did demonstrate that systemic treatment with a thrombolytic agent was feasible and possibly beneficial. This latter point, although far from proven, at least opposed previous work which suggested that coronary occlusion of 30 minutes produced ischaemic damage equivalent to that of permanent coronary occlusion (Blumgart et al., 1941).

In the same year, Fletcher et al., (1959) published on the maintenance of a sustained thrombolytic state in man with streptokinase. They treated 22 patients with myocardial infarction with an intravenous regimen of streptokinase over 30 hours in a dose designed to cause an intense lytic state. Their therapy was initiated very late by modern standards with 19 patients being treated at a mean time delay of 9 hours, and the remaining three receiving therapy from 34 to 65 hours after onset of symptoms. Although this study was essentially observational, and no conclusions could be drawn from it concerning any putative benefits, it was very important in that it demonstrated that a lytic state could be maintained in man without serious haemorrhagic complications.

#### **Early experience of intravenous streptokinase in acute myocardial infarction**

Between 1959 and 1966, there were 21 other feasibility studies of thrombolytic agents in myocardial infarction, with series of 5 to 100 patients, using streptokinase,

urokinase and plasmin (Simon, 1973). The first large-scale controlled trial was completed in 1966. This was a German-Swiss trial comparing streptokinase with heparin in 558 patients treated within 12 hours of onset of myocardial infarction (Schmutzler et al., 1966). They gave a loading dose of streptokinase to overcome resistance to streptokinase, guided by a pretreatment streptokinase resistance test, followed by an infusion lasting 18 hours with a variable dose designed to maintain a thrombolytic state. Although they showed a significant improvement in mortality in the streptokinase treated group (14.1% vs 21.7% at 40 days,  $p < 0.01$ ), their conclusions were flawed by their failure to adhere to their randomisation schedule and their breaking of an intention-to-treat analysis, thereby introducing potential selection biases, severely compromising the conclusions of the study.

The same group set up a second German-Swiss trial (Schmutzler et al., 1966), this time treating 269 patients with a fixed loading dose of 250,000 units, followed by 167,000 unit/hour for 18 hours compared to an alternately assigned, but non-blinded placebo group. All patients recruited were included in the analysis. Clearly, this allocation regimen is far from ideal, and although the baseline patient characteristics in the two groups were similar, a selection bias again cannot be ruled out. Their 40 day mortality was significantly better in the streptokinase treated group (14.5% vs 26%,  $p < 0.01$ ), as was

the 24 hour mortality rate (2% vs 10%,  $p < 0.001$ ). This benefit was confined to those patients treated within 6 hours of onset of symptoms. Interestingly, statistical significance was not achieved in the sub-group of patients treated in a coronary care unit rather than in a general ward.

The First European Working Party trial was established in 1963, and reported in 1969 (Amery et al., 1969) and recruited 167 patients presenting up to 72 hours after onset of symptoms of myocardial infarction. Patients received either heparin or streptokinase as a 1.25 MIU loading dose and 104,000 units/hour for 72 hours. In this study mortality was higher in the streptokinase group, although this trend did not reach statistical significance. Cardiac rupture was higher in the treatment group and there was a high incidence of side effects attributed to streptokinase.

The Second European Working Party trial in 1966 (European Working Party, 1971) recruited a more substantial 730 patients, presenting within 24 hours of chest pain. This study was randomised and double-blind, with patients receiving either streptokinase 250,000 units loading, then 100,000 units/hour for 24 hours, or heparin. On this occasion, mortality was significantly lower in the streptokinase group at 40 days (18.5% vs 26.3%,  $p < 0.01$ ). A contemporary Finnish study addressing the same question

showed no evidence of treatment benefit, but the study was flawed by a number of design faults. In particular, different centres within the study used differing treatment regimens, and generally the doses of streptokinase used were smaller than previously, and again patients were recruited up to 72 hours following onset of symptoms. In addition, there were differences in the pretreatment characteristics of the two groups, with a higher incidence of heart failure in the streptokinase group. Lastly, the control mortality rate was low, and the study lacked the necessary power to detect a treatment benefit.

The first high-dose short duration study of streptokinase in myocardial infarction was performed in Frankfurt in 1972, using an intravenous infusion of 750,000 units of streptokinase over 3 hours in 216 patients within 12 hours of chest pain, in a double-blind randomised protocol. Mortality was markedly improved in the streptokinase group (12.7% vs 27.9%,  $p < 0.01$ ).

Dioguardi et al. published in 1971 the results of a comparison of streptokinase and heparin, administered in a coronary care unit setting. They recruited 321 patients within 12 hours of symptoms, and the treatment group received 250,000 units streptokinase loading, then 150,000 units/hour for 12 hours. Although their study protocol appeared sound, they did not find any treatment effect, with near identical mortality rates in the two groups.

This may in part be accounted for by the relatively low numbers recruited, and a low mortality rate in the control group making a significant improvement difficult to demonstrate. The latter limitation recurs in later studies (v.i.).

The Australian multi-centre trial (Bett et al., 1973) treated 534 patients within 24 hours of onset of myocardial infarction, with the prolonged infusion regimen of streptokinase of 250,000 units loading, then 100,000 units/hour for 17.5 hours. Although none of their differences in mortality reached statistical significance, they found a trend to improvement in the streptokinase group (9.8% vs 12.6% at 3 months, NS) and identified relatively greater benefit in patients in high risk categories by sub-group analysis. Clearly, interpretation of this type of analysis must be qualified. In their discussion, the authors commented on the possibility of a Type II error, and felt that a positive result favouring streptokinase could not be ruled out, and elected to continue to recruit a further 500 patients. The second phase of this study reported in 1977 on a total of 747 patients, followed up for twelve months, but still found no significant difference in mortality between the two groups (Bett et al., 1977).

A United Kingdom coronary care unit based, multi-centre, placebo-controlled, randomised study of streptokinase was performed between 1971 and 1974 (Aber et al., 1976). They recruited 595 patients within 24 hours of onset of pain with a clinical diagnosis of myocardial infarction. The treatment group received 2.5 MIU of streptokinase over 24 hours, and follow-up was for 6 months. Mortality at discharge was 12.6% vs 13.7%, and at six months, 15.9% vs 17.8%, neither difference reaching significance.

A controlled trial of 512 patients randomised to a 24 hour intravenous infusion of either streptokinase (total of 2.65 MIU) or glucose, was carried out by the European Cooperative Study Group in 1979. Mortality was significantly lower in the streptokinase group at six months (15.6% vs 30.6%,  $p < 0.01$ ), this improved mortality becoming apparent statistically only after three weeks. They reported a much higher bleeding complication rate in the streptokinase group, with 18% of the streptokinase group suffering from gastrointestinal haemorrhage or oozing from puncture sites compared to only one of 157 control patients, with 3% requiring cessation of their streptokinase infusion. Two patients had cerebral bleeds, both in the streptokinase group.

Unfortunately, interpretation of the results were a little compromised by a higher prevalence of recognised risk factors in the control group and the fact that only 13.5%

of all their patients with myocardial infarction screened were admitted to the study. Nevertheless, the study did reach a statistically significant result in favour of streptokinase in a good-sized study population.

Ganz et al., in 1984, treated 81 patients within 3 hours of onset of myocardial infarction with a 15-30 minutes intravenous infusion of 0.75 or 1.5 MIU of streptokinase. They assessed reperfusion by immediate non-invasive criteria, with follow-up coronary angiography at 3-7 days. Their results suggested that this intravenous regimen could achieve coronary patency in 78 of the 81 patients although this was almost certainly an over-estimate.

There were several influential experimental papers published at the time of these early studies of prolonged intravenous infusion of streptokinase, which altered contemporary thinking about the use of thrombolytic therapy in acute myocardial infarction. Maroko et al., in 1972, published the results of experiments on dogs undergoing reperfusion after 3 hours of coronary artery occlusion. They demonstrated that reperfusion at this time was associated with myocardial salvage as reflected in less creatine kinase depletion, less marked histological changes and preservation of contractile function. The same group

in similar experiments, showed that reperfusion after three hours resulted in preservation of the subendocardial region of the myocardium, apparent histopathologically a week after occlusion, and reflected by preservation of myocardial creatine kinase activity (Ginks et al., 1972).

In the early 1970s, there was also discussion concerning the "no-reflow" phenomenon. This concept was originally applied to the cerebral and renal circulations and described the failure of even perfusion to a vascular bed following a period of arterial occlusion. This phenomenon was extended to the coronary circulation by Kloner et al., (1974) and led to further confusion regarding whether thrombolytic therapy and coronary reperfusion could possibly be beneficial, or may even be detrimental, on theoretical grounds. The histological study in dogs undergoing temporary coronary occlusion and reperfusion, showed that periods of ischaemia lasting less than 40 minutes were not associated with permanent histopathological features, but that if the duration of ischaemia was extended to 90 minutes, extensive capillary damage and myocyte swelling occurred in the affected area, increasing the tissue pressure and preventing flow of blood into the region - the "no-reflow" phenomenon. The study also confirmed a previous finding which had been a cause of much concern in early thrombolytic experiments, namely the

high incidence of reperfusion arrhythmias in dogs, notably ventricular tachycardia and fibrillation. In this series 24 of 57 dogs died either on coronary occlusion or immediately after sudden reperfusion.

Although this study also showed that the region in which the "no-reflow" phenomenon occurred was entirely contained within an area of irreversible myocardial cell necrosis, and therefore could be considered of little relevance, there followed much debate as to whether the reestablishment of coronary blood patency was worthwhile if no functional perfusion could be obtained. This and similar studies illustrated that relatively short periods of absolute ischaemia resulted in irreversible damage to myocardial cell, and therefore questioned the relevance of previous clinical thrombolytic studies with their exceptionally long treatment window. It also raised doubts as to whether thrombolytic therapy could be feasibly applied to the general population if such a short therapeutic window was available.

Further animal experimental studies by Reimer et al., (1977) provided valuable insight into the realistic time window for salvage of ischaemic myocardium, and demonstrated that restoration of coronary flow did diminish myocardial damage. It was a simple experiment, relating the duration of coronary occlusion in dogs to the degree and distribution of myocardial necrosis. They showed that

longer intervals of ischaemia resulted in a "wavefront" of ischaemic cell death proceeding from the endocardial to the epicardial region, and that reperfusion after 3 hours, and possibly as long as 6 hours resulted in the salvage of a rim of epicardial myocardium. Clearly this time window for intervention might be applicable to worthwhile clinical use, with the possibility of limiting myocardial damage in patients with myocardial infarction, but it also illustrated one explanation as to why the early thrombolytic studies failed to reveal any advantage of streptokinase treatment, namely that many of the patients were recruited to the studies with episodes of myocardial ischaemia which were so long that all vulnerable myocardium had already been irreversibly damaged.

Interpretation of these clinical studies of prolonged intravenous infusion of streptokinase in myocardial infarction was confounded by factors such as the small numbers involved in individual studies, allowing a Type II error; differing dosage regimens of streptokinase, where the benefits of a regimen may have been obscured by the diluting effects of ineffective doses; differing entry criteria, in particular differing duration of symptoms, and inadequate randomisation procedures in some of the studies. In addition to this, the impact of the introduction of coronary care units in the early 1970s confused the issue further by changing the mortality rate of acute myocardial infarction. This influence was cited as an explanation for

the apparent benefits of streptokinase in the earliest studies, which could not be reproduced in later studies, largely based in coronary care units (Simon et al., 1973). Later metanalysis studies (Stampfer et al., 1982, Yusuf et al., 1985) however suggested that many of these discrepancies could be explained by a Type II error (v.i.).

In some of these studies, the mortality rates in the control group were small, and on statistical grounds it would be difficult to show a difference in this context unless the benefit of the agent was immense, or the sample size enormous. In addition, any small benefit present in the sub-groups of patients treated early, would have been swamped by the larger numbers of patients treated who had already suffered a completed myocardial infarction. These small studies however did reach a positive result for streptokinase when grouped together in a metanalysis.

### **Experience with intracoronary streptokinase in acute myocardial infarction**

A seminal paper by deWood and his colleagues in 1980 radically influenced the subsequent development of thrombolytic therapy in the management of acute myocardial infarction. They assessed the prevalence of coronary obstruction in a series of 322 patients admitted within 24 hours of onset of acute transmural myocardial infarction. 87% of patients studied within 4 hours of onset showed coronary occlusion, decreasing to 65% of patients studied

12-24 hours after onset. In 52 of 59 patients with angiographic features of coronary thrombosis, they were able to retrieve thrombus using a Fogarty catheter.

This study established several important landmarks in acute myocardial infarction. Firstly, it demonstrated the central role of coronary thrombosis in the aetiology of myocardial infarction, and determined the incidence of spontaneous coronary recanalisation, thus explaining many of the discrepancies in the previous studies. Moreover, it showed that coronary angiography was feasible and safe so early in the course of myocardial infarction. This established the rationale for thrombolytic therapy beyond doubt, opened the way for intracoronary administration and acute angiographic assessment of efficacy, and emphasised the importance of time in the assessment of patency.

In the early 1980s interest centred on the use of streptokinase in acute myocardial infarction administered by the intracoronary route, and several studies looking at this were published. Unfortunately many of the published studies were not randomised, which although understandable from the ethical viewpoint, confounded the interpretation of the results.

Mathey et al. published a paper in *Circulation* in 1981, treating 41 patients with 2000 units/minute of streptokinase, along with attempted mechanical

recanalisation with a guide-wire, and intracoronary plasminogen. Using this technique, firstly they showed that at presentation, 39 of their 41 patients had occluded coronary arteries, with subtotal lesions in the remaining two. Coronary patency was reestablished in 73% within one hour using this combined treatment approach. Repeat angiography at 7-21 days showed persistent coronary patency in 12 of 15 patients. A significant improvement in left ventricular ejection by left ventriculography was demonstrated in those patients who successfully reperfused.

In a similar study, Ganz et al., in 1981, achieved coronary reperfusion in 19 of 20 patients treated within 3 hours of onset of myocardial infarction with Thrombolysin (streptokinase and plasmin) at an intracoronary subselective infusion rate of 2000-4000IU/minute. They also showed that improvement in left ventricular function was associated with patency. Similar reperfusion rates of 22 of 29 patients (76%) were achieved by Rentrop et al. (1981), and of 74 of 84 (88%) by Timmis et al. (1982). Although there were other similar studies performed at this time, none helped resolve the question of the efficacy or benefits of streptokinase because of the lack of randomisation and placebo control.

Some studies (Smalling et al., 1982, Ganz et al., 1981, Mathey et al., 1981) did however show a difference in left ventricular function between responders and non-responders

to intracoronary streptokinase. In the Smalling paper, reperfused patients showed a progressive improvement in left ventricular ejection fraction with time, which was not apparent in those remaining occluded. It was even more surprising that this observed benefit was observed up to eighteen hours after onset of symptoms. The lack of adequate trial design was emphasised in an editorial by Muller et al., in 1981, which called for placebo-controlled studies in what was increasingly recognised as an important and potentially beneficial area.

The first randomised study was published by Anderson et al., in 1983. It was however not placebo-controlled or blinded, the patients receiving active therapy of either intracoronary streptokinase and intravenous heparin, or control therapy of subcutaneous heparin. This study cannot be said to be an adequate test of intracoronary streptokinase, as the analysis is confused by these other issues. They achieved coronary patency in 19 of 24 (79%) of the streptokinase group, and compared changes in echocardiographic and radionuclide left ventricular function and clinical grading scores in the treated and control groups between days 1 and 10. They demonstrated an immediate and prolonged improvement in ventricular function and in the Killip clinical class following thrombolytic reperfusion.

Khaja et al. (1983) published a placebo controlled, randomised study of intracoronary streptokinase in 40 patients within 6 hours of onset of myocardial infarction. They showed a low 60% reperfusion rate with streptokinase compared to a reperfusion rate of 10% with placebo. They were unable to demonstrate any benefit associated with reperfusion in terms of preservation of left ventricular function.

A similar publication in 1984 (Leiboff et al., 1984) again showed the efficacy of intracoronary streptokinase in achieving coronary artery patency, this time using intracoronary nitroglycerine as a control therapy. Reperfusion of the infarct related coronary artery was achieved in 69% of the streptokinase group compared to only 17% of the nitroglycerine group in 43 patients. However, once again the authors failed to demonstrate any advantage of streptokinase therapy with respect to improvement of left ventricular ejection fraction acutely or at 7-10 days.

#### **Metanalysis of previous studies of streptokinase**

A variety of studies had shown that thrombolytic therapy administered by either the intravenous or intracoronary route was feasible and safe, at least in small numbers within the confines of a study protocol, and that the therapy could successfully reperfuse coronary arteries. However, up to this point although several studies had

shown benefit on mortality, all these studies had been based on small numbers of subjects and equally there were numerous other studies which had failed to show any benefit. Thus the literature was conflicting and it was unclear whether reperfusion was translated into benefit to the patients, either in preserving left ventricular function or in the ultimate gold standard of reduction in mortality. Two review and metanalytic papers published by the same statistically based group of workers helped to clarify the situation and thus stimulate the further investigation and use of thrombolytic agents.

The first article was published in 1982 by Stampfer et al. They pooled the results of eight randomised trials of intravenous streptokinase, where the control group was either placebo or anticoagulation, using mortality at approximately 40 days as their end point. The results of most of these trials have been discussed individually above. They calculated the risk ratio for each group, that is the proportion of deaths in the streptokinase group compared with the proportion of deaths in the control group for each trial, with a risk ratio less than one indicating a beneficial effect of streptokinase. Using this method of analysis, they found that six of their original eight studies showed a benefit of streptokinase, with a tendency for the larger studies to more clearly show benefit. Pooling the results, appropriately weighting the studies, they found an overall risk ratio of 0.8, which was

significantly less than one ( $p=0.01$ ), with a 95% confidence interval of 0.68 to 0.95. Excluding the two studies which recruited patients with duration of symptoms up to 72 hours, the pooled risk ratio became less, and more significant (0.74,  $p=0.001$ , 95% confidence limits 0.62 to 0.89), indicative of a very beneficial effect of intravenous streptokinase in patients treated within 24 hours of onset of symptoms of myocardial infarction, with a reduction in mortality from myocardial infarction by 26%, a figure remarkably similar to that found later in large mortality studies in the late 1980s.

The second publication in 1985 (Yusuf et al., 1985) pooled results from a total of 33 studies using intravenous and intracoronary thrombolytic agents in the treatment of acute myocardial infarction. Of the 24 trials of intravenous thrombolytic therapy they reviewed, only five achieved statistical significance at the 5% level in their own right, 11 showed a non-significant trend in favour of thrombolytic therapy, while the remaining eight showed a non-significant trend toward an adverse effect. Using similar methods as used in their earlier paper, they derived an "average" risk, or odds ratio of 0.78, with a 95% significance interval of 0.68 to 0.89, which suggested a highly significant ( $p<0.001$ ) reduction in mortality of 22%.

They applied similar techniques to nine studies of intracoronary streptokinase, which between them recruited less than 1000 patients. However, there were only 128 deaths in the entire study group, and it is hardly surprising that statistical significance was not achieved even using the pooled results. The calculated pooled average odds ratio was 0.8 or a mortality reduction of 20%.

They also attempted to address the question of the influence of delay in institution of therapy on efficacy of therapy. Dividing the time intervals in the different studies where the information was available, they were unable to show that earlier treatment with intravenous thrombolytic agents was associated with lower mortality. The intracoronary studies all tended to have short intervals between onset of symptoms and treatment, and therefore analysis of this effect was somewhat limited.

Although some studies had suggested that earlier treatment was associated with higher patency rates following intracoronary streptokinase, using their overview techniques, there could not confirm these findings. Embracing the use of streptokinase in coronary care units, they were able to provide evidence that coronary care units did not negate the benefits of thrombolytic therapy. Although this is not surprising, this question had been

seriously raised. It is likely that the apparent negation of the advantages of streptokinase can be attributed to the smaller overall mortality and hence a larger chance of a Type II error.

There are clearly limitations to this statistical overview methodology in the objective assessment of the value of a therapy. Notably, such analyses assume that all the data from all performed studies are included in the metanalysis. Exclusion of any relevant data, or a selection bias can radically influence the conclusions of any overview, and given the tendency in the literature for negative studies to be considered less important than positive studies, this possible influence cannot be ignored. In addition, inevitably this approach must include studies with differing protocols in terms of inclusion and exclusion criteria, and I have alluded to one or two of these differences above. All these considerations lead to difficulties in the interpretation of the finer details of the results, and the population to which these results can reliably be applied. Nevertheless, these papers did provide relatively crude evidence of a beneficial effect of thrombolytic agents in the treatment of acute myocardial infarction, and undoubtedly provided a stimulus to further research into these agents which up until that time, although they had been available for a number of years, were hardly used at all as a routine measure in this context.

### **Intravenous or intracoronary streptokinase?**

It had been at least provisionally established that thrombolytic therapy, at this stage almost exclusively using streptokinase, was beneficial in the treatment of acute myocardial infarction in terms of short term mortality, and possibly in terms of preservation of left ventricular function due to myocardial salvage. However the optimal dosage and route of administration was unclear, questions which remain unresolved to this day.

The intracoronary route offered visualisation of the affected coronary artery, and immediate assessment of therapeutic success in achieving patency, and therefore gave the opportunity to achieve subsequent coronary patency by additional mechanical methods. In addition, there was the attraction of giving a smaller dose of streptokinase, and possibly causing fewer haemorrhagic side effects.

However, there were also obvious drawbacks to this route of administration. Coronary angiography is a highly skilled, invasive and expensive procedure. The necessity of arterial cannulation in this group of patients in a lytic state was associated with an increase in bleeding complications (Merx et al., 1981). The procedure was associated with other risks, such as the provocation of serious arrhythmias and embolic phenomena, and although its risks in patients currently undergoing myocardial infarction had been overestimated, it was felt that such

added risk was detrimental to the patients' welfare. In addition, it was difficult to see how this procedure could be offered on a large scale to the general population. Lastly, although benefits of earlier administration of thrombolytic therapy were yet to be conclusively proven, it was still intuitively felt that therapy should be administered as early as possible in the course of myocardial infarction, and the organisation of coronary angiography must inherently be associated with a delay in the institution of therapy, in most studies of the order of 40-60 minutes.

Therefore, although intracoronary administration of streptokinase could continue as a research tool, it would only be feasible for general application if it were shown to have a major advantage over the intravenous route. In 1984, there was a randomised comparison of intracoronary and intravenous streptokinase published by Alderman and colleagues. In this study intracoronary streptokinase (total dose of 343,375 IU) achieved a reperfusion rate of 73% (11 of 15), taking a mean of 28 minutes, and intravenous streptokinase (total dose 725,505 IU) a reperfusion rate of 62% (8 of 13), taking a mean of 39 minutes. These differences were not significant, which is not surprising in this small sample. This did however show



that in a direct comparison there was no marked difference between the two treatment regimens. These results were comparable to similar published studies (Blunda et al., 1982, Tebbe et al., 1982).

Moreover this study found that the group which achieved reperfusion had significantly lowered fibrinogen levels early in the course of the infusion, immaterial of the route and dose of streptokinase received, and that the two treatment regimens were associated with similar falls in fibrinogen. These results implied that reperfusion was related to the intensity of systemic lytic changes, rather than local concentrations of streptokinase. This would be consistent with streptokinase's known indirect action requiring binding of circulating plasmin or plasminogen. This study also found that bleeding complications were related not to the route of administration and therefore total dose of streptokinase, but to the likelihood of reperfusion, and therefore the fall in fibrinogen and the administration of heparin, which was administered to those patients with patent arteries following angiography.

These findings relating a systemic lytic state to successful coronary reperfusion were supported by Rothbard and colleagues (1985) who found a systemic lytic state to be almost exclusively associated with reperfusion and occurring in most patients receiving "standard" intracoronary doses of streptokinase. It had also been

shown that mean intracoronary doses of streptokinase of 201,000 IU caused a fall in fibrinogen in excess of 70%, in 88% of patients (Cowley et al., 1983). However, there were other studies which did not find a fall in fibrinogen following comparable intracoronary doses of streptokinase (Rentrop et al., 1981).

Thus one major putative advantage was rejected, in that if intracoronary streptokinase caused systemic lytic effects, and these effects were possibly central to the mechanism of reperfusion, then there was no value in using the more invasive and cumbersome intracoronary route.

## **THROMBOLYTIC AGENTS**

In the years following the studies described above, the principle of thrombolytic therapy in acute myocardial infarction became increasingly accepted, until following the very large mortality studies of GISSI (1986), ISIS-2 (1988) and AIMS (1988) the benefits of this treatment were unequivocally proven. However, there remained and remains debate as to the best available thrombolytic agent.

The next section looks at these influential studies in the context of some of the individual thrombolytic agents available, comparing their pharmacological properties, and outlining the evidence available in support of their efficacy in acute myocardial infarction.

Three main drug classes have been developed namely streptokinase, anistreplase and tissue-type plasminogen activator (rt-PA). The pharmacological advantages of each drug and the evidence for their efficacy are discussed below. No mention of urokinase or pro-urokinase is made in this discussion, as this thesis does not contain work relating to these agents.

## **Streptokinase**

Streptokinase is a single chain protein with a molecular weight of 47,000 daltons, which is produced by Group C beta-haemolytic streptococci. In the circulation it combines with plasmin or plasminogen to form a complex which is a potent activator of plasminogen to plasmin (Anderson et al., 1987).

There are one or two major considerations with the clinical use of streptokinase in terms of efficacy and safety. Being a foreign protein, streptokinase is antigenic, and is neutralised by circulating antibodies which may have been stimulated by previous streptococcal infections, and in addition itself will stimulate a further antibody reaction. There is a small incidence of anaphylactoid reactions to streptokinase, and it is possible that their incidence may also be influenced by previously stimulated immunological responses. In many studies, this possibility has been catered for by the routine prophylactic use of chlorpheniramine and hydrocortisone, although the evidence for their efficacy in this context is not strong (Dykewicz et al., 1986, Murray et al., 1986). As a consequence, the immunological aspects of streptokinase treatment are of great interest and their consideration forms part of this thesis.

The administration of high doses of streptokinase is associated with a fall in blood pressure, the degree of which is related to the rate of infusion. This response limits the practical infusion rate of the drug. The mechanism of this blood pressure fall is obscure, and the haemodynamic responses to streptokinase and their possible causes are discussed later in this thesis.

Despite the negative aspects discussed above, streptokinase is effective and safe, as demonstrated in large scale studies (GISSI, 1986), and relatively inexpensive. It is the most commonly used thrombolytic agent in this country.

### **Anistreplase**

Anistreplase is an anisoylated plasminogen streptokinase complex, in which the active serine site on plasminogen is anisoylated with p-anisic acid, and protects the molecule from circulating plasma inhibitors, such as  $\alpha_2$ -antiplasmin and  $\alpha_2$ -macroglobulin. The streptokinase-plasminogen activator complex is still able to bind fibrin at its lysine binding site. In plasma, anistreplase undergoes deacylation by hydrolysis into the active moiety, which is a potent plasminogen activator. The preferential binding of the inactive complex by fibrin prior to activation confers a degree of clot specificity by

encouraging activation of the anistreplase complex at the clot surface. However, doses in excess of 10 units are associated with a systemic lytic state, due to release of plasmin into the circulation (Ferres, 1987).

Dose findings studies with anistreplase have sought a balance between efficacy in terms of a satisfactory reperfusion rate and limited systemic fibrinolysis (Hillis and Hornung, 1985). Dosing up to 15 units allowed successful initial reperfusion, but with a high reocclusion rate (Hillis et al., 1983). The currently recommended dose of 30 units is effective in achieving coronary patency, but is associated with a systemic lytic state reflected in a fall in plasma fibrinogen of approximately 30% (Been et al., 1986).

Although a dose-response relationship has been demonstrated for intracoronary anistreplase in doses of 15-30mg (Jones et al., 1984, Autrey, 1984, Kasper et al., 1984, Been et al., 1984), there is less convincing evidence for a dose-response relationship for intravenous administration (Leizorovicz et al., 1987). Doses in excess of 30 units have not been evaluated.

As anistreplase is a derivative of streptokinase, it can be expected to suffer from the same limitations of immunological and haemodynamic responses, which are also discussed as part of the work of this thesis. Its major

advantage over streptokinase lies in its ability to be administered as a single intravenous injection over 5 minutes, in contrast with the 60 minute continuous intravenous infusion recommended for streptokinase.

### **Tissue-plasminogen activators**

These serine proteases were described as long ago as 1946 (Astrup and Permin), but their lack of availability in any amount from human tissues limited interest until Collen et al. reported the isolation and purification of tissue-type plasminogen activator (t-PA) from a melanoma cell line in 1982. Subsequently, Pennica et al. (1983) cloned the gene responsible for the synthesis of t-PA and expressed it in *E.coli*, allowing the production of recombinant tissue-type plasminogen activator (rt-PA) in sufficient quantity to allow its evaluation and first use in man (van der Werf et al., 1983).

The advantages of rt-PA as a thrombolytic agent are several. Firstly being a human protein, it lacks antigenicity, and although associated with a small fall in blood pressure, is in general very well tolerated (Lew et al., 1985). The compound has a very short half-life in the circulation, of the order of 5 minutes (Garabedian et al., 1986), allowing close manipulation of administration regimens and brief alteration of haemostatic control.

Rt-PA is also relatively clot selective, at least in small doses. The mechanism of its clot specificity is the low affinity of the compound for circulating plasminogen, accompanied by a high affinity for fibrin, to which it binds at specific sites in the kringle structures. Plasminogen has a high affinity for this rt-PA-fibrin complex and binds it avidly, and then is activated at the clot surface to form plasmin.

The ability of rt-PA to lyse thrombi without initiating a lytic state was first demonstrated by van der Werf and co-workers in 1984 at first in animals, and then in patients (van der Werf et al., 1984), with the achievement of coronary reperfusion in 6 of 7 patients without depleting fibrinogen, plasminogen and alpha<sub>2</sub>-antiplasmin. However, early studies were associated with a high incidence of coronary reocclusion. Collen et al., (1984) achieved coronary reperfusion in 75% of patients with an intravenous infusion of 0.5-0.75mg/kg rt-PA, but 5 of a sub-group of 9 patients reperfused with intracoronary rt-PA suffered early reocclusion. Gold et al. (1986) reported a reocclusion rate of 45% in those patients with a persistent high grade stenosis and falling plasma rt-PA concentrations. In contrast, Verstraete et al., (1987) observed a reocclusion rate of only 8% within 24 hours.

Although the clot specificity of rt-PA was thought to be an important asset of this compound at the time of its development, doses of rt-PA necessary to provide satisfactory coronary reperfusion rates in fact cause a systemic lytic state. Rao et al., (1988) compared the degree of systemic fibrinogenolysis induced by 1.5 MIU of streptokinase infused over 1 hour to that following 80mg rt-PA infused over 3 hours. Although rt-PA caused a lesser effect, it did cause a 33% fall in fibrinogen at 5 hours and 57% fall in plasminogen, confirming a significant systemic lytic effect. Similarly Verstraete et al. (1985) showed that fibrinogen fell by 48% following a dose of rt-PA of 0.75 mg/kg. However current opinion now suggests that a degree of systemic activation may be advantageous in limiting early coronary reocclusion, and also reducing plasma viscosity.

The original form of rt-PA developed was a two-chain preparation (duteplase), but subsequently a single strand species was developed (alteplase) which had a higher specific activity (Garabedian et al., 1987, Collen, 1985) and which was able to be produced in greater quantity.

The major advantage of rt-PA remains its lack of antigenicity, although this must be balanced against its expense relative to streptokinase and anistreplase. For this reason, rt-PA is recommended as the drug of choice for patients with significant levels of antibody to

streptokinase, most probably following previous treatment with streptokinase-containing thrombolytic agents. Exactly how long the appropriate interval following treatment with streptokinase or anistreplase remains to a large extent unknown. The current recommendation is one year. In this thesis, further data are presented relevant to the antibody response to streptokinase and anistreplase, and the possible influence of acquired resistance on these agents' effects.

The manufacturer's recommended administration regimen is 100mg alteplase as a 3 hour decremental intravenous infusion, but there remains the possibility of manipulating both the total dose administered and the rate of administration to optimise this drug's thrombolytic efficacy. A large part of this thesis is concerned with the assessment of novel administration regimens of alteplase, with the assessment of the pharmacokinetic properties of the drug administered as boluses.

## **ASSESSMENT OF THROMBOLYTIC EFFICACY**

### **A. CORONARY REPERFUSION**

The original rationale of thrombolytic therapy is to restore coronary artery patency by lysing the occlusive thrombus, thus restoring the blood supply to the ischaemic muscle beyond the occlusion, and so salvaging myocardium under jeopardy.

**Coronary Reperfusion** can be assessed angiographically, in recent times in accordance with the TIMI criteria (Williams et al., 1986), and requires a pretreatment coronary angiogram to exclude a sub-total occlusion as the cause of the myocardial infarction (deWood et al., 1980). Many of the early studies using intracoronary administration of thrombolytic agent assessed reperfusion, but a pretreatment angiogram necessarily delays thrombolytic therapy. Hence, other studies have assessed **coronary patency** at a given time point, conventionally 90 minutes after therapy. The difference between these two measures is the incidence of spontaneous reperfusion, as reported by deWood et al. (1980), being approximately 15-20% at this time point.

The time element is of crucial importance in regard to the assessment and comparison of thrombolytic agents/regimens in achieving coronary patency. Without thrombolytic therapy, occluded coronary arteries reperfuse spontaneously in due course (deWood et al., 1980), and patency rates with all thrombolytic agents increase with time following

therapy. For example the coronary patency rates with 5 units of anistreplase and 1.5 MIU streptokinase assessed at 90 minutes are 55% and 53%, but assessed 24 hours after therapy, patency increases to 81% and 87.5% (Hogg et al., 1990).

In addition the time from onset of myocardial infarction to institution of thrombolytic therapy is critical, as the longer the delay, the lower the reperfusion rate (Smalling et al., 1982). For example, in the early assessment of anistreplase in small patient numbers, an excellent coronary reperfusion rate was achieved (Been et al., 1985, Kasper et al., 1986). When Timmis et al., (1987) studied the same dose in similar numbers, by extending the therapeutic time window from 3 to 6 hours, the reperfusion rate dropped to 56%. When assessed in larger numbers in a multicentre trial (Anderson et al., 1988), the reperfusion rate with a 6 hour time window was 51%, a rate confirmed by Hogg et al. (1990).

The next section and figures illustrate the efficacy of the major thrombolytic agents with respect to restoring coronary patency.

### **Streptokinase**

The ability of streptokinase to achieve coronary reperfusion/patency has been discussed in the earlier part of this chapter, dealing with the historical basis of

reperfusion therapy. In brief, intracoronary doses of streptokinase are associated with reperfusion rates ranging from 60 - 87% (Timmis 1982, Figure 1).

The use of intravenous streptokinase carries obvious advantages in terms of ease of use, and more rapid administration without the need for coronary angiography. As mentioned above, the study by Alderman et al. (1984) compared intracoronary and intravenous streptokinase, and reperfusion, and demonstrated that intravenous administration of streptokinase caused acceptable levels of coronary reperfusion (62%). The advent of intravenous therapy meant that the delay necessary for pretreatment angiography was unacceptable, hence most of the studies of intravenous therapy have assessed coronary patency.

**CORONARY REPERFUSION RATES  
WITH INTRACORONARY STREPTOKINASE**

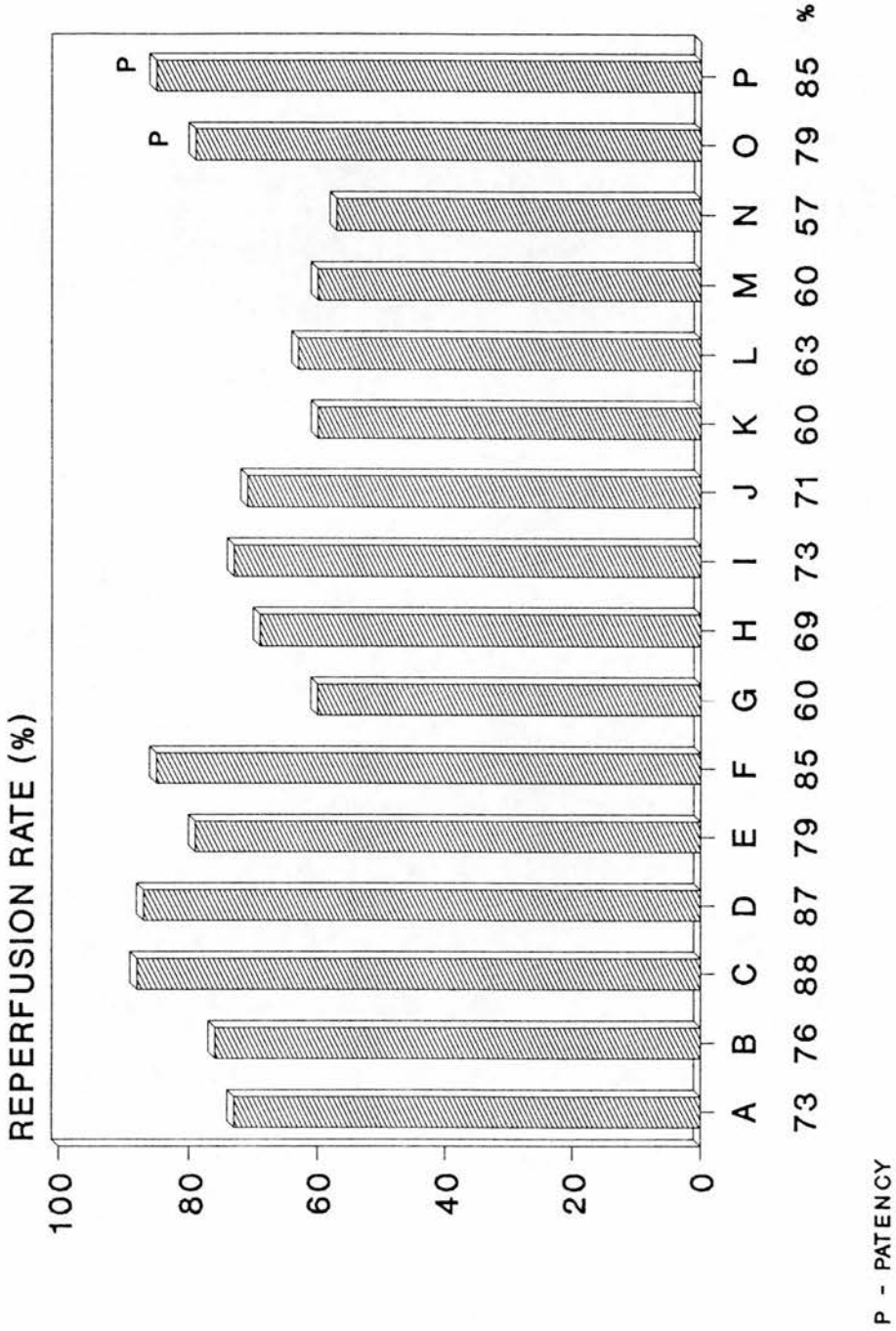


Figure 1. REPERFUSION RATES FOLLOWING INTRACORONARY STREPTOKINASE

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**Code for Figure 1.**

**CORONARY REPERFUSION RATES WITH INTRACORONARY STREPTOKINASE**

<b>CODE</b>	<b>STUDY</b>	<b>REPERFUSION RATE</b>	<b>DOSE COMMENTS</b>
A	Mathey, 1981	73%	2000 u/min, n=30
B	Rentrop, 1981	76%	1000-2000 u/min, n=29
C	Timmis, 1982	88%	mean 211 u, n=84
D	Cowley, 1983	87%	201,00 ± 74,000 u, n=23
E	Anderson, 1983	79%	mean 5000 u/min, n=24
F	Gold, 1983	85%	2000-6000 u/min, n=40
G	Khaja, 1983	60%	15,000 + 5000 u/min, n=20
H	Leiboff, 1984	69%	mean 240,000 u, n=22
I	Alderman, 1984	73%	mean 343,000 u, n=11
J	Kennedy, 1985	71%	4000 u/min, max 350000, n=134
K	Rentrop, 1989	60%	240,000 u, n=67
L	Kaspar, 1987	63%	250,000 U, n=16
M	Anderson, 1988	60%	240,000 U, n=111
N	Tennant, 1984	57%	240,000 U, n=35
O	Anderson, 1983	(P)79%	<300,000 u, n=50
P	Simoons, 1986	(P)85%	<250,000 u, n=533

**P = Patency**

Reported rates for intravenous therapy range from 31-75%, including those studies assessing coronary patency within 2 hours of therapy. The patency rates from these studies are summarised in Figure 2. The studies assessed all use the "standard" streptokinase dose of 1.5 MIU, although the duration of treatment has varied, and in some cases is given in conjunction with other agents.

### **Anistreplase**

Although early studies with intravenous anistreplase suggested that it may be as effective as intracoronary streptokinase in restoring coronary patency, this has not been confirmed in subsequent studies. Figure 3 summarises the available data. 30 U of anistreplase as a 5 minute intravenous injection results in coronary patency rates of 46-83%, although the high patency rates of Kaspar et al. (1986) must be interpreted with caution in view of the wide confidence limits, and patency of the order of 50-70% seems a reasonable interpretation.

**CORONARY PATENCY RATES  
WITH INTRAVENOUS STREPTOKINASE**

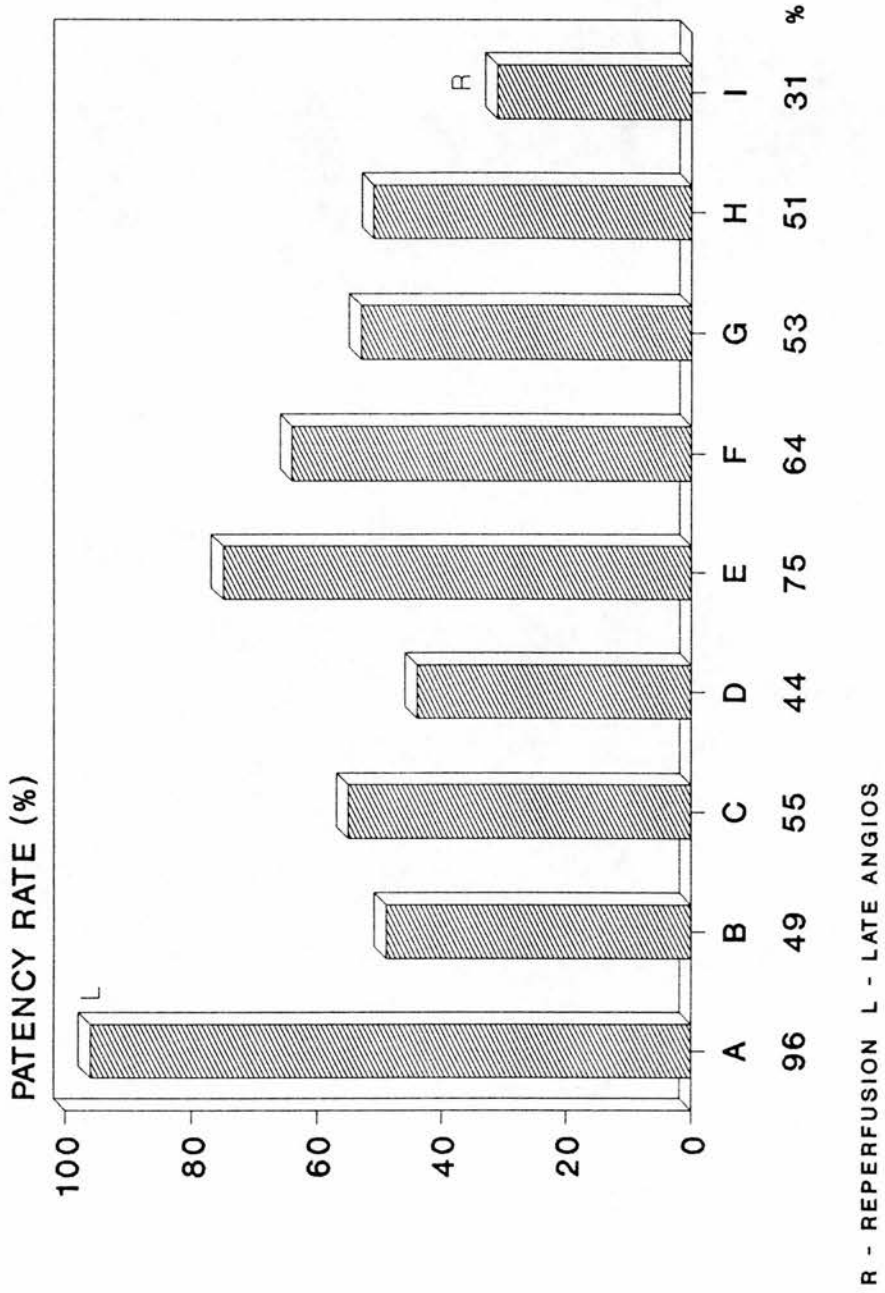


Figure 2. PATENCY RATES FOLLOWING INTRAVENOUS STREPTOKINASE  
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Code for Figure 2.

**CORONARY REPERFUSION RATES WITH INTRAVENOUS STREPTOKINASE**

<b>CODE</b>	<b>STUDY</b>	<b>PATENCY RATE</b>	<b>DOSE COMMENTS</b>
A	Ganz, 1984	96%	.75 or 1.5 MIU, n=78, non-invasive assessment, late angios
B	Spann, 1984	49%	.85 or 1.5 MIU, n=43,
C	ECSG, 1985	55%	1.5 MIU, n=62
D	Stack, 1988	44%	1.5 MIU, n=216
E	Grines, 1989	75%	1.5 MIU+50mg rt-PA, n=40
F	PRIMI, 1989	64%	1.5 MIU, n=203
G	Hogg, 1990	53%	1.5 MIU, n=63
H	Brochier, 1987	51%	1.5 MIU, n=58
I	TIMI-1, 1987	31%(R)	1.5 MIU, n=159

**R = Reperfusion**

**CORONARY PATENCY/REPERFUSION RATES  
WITH ANISTREPLASE**

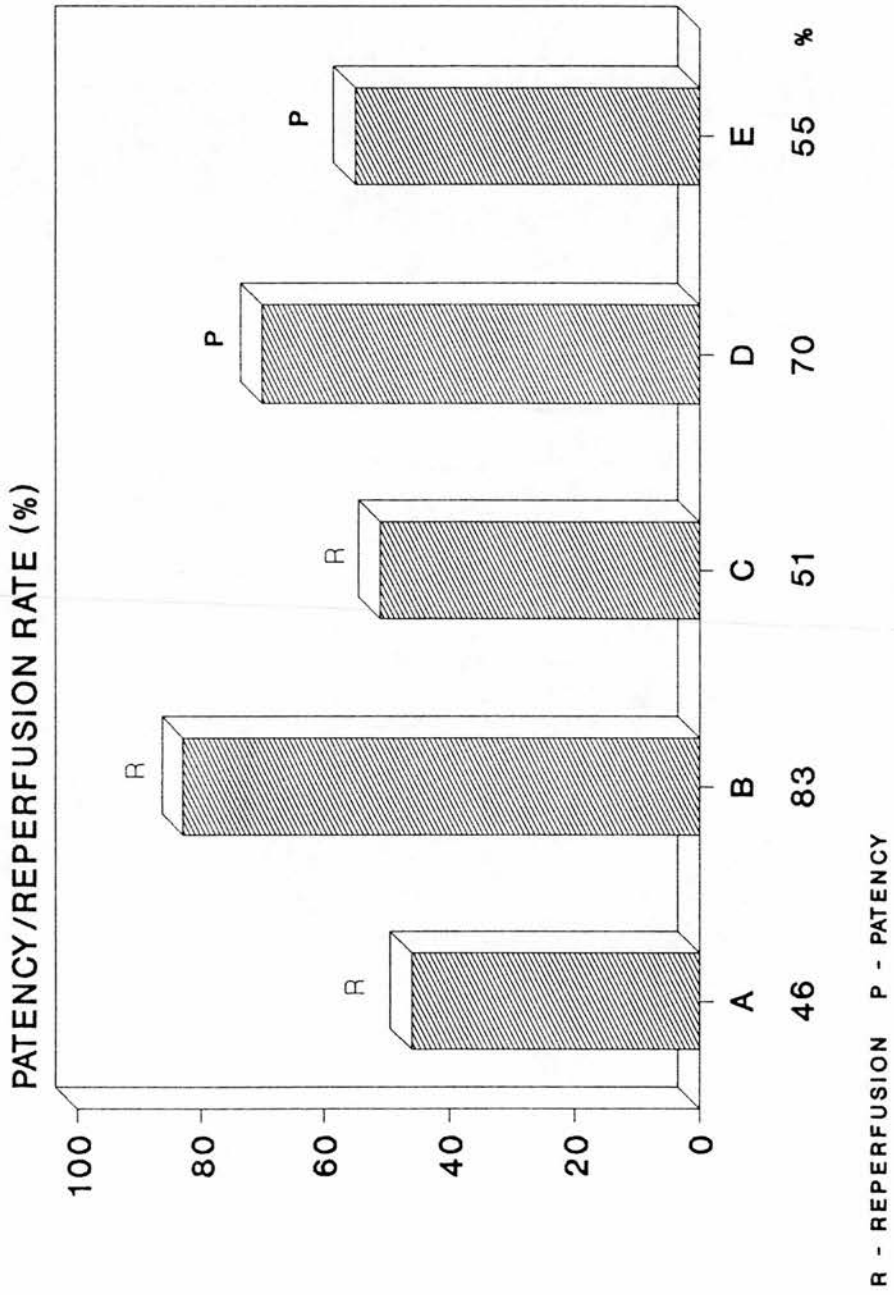


Figure 3. PATENCY RATES FOLLOWING INTRAVENOUS ANISTREPLASE  
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Code for Figure 3.

**CORONARY PATENCY/REPERFUSION RATES WITH ANISTREPLASE**

<b>CODE</b>	<b>STUDY</b>	<b>PATENCY/ REPERFUSION</b>	<b>DOSE COMMENTS</b>
A	Bonnier, 1988	46%	30 U, n=42
B	Kaspar, 1987	83%	30 U, n=8
C	Anderson, 1988	51%	30 U, n=115
D	Brochier, 1987	70%(P)	30 U, n=58
E	Hogg, 1990	55%(P)	30 U, n=65

**P = Patency**

## **Tissue-type plasminogen activators**

There is a considerable literature available on the ability of rt-PA to achieve or restore coronary patency in acute myocardial infarction. The data are however difficult to interpret for reasons discussed below.

Several confounding influences have led to difficulty in the interpretation of the efficacy of alteplase in restoring coronary patency. Early studies were performed with double-chain r-tPA, or duteplase, whereas more recent studies have used single-chain r-tPA, or alteplase, and it is known that the specific activities of these two preparations differ (Garabedian et al., 1987, Muller et al., 1987). In addition, whereas some studies have observed patency, others have observed reperfusion of an artery occluded before treatment. The index artery may not necessarily be completely occluded at time of presentation, and also, spontaneous reperfusion of a previously occluded artery may occur (deWood 1980), therefore patency and reperfusion rates differ. In addition and most importantly, widely varying doses and dosage schedules have been used in different studies based on differing study populations.

Verstraete and co-workers (1985) have demonstrated patency rates of 61% at 75-90 minutes post-therapy using 0.75 mg/kg of double-chain rt-PA over 90 minutes. The same group using a 40mg infusion of double-chain rt-PA over 90

minutes, achieved a coronary patency rate of 66% at 90 minutes (Verstraete et al., 1987). Topol and the TAMI group (Topol et al., 1987, Topol et al., 1989a, 1989b) have achieved a patency rate of 68% patency using 70 mg of double-chain r-tPA over 90 minutes, and 79% using the high dose of 1.5 mg/kg of alteplase over 4 hours, in conjunction with a high dose of heparin. The TIMI group in TIMI-1 (TIMI-1, 1985, Chesebro et al., 1987) used 80 mg of double-chain rt-PA over 3 hours, finding a reperfusion rate of 56%. Williams et al. (1986) using the same dose of 80 mg over 3 hours found a similar reperfusion rate of 68%.

A study by Neuhaus et al. (1989) suggested that accelerated administration of rt-PA may increase coronary patency. They studied a 100mg dose of alteplase over 2.5 hours, given as a 15mg bolus, then 50mg over 30 minutes and 35 mg over the next 90 minutes. They demonstrated a patency rate of 74% (95%CL: 62-84%) at 60 minutes, increasing to 91% (82-96%) at 90 minutes. More recently, a front-loaded regimen of alteplase of 15mg bolus, 50mg over 30 minutes and 35mg over 60 minutes has been studied in comparison with 30 units of intravenous anistreplase, in the TAPS study (von Essen et al., 1991). These results have recently been presented in abstract form only. This regimen achieved 73.4% coronary patency at 60 minutes, and 84.4% patency at 90 minutes, maintained to 24 hours. These

patency rates were significantly higher than that achieved with anistreplase, although there was a higher reocclusion rate with alteplase.

Published experience with bolus doses of alteplase is limited. Verstraete et al. (1989) using single boluses of alteplase, found that doses of 60 mg and 50 mg were associated with reperfusion rates at 90 minutes of 32% and 45% respectively, although a maximum dose of 70 mg achieved 72% reperfusion. However, this study was extended to include 60 patients, using the 70mg bolus dose (80mg in patients weighing more than 90kg) (Tranchesi et al., 1991). The reperfusion rate at 60 minutes was 55% (43-66%), and 48% (37-60%) at 90 minutes, suggesting that this regimen was not ideal for clinical use.

Khan et al., (1990) have evaluated 4 boluses of 25mg over 60 minutes of the double-chain t-PA and demonstrated recanalisation in 11 of 14 patients, and suggested that this regimen achieves coronary patency more rapidly.

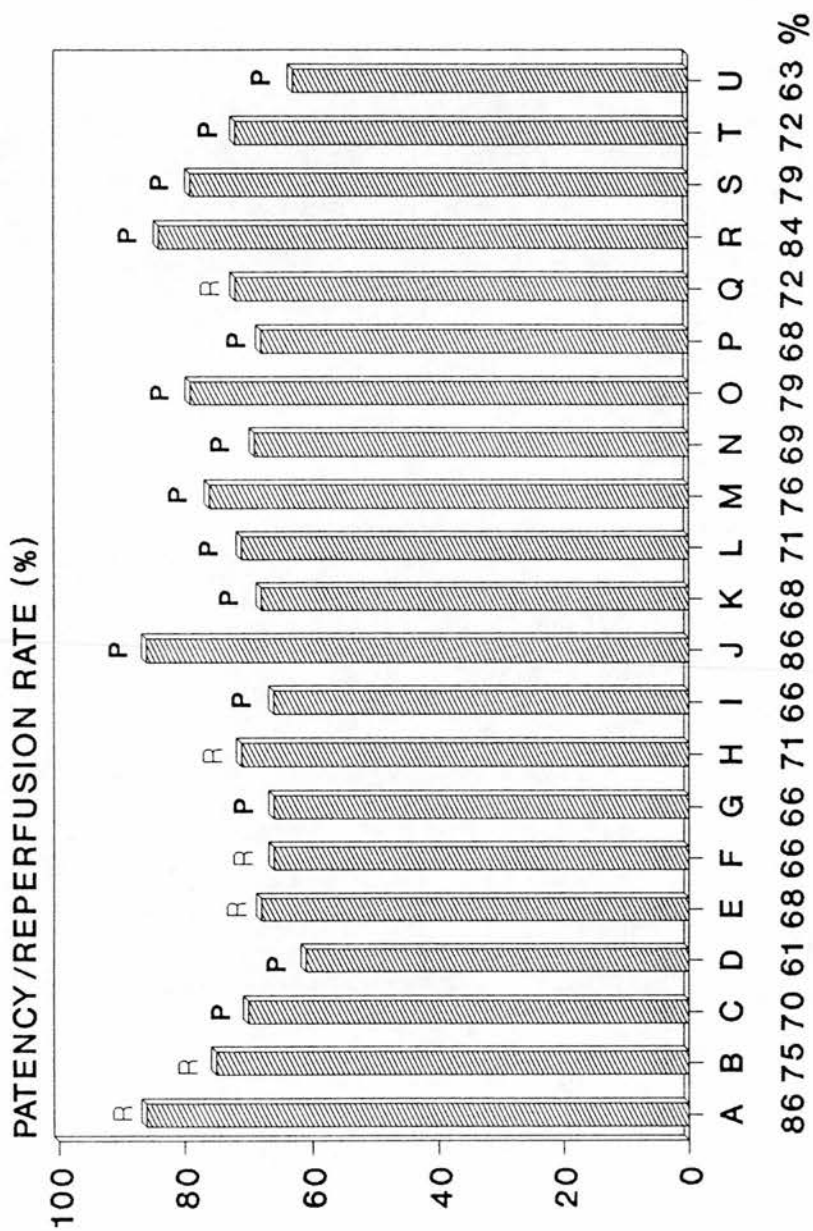
A small study by Smalling et al., (1990) using rapid intravenous infusion of a weight-adjusted dose of alteplase, and a median dose of 145mg, reported a 90 minute patency rate of 84%, which was significantly higher than the control group who received a conventional 3 hour infusion of 100mg.

Some of the studies evaluating coronary reperfusion or patency at 90 minutes are summarised in Figure 4. Overall reported coronary patency rates at 90 minutes after therapy have varied between 53% patency and 86% reperfusion.

#### **B. PRESERVATION OF LEFT VENTRICULAR FUNCTION**

A further aim of thrombolytic therapy is the reduction in left ventricular necrosis, thereby preserving left ventricular function. Several methods are available for the assessment of left ventricular function. Left ventriculography has been used, but because of its invasive nature makes serial estimates of left ventricular function difficult. Although echocardiography is non-invasive and easily repeated, the quality of image obtained may preclude accurate assessment of left ventricular volumes in a proportion of individuals, and there may be considerable inter-observer errors. Many studies have used radionuclide angiography, which is non-invasive reproducible and yields information on both global and regional contractile function.

**CORONARY PATENCY/REPERFUSION RATES  
WITH TISSUE PLASMINOGEN ACTIVATOR**



R - REPERFUSION P - PATENCY

Figure 4. REPERFUSION/PATENCY RATES FOLLOWING INTRAVENOUS rt-PA

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**Code for Figure 4.**

**CORONARY REPERFUSION/PATENCY RATES WITH rt-PA**

CODE	STUDY	PATENCY/ REPERFUSION	DOSE COMMENTS
A	ECSG, 1984	86%	0.54-1.4 x10 <sup>6</sup> IU/30-60min, IC/IV, D, n=7, R
B	Collen, 1984	75%	0.5-0.75mg/kg/30-120min, n=33,D,R
C	ECSG, 1985	70%	0.75mg/kg/90min,D,n=61,P
D	ECSG, 1985	61%	0.75 mg/kg, D, n=62 P
E	Williams,1986	68%	80mg/3h, R, n=37,D
F	TIMI-1,1987	66%	80mg/3h, D,n=157 R
G	ECSG, 1987	66%	40mg/90min,P
H	Muller, 1987	71%	100mg/3h, D,R
I	Verstraete,1987	66%	40mg/90min,n=123,P,D
J	Neuhaus, 1988	86%	85mg/90min,
K	Armstrong, 1989	68%	0.31-0.94 Mu/kg/90min, D,n=223, P
L	Grines, 1988	71%	1.25mg/kg/3h,?A,n=38,P
M	Johns, 1988	76%	1mg/kg/90min,n=68,P,?D
N	GAUS, 1988	69%	70mg/90min, n=125 P
O	Topol,TAMI-3,1989	79%	1.5mg/kg/4h,n=131,P,A
P	TOPOL,TAMI,	68%	70mg/90min,P
Q	Verstraete, 1990	72%	70mg bolus, R, n=25
R	Smalling,1990	84%	median 145mg,rapid inf,?A,P
S	Khan, 1990	79%	100mg,4 boluses/60min, D,n=14,P
T	Tranchesi, 1990	72%	70mg, bolus A, P,n=28
U	Koster, 1990	53%	100mg/90min,n=32,D,P

Code for Dose

**A = alteplase**  
**D = duteplase**  
**R = reperfusion**  
**P = patency**  
**IC = intracoronary**  
**IV = intravenous**

It is important when assessing left ventricular function in the post infarct period to take into consideration several factors. Firstly, it is well documented that left ventricular ejection fraction in patients with acute myocardial infarction shows spontaneous improvement even when not treated with thrombolytic therapy (Dewhurst & Muir, 1983, Schwarz et al., 1982). Schwarz and co-workers showed the time course of the improvement to be two weeks and correlated with the amount of residual flow to the infarct zone, with the mean change in ejection fraction for patients with residual flow being  $6.9 \pm 2.3\%$  compared to  $-2.2 \pm 1.7\%$  without ( $p < 0.01$ ). Clearly in the absence of adequate controls this would make data on improvement in left ventricular function difficult to interpret.

Secondly, it is probably necessary to consider not only global ejection fraction, but to examine changes in regional wall motion. Rogers et al. (1984) showed that in those patients with no flow to the infarct zone, either anterograde or via collaterals, there was a fall in global ejection fraction over the two weeks following infarction, which was due to a reduction in systolic contribution from the non-infarct zone, most likely due to a non-sustained initial hyperkinesis of the non-infarct zone. It might be expected that this compensatory hyperkinesis may vary between different individuals, and be modified by previous myocardial infarction.

Thirdly, in many of the studies assessing left ventricular function after thrombolytic therapy, the ejection fraction data is incomplete in up to 30%, possibly introducing a systematic error. This is particularly likely, as those patients in whom left ventricular function data is incomplete, are more likely to be those who are haemodynamically unstable, that is those with larger infarcts, and/or a poorer response to therapy.

Early publications by Reduto et al. (1981) and Smalling et al. (1982) suggested that thrombolytic therapy in acute myocardial infarction could preserve left ventricular function. These early studies were controlled but not randomised, and the randomised studies of Anderson et al. (1983) and Khaja et al. (1983) published conflicting results. Ritchie et al., (1984) were unable to demonstrate a benefit of intracoronary streptokinase on infarct size or left ventricular function. However they were subsequently able to show that intravenous streptokinase reduced infarct size and preserved left ventricular ejection fraction (Ritchie et al., 1988). The benefits in the intravenous study were confined to those patients with anterior infarction treated within 3 hours of onset of symptoms, and they explained the discrepancy between their studies on the basis that the time to treatment in the intravenous study was on average one hour shorter.

Overall, despite the limitations discussed above, benefit in regard to left ventricular function has been demonstrated for the available thrombolytic agents. Figure 5 illustrates the available data from controlled trials of the difference in ejection fraction of those patients receiving different forms of thrombolytic therapy. In 11 of the 16 data sets, the 95% confidence limits fail to cross the zero line, and it can be concluded that thrombolytic therapy preserves myocardial function, presumably by limiting infarct size.

CHANGE IN EJECTION FRACTION  
MEAN (95% CONFIDENCE LIMITS)

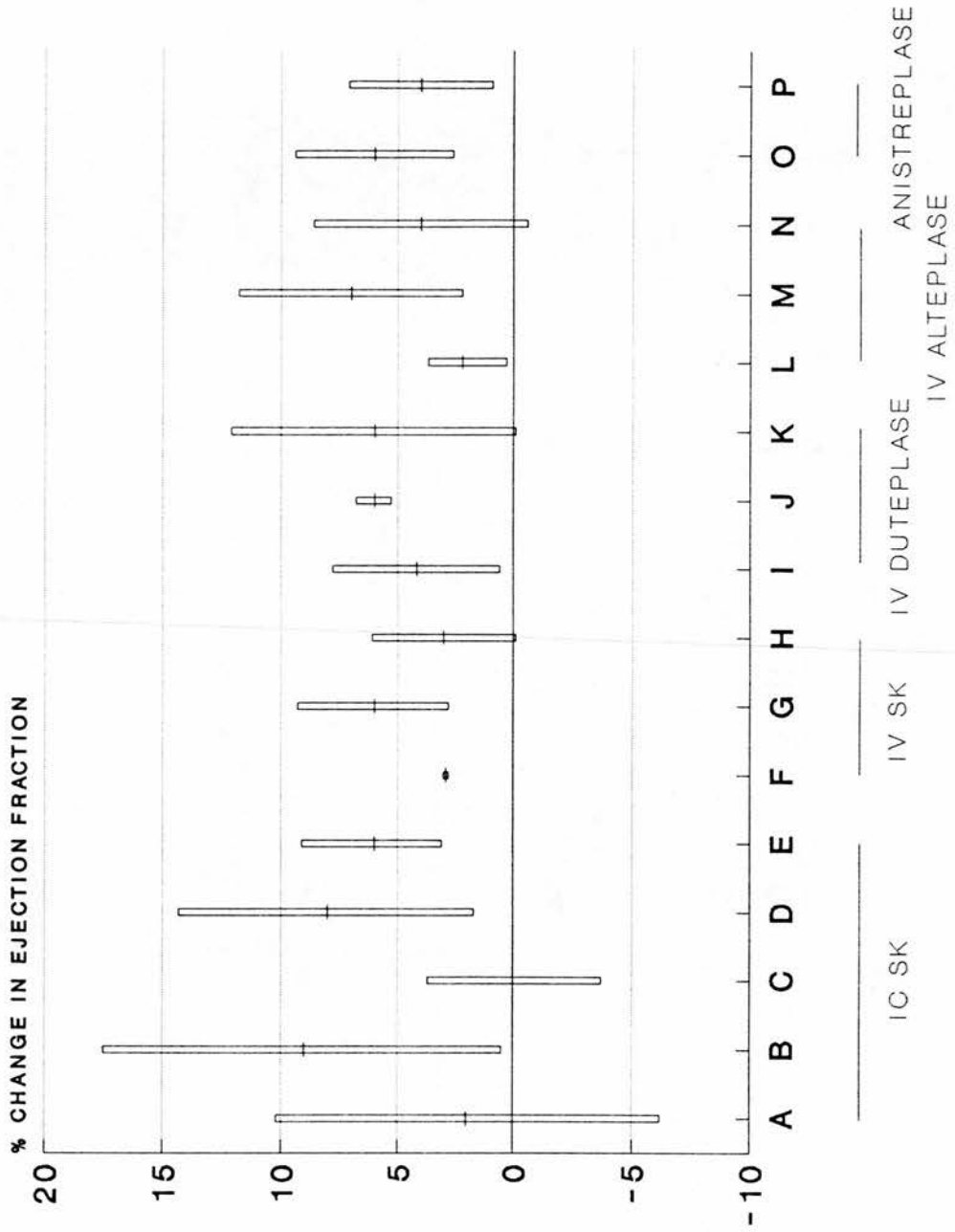


Figure 5. CHANGE IN LEFT VENTRICULAR FUNCTION RELATIVE TO PLACEBO WITH DIFFERENT THROMBOLYTIC AGENTS  
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**Code for Figure 5.**

<b>CODE</b>	<b>STUDY</b>	<b>NUMBER OF PATIENTS</b>	<b>TREATMENT</b>	<b>TIME OF EVALUATION</b>
A	Khaja, 1983	40	IC SK	1 hour
B	Khaja, 1983	40	IC SK	2 days
C	Ritchie, 1984	250	IC SK	8-9 wks
D	Anderson, 1983	50	IC SK	10 days
E	Serruys, 1986	533	IC SK	2 weeks
F	Schroeder, 1989	1741	IV SK	1 month
G	White, 1987	219	IV SK	3 weeks
H	Kennedy, 1988	368	IV SK	3 weeks
I	Armstrong, 1989	118	IV alteplase	4 hours
J	Armstrong, 1989	118	IV alteplase	9 days
K	NHFAustr, 1988	144	IV alteplase	1 week
L	van der Werf, 1988	721	IV alteplase	10-22d
M	O'Rourke, 1988	147	IV alteplase	3 weeks
N	O'Rourke, 1988	147	IV alteplase	3 weeks
O	Bassand, 1989	231	IV anistreplase	2-7 d
P	Bassand, 1989	231	IV anistreplase	2-3 wks

**Code for Treatment**

**IV = intravenous**

**IC = intracoronary**

**SK = streptokinase**

### **C. MORTALITY STUDIES**

In many respects, the acid test of the efficacy of thrombolytic therapy is whether the prognosis of acute myocardial infarction is altered in terms of improved survival. There have now been several large mortality studies performed which allow us to reliably answer this question (Figure 6).

As has been already discussed, early small studies of streptokinase in acute myocardial infarction showed variable effects on mortality, and it was the metanalysis of the combined results which first implied that there was a consistent improvement in mortality.

The first major mortality study was GISSI, which randomly assigned 11806 patients to either intravenous streptokinase or standard therapy, assessing mortality after 3 weeks (GISSI, 1986) then again at 12 months (GISSI, 1987). These papers, irrevocably establishing the benefits of intravenous streptokinase, showed an 18% relative reduction in mortality at 21 days (an absolute reduction of 2.3% mortality,  $p=0.0002$ , relative risk 0.81), which remained a 9% relative reduction in mortality at 12 months (an absolute reduction of 2.8% mortality in 11712 patients,  $p=0.008$ , relative risk 0.90).

CHANGE IN MORTALITY  
MEAN (95% CONFIDENCE LIMITS)

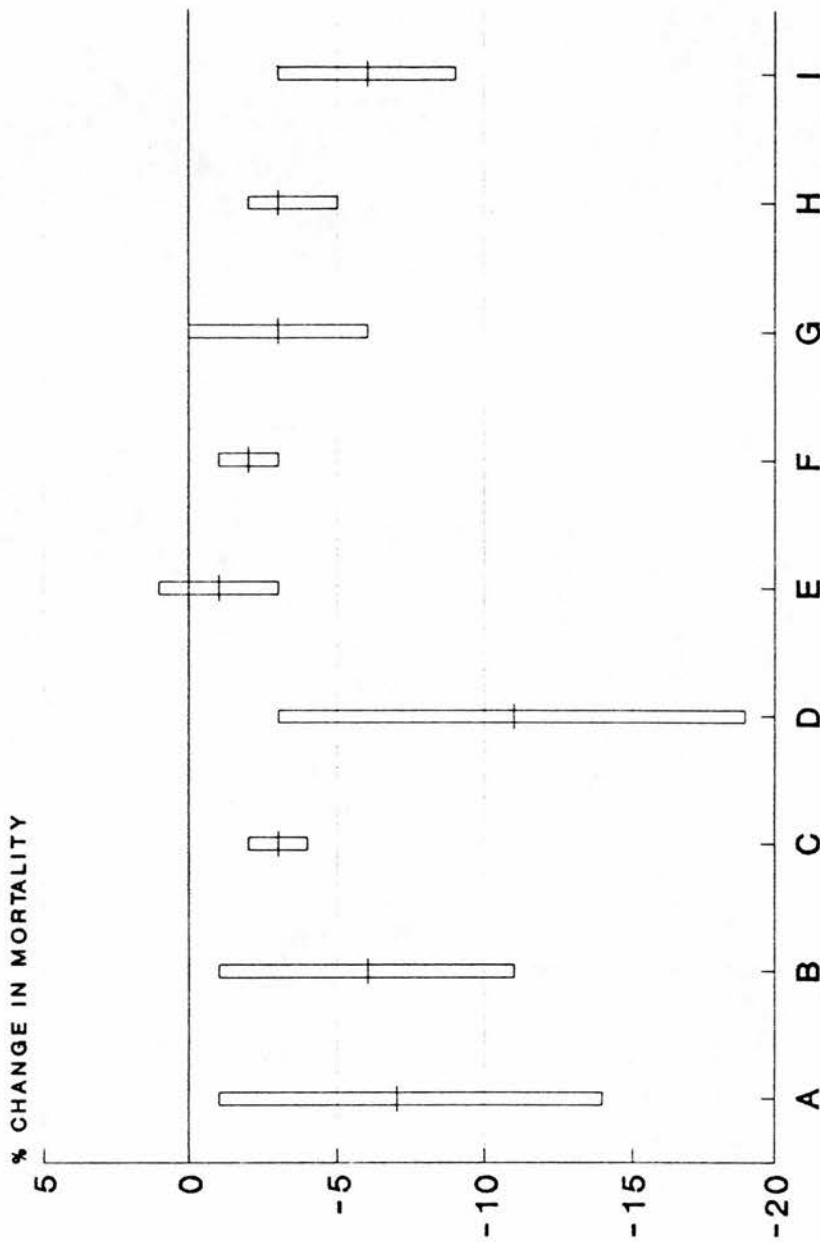


Figure 6. CHANGE IN MORTALITY RELATIVE TO PLACEBO WITHIN THREE MONTHS FOLLOWING THROMBOLYTIC THERAPY  
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**Code for Figure 6.**

**CHANGE IN MORTALITY RELATIVE TO PLACEBO WITHIN THREE MONTHS FOLLOWING THROMBOLYTIC THERAPY.**

<b>CODE</b>	<b>STUDY</b>	<b>DRUG</b>	<b>DIFFERENCE IN MORTALITY</b>	<b>COMMENTS</b>
A	Western Washington	IC SK	-7%	n=250, <12h
B	ICIN	IC SK	-6%	n=533, <4h
C	ISIS-2	IV SK	-3%	n=17187, <24h
D	White	IV SK	-11%	n=219, <6h
E	ISAM	IV SK	-1%	n=1741, <6h
F	GISSI-1	IV SK	-2%	n=11806, <12h
G	ECSG-4	IV D	-3%	n=721, <5h
H	ASSET	IV A	-3%	n=5011, <5h
I	AIMS	IV AN	-6%	n=1258, <6h

Code for Dose  
**A = alteplase**  
**D = duteplase**  
**R = reperfusion**  
**P = patency**  
**IC = intracoronary**  
**IV = intravenous**

The greater benefits of early therapy were also clearly described. The relative risk of death following streptokinase received within 3 hours of onset of symptoms of myocardial infarction was 0.74, rising to 0.87 for 6-9 hours, and 1.19 for 9-12 hours. However, the benefits from treatment beyond 6 hours were not clear, as the relative risks for these delays were not significantly different from unity. The relatively few patients who received thrombolytic therapy within 1 hour of onset of symptoms had an extraordinarily low relative risk of 0.49, with 95% confidence intervals of 0.34-0.69. This pattern persisted in the mortality improvement at 12 months, with the relative risk of the subgroup of patients being treated within 1 hour being 0.73, although the confidence intervals were widened to embrace unity.

This study also established the safety of intravenous streptokinase in this population, with the complication rate in the streptokinase group being low, with an incidence of stroke of 0.2%, which was comparable for the two groups, of major bleeds of 0.3%, and of anaphylactoid reactions of 0.1%. As this study was unblinded, it must be considered that this was an over-estimate of the true incidence.

The second large scale study of intravenous streptokinase was the Second International Study of Infarct Survival (ISIS-2, 1987). This study was based on 17187 patients with a clinical diagnosis of acute myocardial infarction who were randomised to receive intravenous streptokinase, oral aspirin, both or neither, in a placebo controlled design. Mortality was assessed at 5 weeks, and there was a 25% relative reduction in vascular deaths at this time point in those receiving streptokinase (95% CL 18-32%, 9.2 vs 12.0%, absolute reduction 2.8%,  $p < 0.00001$ ). In addition aspirin alone conveyed considerable advantage, with a 23% relative reduction in mortality compared to placebo (9.4 vs 11.8%, absolute reduction 2.4%,  $p < 0.00001$ ). The combination of streptokinase and placebo achieved an unparalleled reduction in mortality of 42% (95% CL 34-50%, absolute reduction 4.8%, 13.2 vs 8.0% ,  $p < 0.00001$ ), establishing the gold standard for thrombolytic therapy in the future.

Again the beneficial influence of early treatment was confirmed, with those treated within 4 hours of symptoms gaining a reduction in odds ratio of vascular death of 35%, compared to 16% and 21% for those receiving treatment within the 5-12 and 13-24 hour windows respectively. The reduction in odds ratio achieved statistical significance in the 5-12 hour group but not in those receiving therapy after 12 hours.

Study of the adverse event profile in this large blinded, placebo controlled trial was also rewarding. Hypotension and bradycardia was considerably more common in the streptokinase group (10.0 vs 2.0%), as were reported allergic reactions (4.4 vs 0.9%) although most of these events were minor and of little clinical significance. There was a significant excess of bleeding events in the streptokinase treated group, with a minor bleeding rate of 3.5 vs 1.0%, and a major bleeding rate of 0.5 vs 0.2% ( $p < 0.001$ ). There was also a significant excess of confirmed cerebral haemorrhage with streptokinase, although with a total number of only 7 events (0.08% vs 0,  $p < 0.02$ ). The overall stroke rate was comparable overall, reflecting a diminution of late stroke rate in the streptokinase group compared to placebo, presumably due to a lower incidence of embolic events. The incidence of haemorrhagic events was increased by the concomitant use of heparin, but not of aspirin.

One interesting aspect of the ISIS-2 study was that it was based on patients who were thought by their attending physician to be suffering from acute myocardial infarction. There were no rigid electrocardiographic entry criteria indicative of myocardial infarction required. Nevertheless 56% of the patients randomised had ST elevation diagnostic of acute myocardial infarction. However, 8% had electrocardiographic (ECG) changes of ST depression only, and a further 27% had abnormal ECGs but which were not

necessarily confirmatory of acute myocardial infarction. Two percent had normal ECGs. Sub-group analysis suggested that those patients with normal ECGs or ST depression did not receive obvious benefit from thrombolytic therapy, although this retrospective subgroup analysis must be interpreted with caution.

That this improvement in mortality could be translated into similar success with other thrombolytic agents was demonstrated by the studies ASSET and AIMS.

The Anglo-Scandinavian Study of Early Thrombolysis (ASSET, 1988) was based on 5011 patients treated within 5 hours of onset of symptoms with alteplase as a decremental intravenous infusion of 100 mg over 3 hours, compared with placebo. Concomitant therapy with heparin, as a 5000 IU bolus, then an intravenous infusion of 1000 IU/hour was given for 21 hours. Aspirin was not given. The results showed a relative reduction in mortality at one month of 26% (95% CL 11-39%, 7.2 vs 9.8%). On this occasion, it appeared that delay in treatment had no apparent detrimental effect, in that groups treated before and after 3 hours of onset of symptoms had similar mortality at one month. Again there were no strict ECG criteria for entry, and 18% of patients had a normal ECG at presentation. Overall 72.8% proved to have an in-hospital diagnosis of myocardial infarction. The benefits on mortality were maintained to 6 months (relative reduction 21%, 95% CL 8-

32%, mortality 10.4 vs 13.1%), with a more pronounced benefit for those with a confirmed diagnosis of myocardial infarction (relative reduction 26%, 95% CL 14-37%, 12.6 vs 17.1%), and one year (relative reduction 12.6%, 13.2 vs 15.1%). (Wilcox et al., 1988, Wilcox et al., 1989, Wilcox et al., 1990).

The Anistreplase in Myocardial Infarction (AIMS, 1988) study assessed the effect on mortality of 30 U of anistreplase as a five minute intravenous bolus, administered to 1258 patients in a randomised double-blind placebo-controlled study within 6 hours of onset of symptoms of acute myocardial infarction, confirmed by ST segment elevation on the presentation ECG. In addition all patients received heparin followed by warfarin for at least 3 months, and timolol in the absence of contraindications (AIMS Trial Study Group, 1988 and 1990). Mortality at 30 days was reduced from 12.1 to 6.4% (relative reduction 47%, 95% CL 21-65%), and at one year follow-up the mortality was 11% in the anistreplase group and 18% in the placebo group (relative reduction 43%, 95% CL 21-59%). Again this study failed to show any differential benefit of treatment before 4 hours or between 4-6 hours.

Following the clear-cut evidence from these placebo-controlled large scale mortality studies, the benefits of thrombolytic therapy were clearly established, and any further placebo-controlled trials would be ethically unjustified. There are however two further large scale mortality studies which merit mention.

GISSI-2 (1990) assessed the effects of intravenous streptokinase and 100 mg of alteplase as a 3 hour decremental infusion of 100mg, each with or without subcutaneous heparin, in a total of 20749 randomised patients. All patients received  $\beta$ -adrenoceptor antagonists unless contraindicated. In-hospital mortality in the two groups was similar at 8.7%, alteplase and heparin 9.2%, streptokinase 9.2%, and streptokinase and heparin 7.9%. The conclusion was that there was no difference in hospital mortality between the treatment strategies. Although there has since been discussion regarding the most appropriate concomitant regimen for heparin, it must be considered that the conclusion drawn is valid, within the confines of the study design (GISSI-2, 1990).

ISIS-3 has recently been published (ISIS-3, 1992). Patients with suspected myocardial infarction were recruited, and concomitant therapy was 162mg aspirin and subcutaneous heparin 12500 IU bd, starting at 4 hours. The results indicate that there was no material difference between streptokinase, anistreplase and alteplase, as regards mortality assessed at 5 weeks, with mortality rates of

10.5%, 10.6% and 10.3% respectively. There was however a significant difference in the rate of cerebral bleeds, being 0.3%, 0.6% and 0.7% respectively ( $p < 0.001$ ), and the overall stroke rates were 1.1%, 1.4% and 1.5% ( $p < 0.001$ ). Thus in this study population of patients with suspected myocardial infarction, and receiving the standard treatment regimens of the three thrombolytic agents available in this country, and with the concomitant therapy described, there is no difference between the agents with regard to efficacy, but there is a lower incidence of stroke with streptokinase.

#### CONCLUSIONS

It is possible that the use of thrombolytic therapy in acute myocardial infarction has been the most extensively researched and assessed drug treatment in modern medicine. It is also probably the single most important innovation in the management of acute myocardial infarction, certainly since the introduction of the coronary care unit in the 1960s.

The early studies of the benefits of intravenous streptokinase were misleading, largely due to their small numbers and lack of adequate randomisation, and almost led to the discarding of this therapy. However, since the pioneering metanalysis of Stampfer and Yusuf suggested the possibility of a benefit concealed within these conflicting studies, many well designed studies of the different thrombolytic agents have been performed, and we can now

confidently state that streptokinase, tissue-type plasminogen activator, and anistreplase are all capable of recanalising occluded coronary arteries, preserving left ventricular function, and improving mortality if administered early in the course of acute myocardial infarction.

However, there remain many questions which cannot be answered from the currently available data. For example, streptokinase is the most efficacious agent. Most of the studies have been performed using "standard" doses and administration regimens of the thrombolytic agents. The accepted standard dose of streptokinase has been 1.5 MIU administered as a continuous intravenous infusion over 60 minutes. This dose is essentially empirical. Although 1.25 MIU has been shown to establish a lytic state in 97% of the normal population (Verstraete et al., 1985), no adequate dose finding studies have been performed, nor have the pharmacokinetic properties or pharmacodynamic effects of this infusion been fully assessed. For example, from first principles a "front-loaded" regimen of streptokinase might appear attractive, achieving an early high concentration of agent, possibly maximising the chances of achieving early reperfusion. Similarly, the dose finding studies of anistreplase have been incomplete and doses of anistreplase in excess of the standard 30 units have never been administered to man. The studies involving tissue-type plasminogen activator have been confused by using different doses of different preparations of rt-PA which are known to

have different specific activities. Although patency rates have been shown to be dose-dependent, so also have the complication rates, in particular of intracerebral haemorrhage (TIMI-2, 1989). However, the manipulation of doses and plasma concentrations of rt-PA remains relatively unexplored.

The possibilities of combining different drugs in the different regimens also remains an intriguing area. Although some preliminary studies have been performed with combinations of rt-PA and scupa, there remains much scope for research in this area.

The latest of the large scale mortality studies have confirmed the high efficacy and safety of the simple regimen of streptokinase and aspirin. In the context of the high cost of the other thrombolytic agents, it is also the most cost-effective treatment. However, as has been discussed above, the streptokinase-containing thrombolytic agents are not without their problems. In particular, the role of antibodies and acquired resistance to these agents remains largely undefined. In the light of the increasing use of the streptokinase-containing thrombolytic agents in clinical practice in this country, the question of when is the "safe" interval for readministration of streptokinase, and what are the possible effects of antibodies to streptokinase on the efficacy and safety of thrombolytic therapy, are probably the two most important questions in contemporary cardiology.

In this thesis, I have attempted to address some of these questions. I have compared the pharmacokinetic properties of the two streptokinase-containing thrombolytic agents, streptokinase and anistreplase. I have studied the influence of acquired resistance to streptokinase on the thrombolytic efficacy of the streptokinase-containing thrombolytic agents, observed the haemodynamic changes following thrombolytic therapy, and related these changes to parameters both of streptokinase resistance and of fibrin- and fibrinogenolysis. Finally, bearing in mind the limitations of the streptokinase-containing thrombolytic agents in certain circumstances, I have attempted to evaluate novel regimens of rt-PA, given as intravenous boluses, by observing their effects on coronary patency.

**CHAPTER THREE**

**COMPARISON OF THE PHARMACOKINETIC PROPERTIES OF  
STREPTOKINASE AND ANISTREPLASE IN ACUTE MYOCARDIAL  
INFARCTION**

## **INTRODUCTION**

In view of the established advantage of the early institution of thrombolytic therapy in acute myocardial infarction in terms of improved clinical benefits of higher coronary patency, left ventricular preservation and decreased mortality, (GISSI, 1986, ISAM, 1986, ISIS II, 1987, AIMS, 1988), the ideal thrombolytic agent would have the following pharmacological properties. It would be easily and rapidly administered by the intravenous route, and once given would achieve high early concentrations in the circulation, giving an early peak of thrombolytic activity, and have pharmacokinetic properties such that thrombolytic activity was maintained for a long enough period to prevent early coronary reocclusion. Currently available agents and regimens are not ideal, but the study of the pharmacokinetic properties of these agents will allow the development of optimal administration regimens.

Streptokinase binds with plasminogen (or plasmin) in the blood to form the complex streptokinase-glu-plasminogen (-plasmin) which is an effective plasminogen activator (Anderson et al., 1987). Anisoylated lys-plasminogen streptokinase activator complex (APSAC, anistreplase) is a pro-enzyme giving rise to the plasminogen activator complex streptokinase-lys-plasminogen by deacylation within the plasma. The activator complexes of the two agents are of comparable potency in the activation of plasminogen. Deacylation of anistreplase occurs with a half-life of 105

minutes in vitro and is thought to be rate-limiting for the removal of anistreplase from the circulation (Ferres et al., 1987, Standring et al., 1987), and to be slower than the elimination of streptokinase-plasminogen or streptokinase (Fears, 1989).

Previous studies addressing the pharmacokinetic properties of the streptokinase-containing thrombolytic agents are relatively few in number (Been et al., 1986, Kohler et al., 1987, Mentzer et al., 1986). The studies have used different assay techniques for streptokinase, measuring different species of lytic activity, largely dependent on whether human or bovine plasminogen and fibrinogen were used in the assay, and the concentrations in the assay media, these technical differences making direct comparison difficult. In addition, the timing and number of their sampling time points were not specifically tailored to the estimation of pharmacokinetic parameters, and compromise the estimation of these parameters. Despite these limitations, they have suggested that streptokinase is rapidly eliminated and its concentration-time profile fit either a mono- or bi-exponential pharmacokinetic model (Kohler et al., 1987, Mentzer et al., 1986). Previous work in man has suggested that anistreplase is eliminated from the circulation much more slowly than streptokinase (Been et al., 1986, Kohler et al., 1987). In the present study, the pharmacokinetic

properties of these agents have been directly compared with a protocol of sampling times tailored for this purpose, using a functional bioassay, to allow definitive conclusions to be drawn.

## **METHODS**

Twenty-four consecutive patients (18 male, 6 female, age range 48 to 72 years) with acute myocardial infarction, as judged by strict ECG criteria of at least 2mm ST elevation in two praecordial leads or at least 1 mm ST elevation in two limb leads, presenting within 6 hours of onset of pain, and without any of the standard contraindications to thrombolytic therapy were treated with either a conventional dose of  $1.5 \times 10^6$  I.U. of streptokinase infused over 60 minutes, or 30 U of anistreplase as a 5 minute continuous intravenous injection. The study protocol is detailed in Appendix III. The patient population was a sub-group of the 128 patients recruited in a comparative study of streptokinase and anistreplase, and whose details are listed in Appendix I.

Blood samples were obtained from an indwelling venous catheter at frequent intervals up to 24 hours post dosing (0, 6, 10, 20, 30, 45, 60, 75, 90 minutes, 2, 4, 6, 9, 12 and 24 hours), and collected into 0.1 volume of 3.8% sodium citrate, separated immediately at 4°C and the plasma stored at -70°C. Total fibrinolytic activity was measured by a described technique (Been et al., 1986, Nunn et al., 1987) and used a functional bioassay of the plasma concentrations of the

thrombolytic agents. The preparation of euglobulin fractions has been reported in detail elsewhere (Standring et al., 1988) but, in brief, 50 $\mu$ l plasma samples were diluted into 910 $\mu$ l 0.011% acetic acid and the resulting precipitates solubilized in 1.5ml 0.05M sodium phosphate/0.1M sodium chloride/0.01% Tween 80 buffer, pH 7.4, to give a 30 fold dilution of the original plasma. This dilution factor was found, during preliminary validation studies on the fibrin plate assay, to abolish the interference of variable endogenous plasminogen and plasmin in the samples (Nunn et al., 1987). Fibrinolytic activity was assayed by the lysis of fibrin plates, prepared from human fibrinogen (containing 2  $\mu$ g plasminogen/mg fibrinogen), incubated at 37 $^{\circ}$ C for at least 18 hours. The plates were stained with bromophenol blue and lysis zones measured on an AMS image analyser. Quadruplicate determinations were performed at each time point and typical coefficients of variation ranged from 0.44 to 2.6% (mean 1.6%, n=11) for anistreplase and from 0.36 to 6.6% (mean 2.4%, n=10) for streptokinase. The concentration of activator was calculated for each patient employing the appropriate standards diluted into autologous predosing plasma i.e. streptokinase for streptokinase treated patients and anistreplase for anistreplase treated patients. Standards were prepared in the patient's own pretreatment plasma to allow for any interpatient variability; the slopes of the standards ranged from 1.57 to 3.26 (mean 2.42) for streptokinase, and 1.78 to 3.07 (mean 2.47) for anistreplase. Both streptokinase and anistreplase

standards gave linear responses with correlation coefficients of 0.9946 (percentage coefficient of variation, %CV 0.34) for streptokinase (n=12) and 0.9938 (%CV 0.21) for anistreplase (n=12).

Internal standards were also included in each assay, in pretreatment, and 10 minutes to 2 hours post treatment samples, and processed concurrently. The average recovery for the entire group of 12 patients was 91% for streptokinase and 94% for anistreplase. The limit of reliable determination was  $0.08 \times 10^{-8} \text{M}$  (3.91 IU/ml) for 10 streptokinase treated patients and  $0.04 \times 10^{-8} \text{M}$  (1.95 IU/ml) for the remaining two. For anistreplase the lower limit of determination was  $0.06 \times 10^{-8} \text{M}$  ( $7.81 \times 10^{-5} \text{U/ml}$ ).

Plasma concentration of streptokinase- and anistreplase - time data were fitted to a one-compartment or two-compartment model by non-linear regression as appropriate to each individual data set. Modelling was performed using a computer program, MODFIT, which employs a modified Danielson-Fletcher-Powell algorithm (Allen, 1989). The same data sets were subjected to model-independent analysis, calculating area under concentration-time curves from zero to the last data point ( $\text{AUC}_L$ ) using a linear trapezoidal method. The area under the curve extrapolated from the last data point to infinity was estimated using the last data point and the rate constant of the terminal log-linear phase, as determined by non-linear regression analysis of the terminal phase. These data were used to calculate clearance

(CL) and volume of distribution (V), according to established methods (Gibaldi and Perrier, 1982). The terminal phase half-life ( $t_{1/2}$ ) was also determined by regression analysis and, in addition, the time to loss of half the maximal fibrinolytic activity from the end of the dosing period was calculated by linear regression analysis. Instantaneous mean residence times (MRT), which compensate for the differences in the duration of dosing between streptokinase and anistreplase, were determined by moment analysis (Riegelman and Collier, 1980).

Pharmacokinetic parameters for anistreplase and streptokinase (V,  $t_{1/2}$  and CL) were compared by one-way analysis of variance.

## RESULTS

### a) STREPTOKINASE

Derived pharmacokinetic parameters are summarised in Table 1 and mean concentration-time curves represented in Figure 7. Concentration time curves following a 60 minute infusion of 1.5 MIU of streptokinase were analysed by model-independent methods only as the data could not be adequately fitted to a compartmental model. The terminal phase rate constant was determined using non-linear regression analysis with a weighting concentration<sup>-2.0</sup>.

Maximum plasma concentration ( $C_{\max}$ ) occurred within 1.25 hours ( $t_{\max}$ ) of the start of the infusion of 1.5 MIU of streptokinase over a 1 hour period (mean 0.9 hours). Concentrations subsequently declined rapidly to less than 15% of  $C_{\max}$  in all subjects by 4 hours after start of infusion, and in most subjects were below the limit of reliable determination. The post peak decline in concentration approximated to a mono-exponential fall (maximum % CV of the regression lines was 26%) as determined by MODFIT (Allen, 1989).

The volume of distribution of streptokinase was small, approximating to that of plasma proteins, and this in conjunction with a moderately rapid clearance defines a relatively rapid terminal phase elimination constant and a short terminal phase half-life.

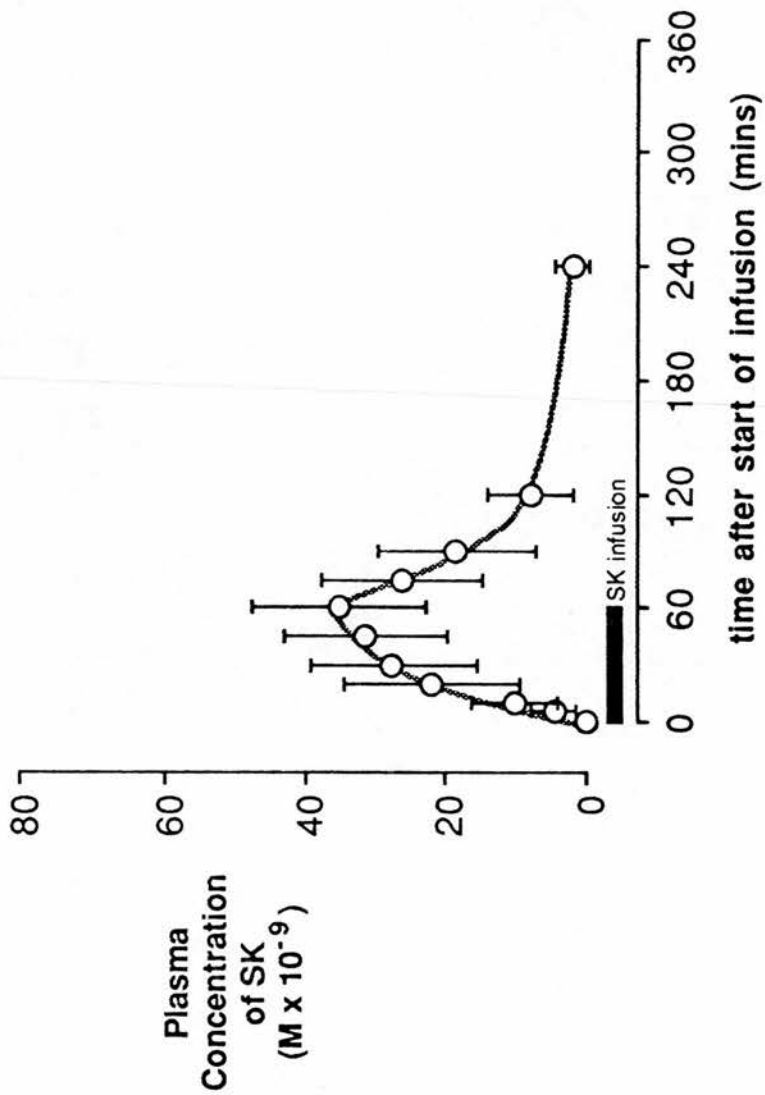


Figure 7. PLASMA CONCENTRATIONS OF STREPTOKINASE (MEAN + SD) FOLLOWING INTRAVENOUS INFUSION OF 1.5 MIU OF STREPTOKINASE OVER 60 MINUTES

**TABLE 1****PHARMACOKINETIC PARAMETERS**

	Anistreplase (n=12) MEAN (SD)	SK (n=12) MEAN (SD)
<b>t<sub>max</sub> (h)</b>	0.154 (0.07)	0.9(0.21) **
<b>C<sub>max</sub> (M x 10<sup>-8</sup>)</b>	5.59 (2.22)	3.85 (1.18) +
<b>Volume of Distribution (l)</b>		
Model-independent (V)	5.90 (1.91)	5.68 (2.29)NS
Computer-modelled (V <sub>SS</sub> )	5.25 (1.49)	-
<b>Clearance (l/h)</b>		
Model-independent (CL)	3.87 (1.52)	7.08 (2.91) *
Computer-modelled (CL)	3.72 (1.35)	-
<b>Terminal phase elimination half-life (h)</b>		
Model-independent t <sub>1/2</sub>	1.16 (0.38)	0.61 (0.24) **
Computer-modelled t <sub>1/2</sub>	1.15 (0.38)	-
<b>Time to half maximal fibrinolytic activity (h)</b>		
	1.12 (0.31)	0.48(0.14)**
<b>Mean residence time (h)</b>	1.55 (0.48)	0.76 (0.31) **

+ P<0.05 \* P<0.01 \*\* P<0.001

NS = Non significant

## **b) ANISTREPLASE**

The maximum total activity of anistreplase ( $C_{\max}$ ) occurred within 20 minutes ( $t_{\max}$ ) of the start of the 5 minute intravenous injection of 30 units of anistreplase (mean 0.15 hours).

Activity fell to less than 15% of maximum by 4 hours. In 6 of 12 patients, an early rapid decline phase of the concentration-time curve could be delineated, conforming to a two-compartment model. In 5 subjects the data sets conformed to a one-compartment model. In one subject, neither model provided a satisfactory fit and these data were analysed by model-independent methods only. The derived parameters were similar regardless of which model was most appropriate, and were comparable with parameters derived from model-independent analysis.

Pharmacokinetic parameters are summarised in Table 1 and mean total activity-time curves represented in Figure 8.

The volume of distribution of anistreplase and its deacylated product is relatively small, consistent with anistreplase and its metabolite being largely confined to the systemic circulation. Clearance is modest and in conjunction with a small volume of distribution allows a relatively long elimination phase half-life compared to streptokinase.

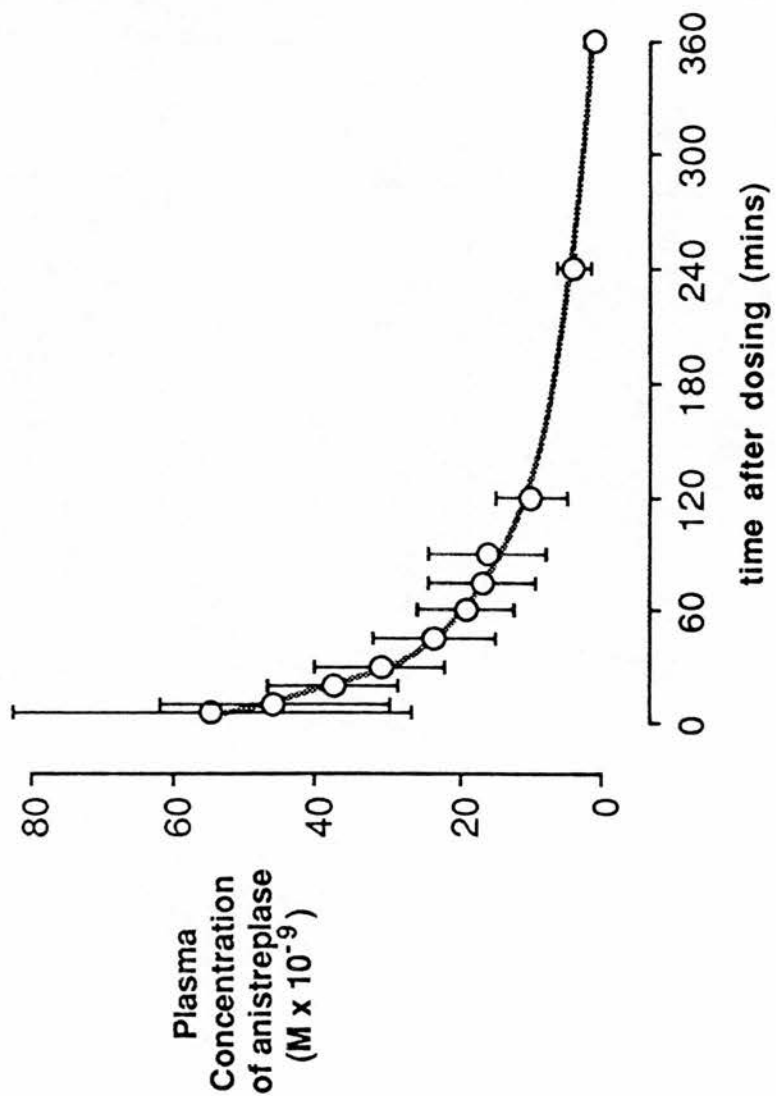


Figure 8. PLASMA CONCENTRATIONS OF ANISTREPLASE (MEAN  $\pm$  SD) FOLLOWING INTRAVENOUS INJECTION OF 30 U OF ANISTREPLASE OVER 5 MINUTES

## DISCUSSION

Coronary reperfusion rates are highest when thrombolytic therapy is administered early in the course of myocardial infarction (Lew et al., 1986, Kennedy et al., 1985, Weinstein, 1982, Khalilullah et al., 1984), and is associated with greatest clinical benefits (AIMS, 1988, GISSI, 1986, ISIS II, 1987, Simoons et al., 1985 & 1986, Anderson, 1984, ISAM, 1986). For earlier institution of therapy, a suitable thrombolytic agent must be administered in a simple regimen allowing easy administration in difficult circumstances, and with the increasing interest in the possibility of thrombolytic therapy at home, applicable even outside the hospital environment. The ideal regimen should therefore achieve a high early concentration, and resulting fibrinolytic activity (short  $t_{max}$  and high  $C_{max}$ ). A slow elimination phase, allowing maintenance of adequate concentrations from a single "bolus" injection (long  $t_{1/2}$ , low CL) for an appropriate period of time, would be expected to minimise early coronary reocclusion.

Early recommendations for the use of streptokinase were for a prolonged low dose infusion, but more recent usage in myocardial infarction has adopted the now standard, but essentially empirical dose of 1.5 MIU over 60 minutes. The recommended dose of anistreplase was based on being equivalent to 1-1.5 MIU of streptokinase. Following the administration of streptokinase and anistreplase, both in their standard treatment regimen for myocardial infarction, anistreplase achieves a significantly earlier, high

concentration, with a peak plasma concentration of  $5.59 \times 10^{-8} \text{M}$  within 4.5 minutes of the end of its infusion period, as would be predicted from its more rapid infusion.

In this study, it has been shown that both streptokinase and anistreplase have a small volume of distribution, approximately twice that of plasma volume and similar to the volume of distribution of plasma proteins (Rowland & Tozer, 1980). This would be consistent with both agents behaving as proteins with no specific carrier mechanism, being largely confined to the systemic circulation.

The elimination phase half-life of streptokinase of 0.61 hours is very similar to previous estimates based on fibrin plate lysis assay in acute myocardial infarction (Kohler 1987) and slightly longer than estimates based on other functional assays of 0.3 hours (Martin 1982) and 0.38 hours (Mentzer et al., 1986). These small differences may be accounted for by variation in assay specificity and pharmacokinetic analysis. Claims of a considerably longer terminal phase, with a half-life of 1.38 hours (Grierson & Bjornson, 1987, Fletcher et al., 1958) are based on radioimmunoassay or amidolytic assay methods for streptokinase. These methods do not differentiate between active and inactive streptokinase fragments, or take account of in vivo de-iodination. Similarly, the amidolytic assays based on the lysis of chromogenic substrate do not evaluate the fibrinolytic sites of the activator molecule, and also

therefore cannot differentiate active and inactive streptokinase fragments, or fragments bound by circulating inhibitors such as  $\alpha_2$ -antiplasmin, neither is it entirely specific for streptokinase. Previous studies based on the lysis of bovine fibrin are also problematic, in that this assay is particularly sensitive to endogenous human plasminogen. Some previous studies have been based on inadequate sampling protocols, and because of these methodological problems, these studies are not entirely comparable.

In this study, both drugs have been assayed by the same fibrinolytic bioassay based on human fibrin plate lysis. It has been shown that administration of this dose of either thrombolytic agent does not completely deplete circulating plasminogen, therefore all the streptokinase present would be in its fibrinolytically active complexed form. Dilution of the euglobulin fractions thirty-fold eliminates the influence of endogenous plasminogen on the assay procedure (Fears et al., 1989). Therefore this assay method measures the functional moiety and is relevant to the clinical application of these drugs.

It has been shown that streptokinase and anistreplase have similar small volumes of distribution, but that streptokinase is effectively cleared from the circulation twice as quickly as anistreplase, (7.08 vs 3.87 l/hours,  $p < 0.01$ ). In vitro studies of deacylation of anistreplase in human blood or plasma have shown a deacylation half-life of

1.76 hours (Ferres 1987). The shorter half-life of elimination of anistreplase activity (1.16 hours) would therefore support the concept that the deacylation of anistreplase is the rate-limiting step in its elimination.

Both streptokinase as a 60 minute infusion of 1.5 MIU (Verstraete et al., 1985) and anistreplase as a 5 minute injection of 30 U (Been et al., 1985, Hillis et al., 1987, Ikram et al., 1986, Timmis et al., 1987) are well-tolerated and effective in restoring coronary patency in acute myocardial infarction. In this study, it has been confirmed that anistreplase in this dosage schedule achieves higher levels of total thrombolytic activity, which are attained earlier following administration and are maintained for longer.

**CHAPTER FOUR**

**PRE-DOSING ANTIBODY LEVELS AND EFFICACY OF  
STREPTOKINASE-CONTAINING THROMBOLYTIC AGENTS**

## **INTRODUCTION**

The effectiveness of streptokinase and anistreplase in the treatment of acute myocardial infarction has already been discussed in detail. Their efficacy in achieving coronary patency (Anderson et al., 1983, Kennedy et al., 1983, Been et al., 1986), preserving left ventricular function (ISAM Study Group, 1986, Bassand et al., 1989) and improving mortality (GISSI, 1989, AIMS, 1988) has been well demonstrated. The thrombolytic effects of both these agents are mediated by the activation of plasminogen by a streptokinase-activator complex, in the case of streptokinase following the combining of streptokinase with circulating plasmin(-ogen) (Anderson et al., 1987), and of anistreplase following the rate-limiting hydrolysis of the compound in the circulation liberating streptokinase-lys-plasminogen (Ferres, 1987).

In some patients however, successful thrombolysis is not achieved. It has been suggested that high levels of resistance to streptokinase, probably acquired by previous exposure to streptococci, can compromise the therapeutic response to streptokinase (Hirsh et al., 1970, James, 1973). To overcome this, the "standard" dose of 1.5 MIU of streptokinase aims to achieve a lytic state in a high proportion of the population. A dose of 1.25 MIU has been shown to achieve a lytic state in 97% of the population (Verstraete et al., 1966). However previous clinical studies have not shown any relation between pretreatment anti-SK IgG concentration and efficacy as assessed by

reperfusion rate or time to reperfusion after intracoronary streptokinase or intravenous anistreplase (Hoffmann et al., 1988).

This study extends previous studies by examining the relationship between the streptokinase resistance titre (SKRT), and the concentration of specific antibody to streptokinase of the IgG class, and therapeutic response to the streptokinase-containing thrombolytic agents, streptokinase and anistreplase assessed by coronary angiography. The streptokinase resistance titre is a measure of the total inhibitory capacity of the patient's plasma to streptokinase, and reflects not only the contribution of anti-streptokinase antibodies, but in addition the effects of circulating plasma inhibitors such as  $\alpha_2$ -antiplasmin and macroglobulins, and is influenced by changes in other plasma proteins such as fibrinogen and plasminogen, which show large changes following thrombolytic therapy. Unlike the streptokinase resistance titre, the anti-SK IgG concentration is a direct measure of the immune response reflecting previous exposure to streptococcal protein, and is independent of circulating non-specific inhibitors.

## **PATIENTS AND METHODS**

The study group consisted of a cohort of 128 consecutive patients the details of which are recorded in Appendix I. The study protocol is included in Appendix IV. All patients presented within 6 hours of onset of symptoms of myocardial infarction, having had chest pain for at least 30 minutes, and without any of the standard contraindications to thrombolytic therapy (TIMI, 1985) or coronary angiography. All patients had at least 1mm of ST elevation in two or more limb leads, or at least 2mm ST elevation in two or more praecordial leads. We excluded patients over the age of 70 years, those with a systolic blood pressure less than 95 mmHg, or those who had experienced a previous myocardial infarction in the same anatomical distribution, or who had previously received thrombolytic therapy. Following discussion with the patient and attending relatives, written informed consent was obtained.

The mean age (SD) of the patients was 55.6 (8.3) years, with a range of 31-70 years. Mean time from onset of symptoms to commencement of thrombolytic therapy was 204 (79) minutes. There were 101 men and 27 women. Patients were treated with either 1.5 MIU streptokinase (65 patients) as a continuous intravenous infusion over 60 minutes, or 30 units of anistreplase (63 patients) as a 5 minute intravenous injection, in a double-blind, double-dummy study design.

All patients received 2mg/hour isosorbide dinitrate as a continuous intravenous infusion for at least 30 minutes prior to the commencement of thrombolytic therapy, and 100mg hydrocortisone and 10mg chlorpheniramine intravenously immediately before thrombolytic treatment.

Coronary angiography was performed from the femoral route by Judkin's method, at 90 minutes and 24 (18-30) hours after treatment, injecting the infarct related artery first as indicated by the admission electrocardiogram. Coronary perfusion was scored on the TIMI scale (TIMI, 1985 and Appendix II), by an independent, experienced cardiologist, blinded to the study protocol.

Venous blood samples were collected in all patients prior to the administration of thrombolytic therapy. In 96 patients (48 streptokinase, 48 anistreplase) streptokinase resistance titres were measured. In 124 patients (60 streptokinase, 64 anistreplase) specific anti-streptokinase antibodies of the immunoglobulin G (IgG) class were assayed, using a specific micro-radioimmunoassay which has been described previously (Moran et al., 1985).

The data for each treatment group were analysed separately, then jointly using non-parametric descriptive statistics and tests of location, complying with the conventional level of significance ( $p < 0.05$ ). Linear regression analysis was performed on normally transformed data. Normality was confirmed using the Shapiro-Wilks test.

## RESULTS

In the study population neither SKRT nor anti-SK IgG concentrations were normally distributed, with marked skewing towards higher values. However,  $\log_{10}$  of the anti-SK IgG concentrations, and the fourth root of the streptokinase resistance titres were normally distributed. Comparing the two treatment groups, it was found that the group treated with anistreplase had significantly higher SKRT than the streptokinase group (median 50 vs 20 IU.SK.ml<sup>-1</sup>,  $p < 0.05$ , Fig 9). No significant difference was found between the two groups for anti-SK IgG concentrations.

Regression analysis of the distribution of anti-SK IgG concentration revealed a weak but statistically significant negative correlation between  $\log_{10}$ -IgG values and age (Figures 10 & 11). The regression equation was:

$$\text{Log}_{10}(\text{IgG}) = 0.953 - 0.0166 (\text{age})$$

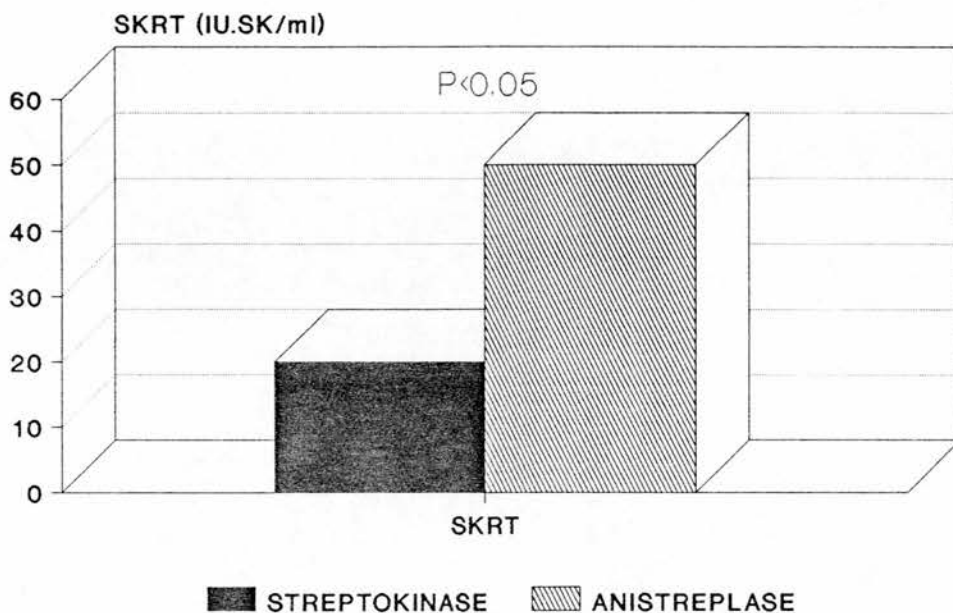
$$p=0.02, R\text{-sq} = 7.3\%.$$

There was a similar relationship between  $\sqrt[4]{}$ (streptokinase resistance titre) and age. The regression equation was:

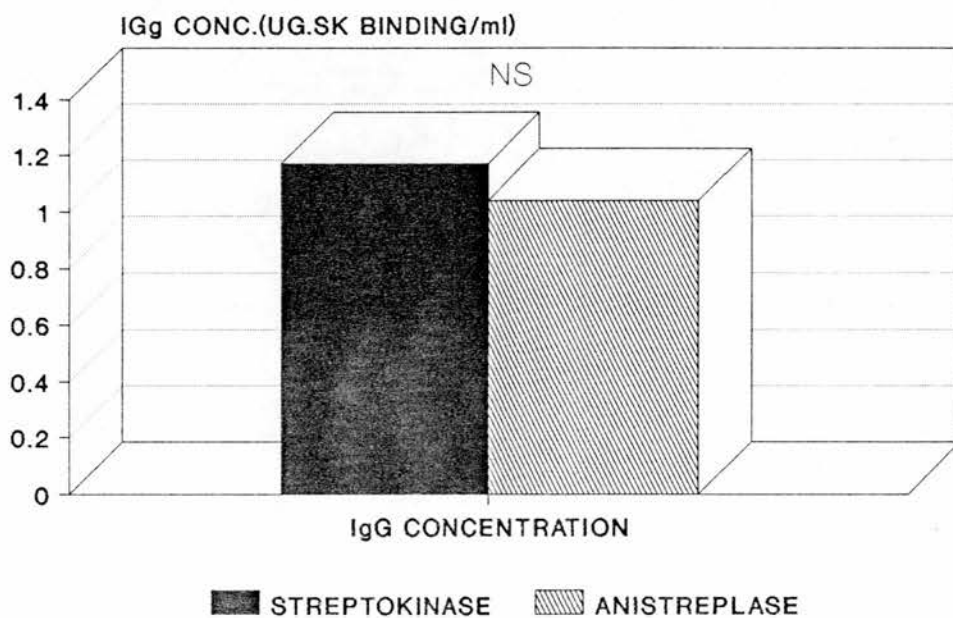
$$\sqrt[4]{(\text{SKRT})} = 3.34 - 0.014 (\text{age})$$

$$p=0.41, R\text{-sq} = 4.3\%.$$

**BASELINE MEDIAN SK RESISTANCE TITRE  
IN SK AND ANISTREPLASE TREATMENT GROUPS**



**BASELINE MEDIAN IgG CONCENTRATION  
IN SK AND ANISTREPLASE TREATMENT GROUPS**



**Figure 9. BASELINE STREPTOKINASE RESISTANCE TITRE FOR THE STREPTOKINASE AND ANISTREPLASE TREATED PATIENT GROUPS**



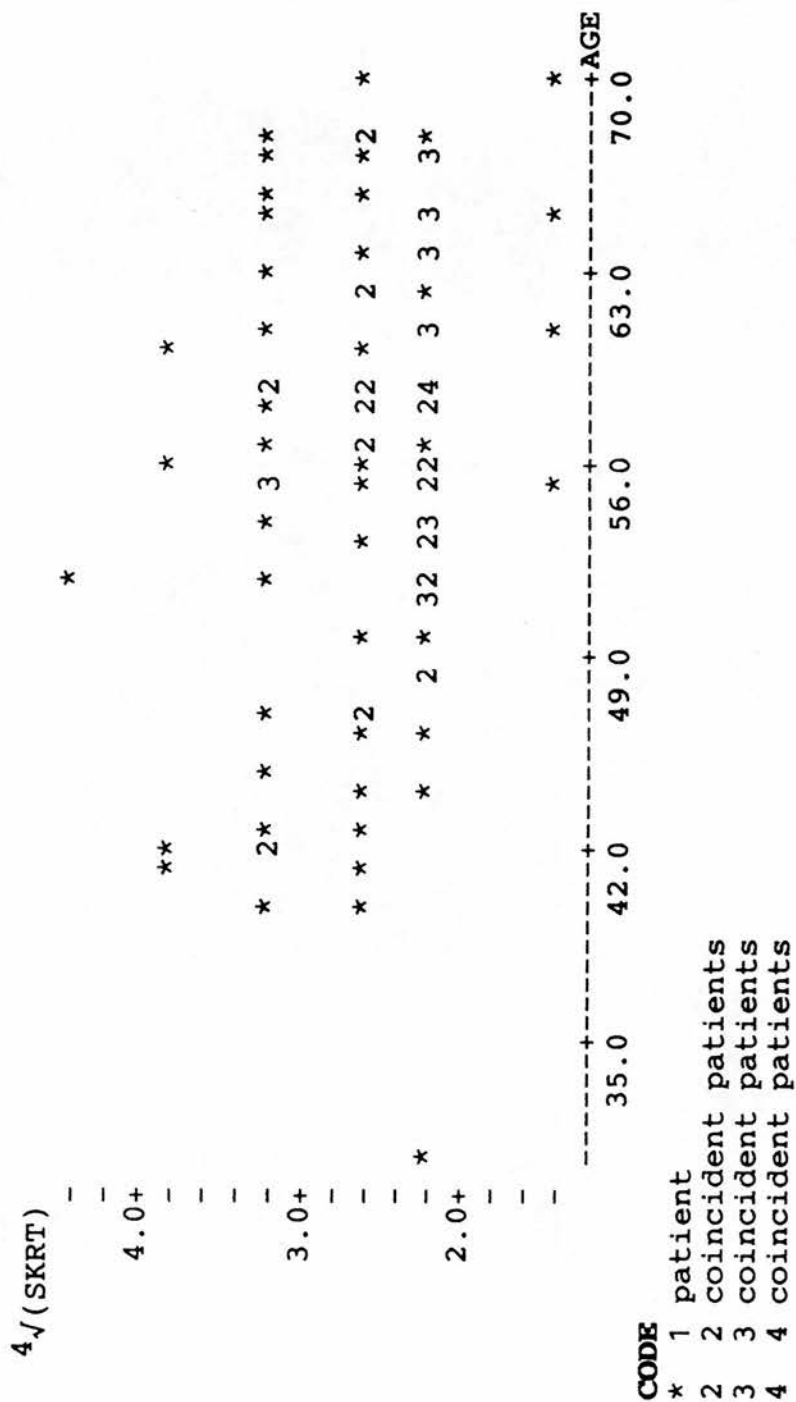


Figure 11. SCATTER DIAGRAM OF DISTRIBUTION OF STREPTOKINASE RESISTANCE TITRE (FOURTH ROOT TRANSFORMED) AGAINST AGE

Details of the coronary patency rates in the treatment groups have been previously published (Hogg et al., 1990). In summary, the streptokinase group achieved a patency rate of 53% at 90 minutes and 87.5% at 24 hours, and the anistreplase group a patency of 55% and 81% respectively. There were no significant differences between the patency rates of the two treatment groups at either time point.

There were no significant differences in the streptokinase resistance titres or anti-SK IgG concentrations for the streptokinase or anistreplase treated groups analysed separately with regard to coronary patency at either 90 minutes or 24 hours. Considering the patients in both treatment groups together, there was a significant difference in streptokinase resistance titre between those patients with TIMI grades 0&1 versus TIMI grades 2&3 at 24 hours (median 100 vs 50 SK IU ml<sup>-1</sup>, p=0.015). In addition, there were non-significant trends towards a higher streptokinase resistance titre in those patients with an occluded index coronary artery at 90 minutes (TIMI grade 0 or 1, vs grade 2 or 3, median 50 vs 20 SK IU ml<sup>-1</sup>, p=0.089, Table 2) and towards a higher anti-SK IgG concentration in these groups with TIMI grades 0&1 at both 90 minutes and 24 hours, (1.53 vs 0.925, p=0.050, and 1.65 vs 1.04 µg SK binding ml<sup>-1</sup>, p=0.075, Table 3).

Figures 12 & 13 show streptokinase resistance titres and anti-SK IgG concentrations of the combined groups related to angiographic patency scores at 90 minutes and 24 hours.

**Table 2****Median SKRT values (SK IU ml<sup>-1</sup>) according to TIMI grading (all patients)**

<b>Time of angiogram</b>	<b>90 minutes</b>	<b>24 hours</b>
TIMI 0&1	50(n=41)	100(n=13)
TIMI 2&3	20(n=47)	50(n=75)
p value	0.089	0.015

**Table 3****Median anti-SK IgG levels µg SK binding ml<sup>-1</sup> according to TIMI grading (all patients)**

<b>Time of angiogram</b>	<b>90 minutes</b>	<b>24 hours</b>
TIMI 0&1	1.53(n=52)	1.65(n=18)
TIMI 2&3	0.925(n=62)	1.040(n=95)
p value	0.050	0.075

**MEDIAN SKRT GROUPED ACCORDING  
TO CORONARY ANGIOGRAPHIC SCORES**

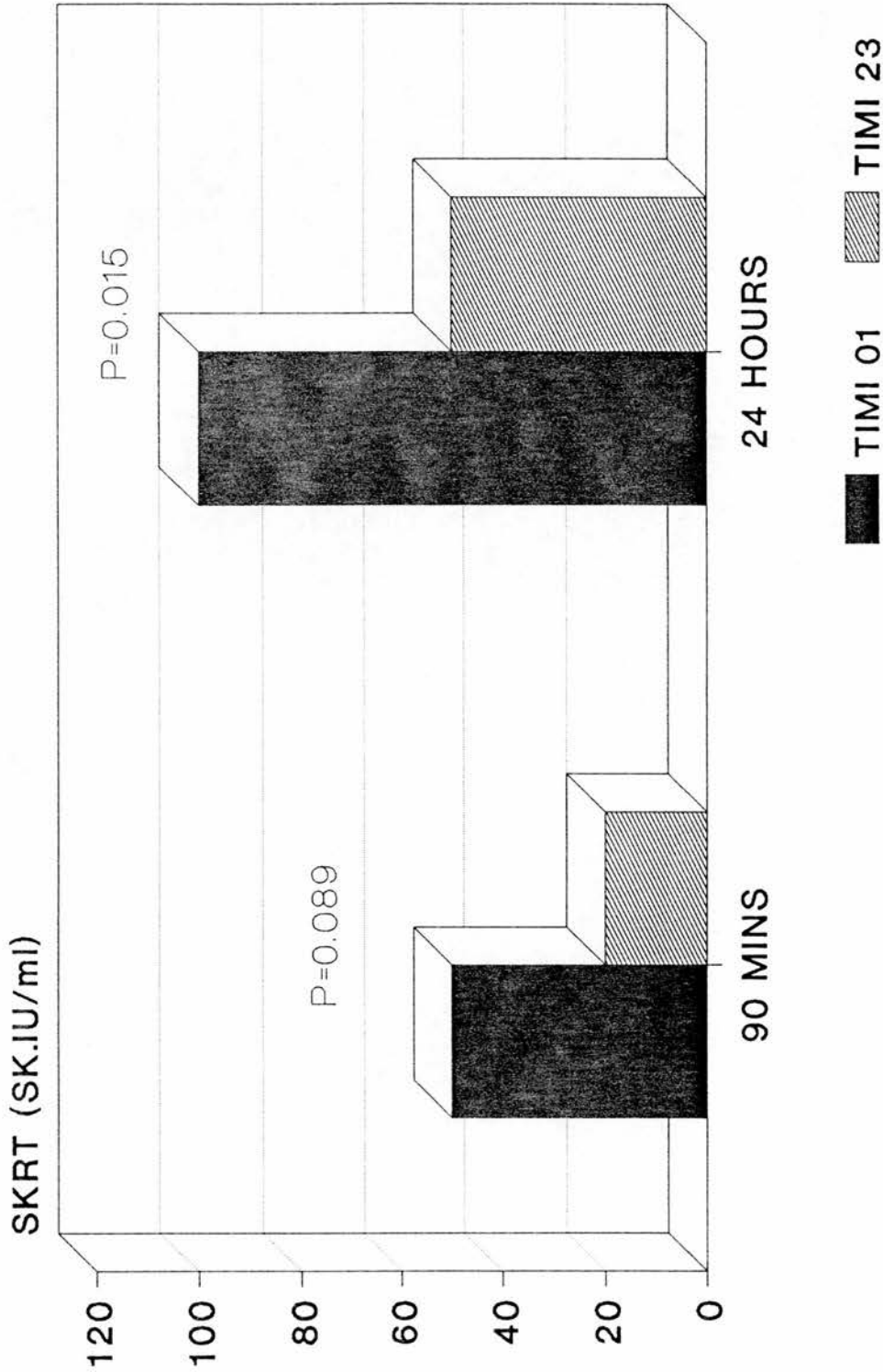


Figure 12. MEDIAN STREPTOKINASE RESISTANCE TITRES RELATED TO CORONARY PATENCY GRADES, FOR BOTH STREPTOKINASE AND ANISTREPLASE, ASSESSED AT 90 MINUTES AND 24 HOURS

# MEDIAN IgG GROUPED ACCORDING TO CORONARY ANGIOGRAPHIC SCORES

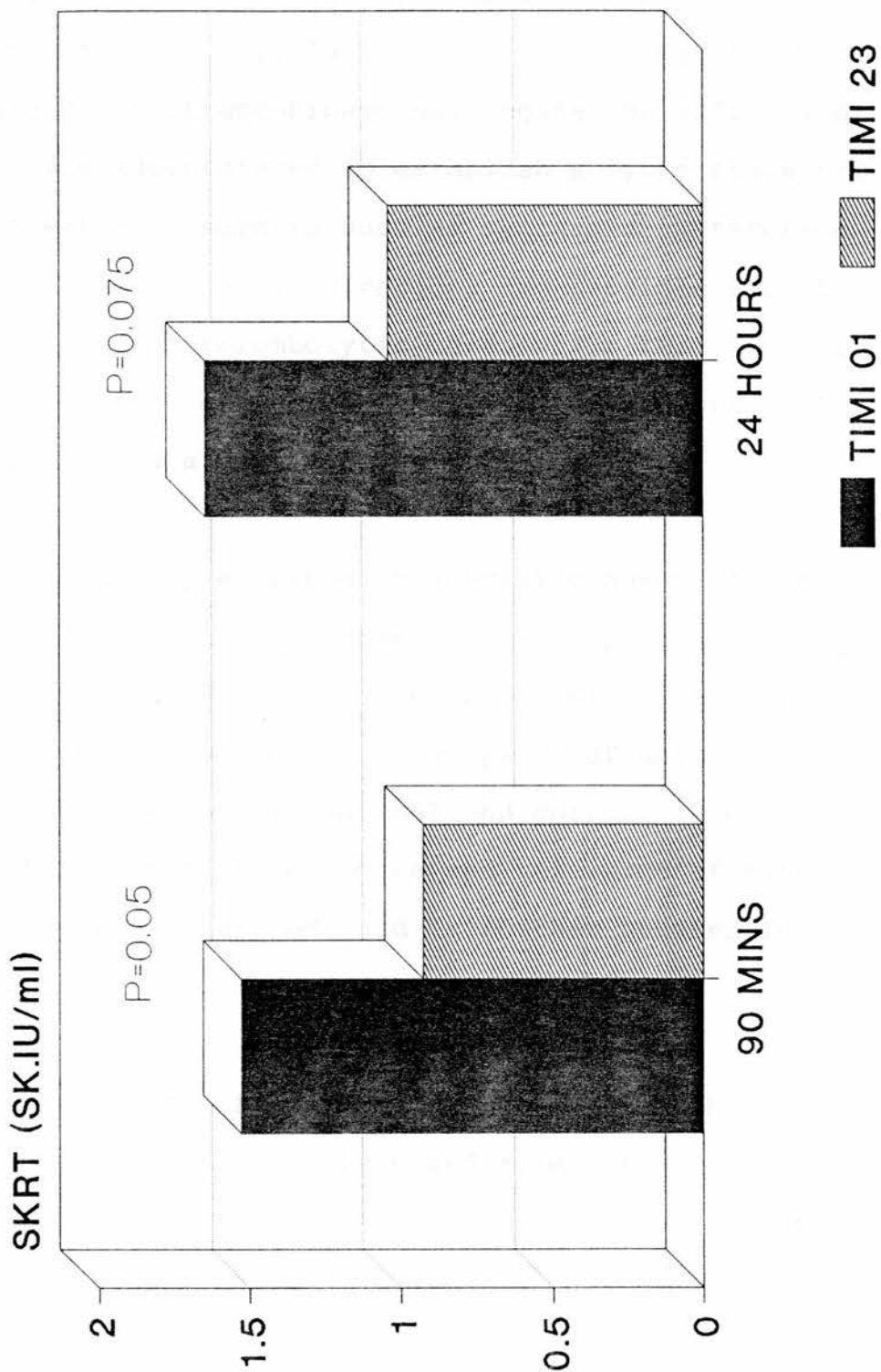


Figure 13. MEDIAN ANTI-SK IgG CONCENTRATION RELATED TO CORONARY PATENCY GRADES, FOR BOTH STREPTOKINASE AND ANISTREPLASE ASSESSED AT 90 MINUTES AND 24 HOURS

## **DISCUSSION**

The exact prevalence of antibodies to streptococcal protein and resistance to streptokinase among the community remains to a large extent undefined. It has previously been suggested (Hirsh et al., 1970, James, 1973) that high levels of resistance to streptokinase may negate the efficacy of streptokinase administered to establish a lytic state for the treatment of disorders such as myocardial infarction. With the increasing administration, and possible repeat administration, of thrombolytic therapy for this indication, it is important to define the relevance of resistance to streptokinase-containing thrombolytic agents.

The most commonly prescribed thrombolytic agent in the United Kingdom is streptokinase, and therapeutic administration of this agent and anistreplase (see Chapter 6) is known to stimulate the development of antibodies to the streptokinase moiety (Jalihal and Morris, 1990). However, the extent, time course and relevance of this response remains undefined, and is further studied in Chapter 6.

In this study, the influence of IgG anti-streptokinase antibody and the functionally based assay of streptokinase resistance titres on the ability of streptokinase-containing thrombolytic agents to achieve coronary patency following acute myocardial infarction has been studied. The streptokinase resistance titre is a well-established functional assay used to assess in vivo global resistance to

streptokinase. The specific anti-streptokinase IgG assay is relatively new, and the exact relevance of this antibody to the mechanism of acquired resistance to streptokinase remains to be established. It is suggested that it is a reliable marker of previous exposure to streptococcal protein.

These data demonstrate that there is a wide range of SKRT and anti-SK IgG concentrations among the population presenting with acute myocardial infarction. The distribution of these markers is markedly skewed, with the majority of the relevant population having a low resistance to streptokinase. We have also shown that resistance to streptokinase and anti-SK IgG concentration decrease with age.

Within the range of these measured parameters, streptokinase resistance titre has a small, but demonstrable, influence on the efficacy of the streptokinase-containing thrombolytic agents, streptokinase and anistreplase. There is a significantly higher streptokinase resistance titre among those patients with TIMI grades 0&1 at 24 hours, and trends toward higher streptokinase resistance titres among the comparable group for TIMI grades at 90 minutes. There were also trends towards higher anti-SK IgG in these groups. The

differences are relatively minor and consistent with some previous studies which have suggested that the efficacy of these agents are not substantially influenced by resistance to streptokinase (Rothbard, 1985, Hoffmann, 1988).

These differences were only apparent when the two treatment groups were analysed together. Although these two thrombolytic agents are distinct pharmacological compounds, their thrombolytic effects are both mediated by an extremely similar streptokinase-plasminogen activator complex. There are theoretical reasons why the two agents might be affected differently by humorally mediated resistance. The streptokinase molecule in anistreplase is already bound to lys-plasminogen, and therefore may be less susceptible to interference by circulating antibodies directed at this binding site. The obscuration of the catalytically-active centre in the administered inactive form of anistreplase may diminish the potency of antibodies directed at the active centre of the streptokinase moiety, and the higher affinity of the anistreplase for fibrin relative to that of streptokinase, may make it less effectively bound by competing antibodies. Nevertheless, the presence of non-significant trends in the two smaller treatment groups which when combined achieve conventional statistical significance is highly suggestive of a Type II error attributable to the relatively small numbers in some of the sub-groups.

In the range measured in the population prior to receiving thrombolytic therapy for acute myocardial infarction, the streptokinase resistance titre and anti-SK IgG concentrations as gauges of previous exposure to streptococcal protein, have a small, but significant, negative influence on the efficacy of the thrombolytic agents streptokinase and anistreplase as judged by their ability to achieve coronary patency. It remains important to document the influence of higher levels of resistance which are found following previous therapy with streptokinase, and the time course of these immunological reactions. Such studies may allow the development of flexible dosing regimens of thrombolytic agents aimed at saturating the patient's resistance to streptokinase, allowing the establishment of a lytic state. In this context, the role of high antibody levels in the mechanism of blood pressure changes and allergic reactions to streptokinase requires elucidation, and this question forms the basis of the next chapter in this thesis.

**CHAPTER FIVE**

**HYPOTENSIVE RESPONSES FOLLOWING THROMBOLYTIC THERAPY WITH  
STREPTOKINASE-CONTAINING AGENTS IN ACUTE MYOCARDIAL  
INFARCTION.**

## **INTRODUCTION**

Streptokinase has been in clinical use for many years (Fletcher et al., 1959), and its propensity for causing systemic hypotension has been long documented (Ganz et al., 1984, Alderman et al., 1984). The mechanism of this fall in blood pressure has not been elucidated. Several possible mechanisms have been suggested, including an immune-mediated "allergic" response, possibly due to IgE class antibodies (Dykewicz et al., 1988); plasmin activation by streptokinase, with resulting activation of the bradykinin-kallikrein or complement systems (Green et al., 1984); direct vasodilatation due to streptokinase itself; reduction in blood viscosity (Lew et al., 1985); or activation of endothelium-dependent systems such as the endothelium derived relaxing factor, EDRF or prostaglandin systems.

In this study the incidence, time course and magnitude of hypotensive episodes following treatment with the streptokinase-containing thrombolytic agents, streptokinase and anistreplase, have been documented. An association between hypotension and prior exposure to streptococcal antigen, as measured by streptokinase resistance titre and anti-SK IgG concentration, was sought. In addition, the relationship to plasmin activation, as measured by changes in plasma fibrinogen and the release of fragments of fibrin(-ogen), and changes in blood viscosity was investigated.

## **METHODS**

The same study population as for the previous chapter were used. Patient details are summarised in Table 4, and individual demographic data recorded in Appendix I. The mean ( $\pm$  SD) time from onset of symptoms to commencement of thrombolytic therapy was 204 ( $\pm$  79) minutes. There were 101 men and 27 women, with a mean age of 55.6 years. Patients were treated with either 1.5 MIU streptokinase (63 patients) as a continuous intravenous infusion over 60 minutes, or 30 units of anistreplase (65 patients) as a 5 minute intravenous injection, in a double-blind, double-dummy design.

All patients received 2mg/hour isosorbide dinitrate as a continuous intravenous infusion for at least 30 minutes prior to the commencement of thrombolytic therapy, and prophylactic treatment with 10mg chlorpheniramine and 100mg hydrocortisone intravenously. Patients were allowed other drugs, including opioid analgesics as clinically indicated. Those patients with a history of prior exposure to streptokinase-containing thrombolytic agents, a previous myocardial infarction in the same anatomical distribution or a baseline systolic blood pressure of less than 95 mmHg were excluded. The study protocol is included in Appendix III.

Venous blood samples were taken, prior to the administration of thrombolytic therapy. Streptokinase resistance titres were measured in 96 patients (48 streptokinase, 48 anistreplase). In 124 patients (60 streptokinase, 64 anistreplase), specific anti-streptokinase antibodies of the immunoglobulin G (IgG) class were assayed, as has been described in the preceding chapter (Moran et al., 1985).

Venous blood samples were also taken from 78 patients at 0, 6, 10, 15, 30, 45, 60, 90 minutes, 2,4,6,9,12 hours, 1, 2, 3, 4, and 5 days after thrombolytic therapy and subsequently assayed for plasma fibrinogen (Clauss assay), B-B 15-42 peptide (RIA, IMCO), D-dimer (ELISA, AGEN) and plasma viscosity corrected for haematocrit (Coulter-Harkness capillary viscometer, at 37<sup>o</sup>c). From these profiles, individual areas under the concentration-time curve (AUC) were calculated over the first 90 minutes for B-B 15-42, the falls in fibrinogen from baseline and corrected plasma viscosity, and over 5 days for D-dimer in view of the latter's prolonged release curve.

**Table 4.**

**Details of study patient population**

Total No. of patients	128
Sex	
Male	101
Female	27
Age	
Mean	55.6 ± 8.3 years
Range	31 - 70
Mean time to therapy ( ± SD)	204 ± 79 minutes

Blood pressure was monitored at 2 minute intervals by a semi-automatic sphygmomanometer cuff (Sentron) and permanent hard copies stored. The blood pressure was observed for at least 10 minutes prior to the start of thrombolytic therapy, on a stable dose of intravenous nitrates, and the final recording taken as the baseline recording. A hypotensive response was arbitrarily defined as a fall in systolic blood pressure of at least 20mmHg lasting for at least three cycles of the automatic cuff (6 minutes), and were classified as early (within 30 minutes of start of thrombolytic therapy), late (30 - 90 minutes) or both (ie: persistent).

#### **STATISTICAL METHODS**

Differences in frequency of hypotensive episodes between the agents were compared by the Chi-squared test. The AUCs of change in fibrinogen, B- $\beta$  15-42 plasma viscosity and d-Dimer for the different groups, according to presence or absence of hypotensive episodes were compared using the non-parametric Kruskal-Wallis and Chi-squared tests on independent samples. Blood pressure changes were calculated as changes from the stable baseline value, and changes from baseline expressed as mean and 95% confidence limits. The conventional level of significance was adopted. For clarity of presentation, means and standard deviations have been used for the graphical representation of the data.

## RESULTS

As has been discussed previously, neither streptokinase resistance titre nor anti-SK IgG concentrations were normally distributed, with marked skewing toward a few high values.

52 patients had a myocardial infarction affecting the anterior distribution, 21 (40.4%) of whom suffered a hypotensive response; of these 17 were early, 7 late and 3 both. 73 patients had an inferior myocardial infarction, and 34 suffered a hypotensive response (46.5%); of these, 29 were early, 14 late and 9 both. Those patients in whom the hypotensive episodes were attributable to a primary cardiac arrhythmia (7 patients) were excluded. There were no significant differences between the site of infarction and frequency of hypotension following thrombolytic therapy (Table 5 and Figure 14).

There was no significant difference in baseline blood pressures between the two treatment groups. The mean baseline systolic blood pressure was  $131.1 \pm 23.1$  mmHg, and the mean diastolic blood pressure  $80.5 \pm 13.5$  mmHg. Following thrombolytic therapy there was a fall in both systolic and diastolic blood pressure. The amplitude and duration of the blood pressure changes were identical for streptokinase and anistreplase (Figures 15-18). The maximum mean fall in systolic blood pressure was 16.9 (95%CL 12.2 to 24.54, range -105 to +20) mmHg, and the

**Table 5.**

**Hypotensive episodes in relation to site of infarct and thrombolytic agent.  
Also Figure 14.**

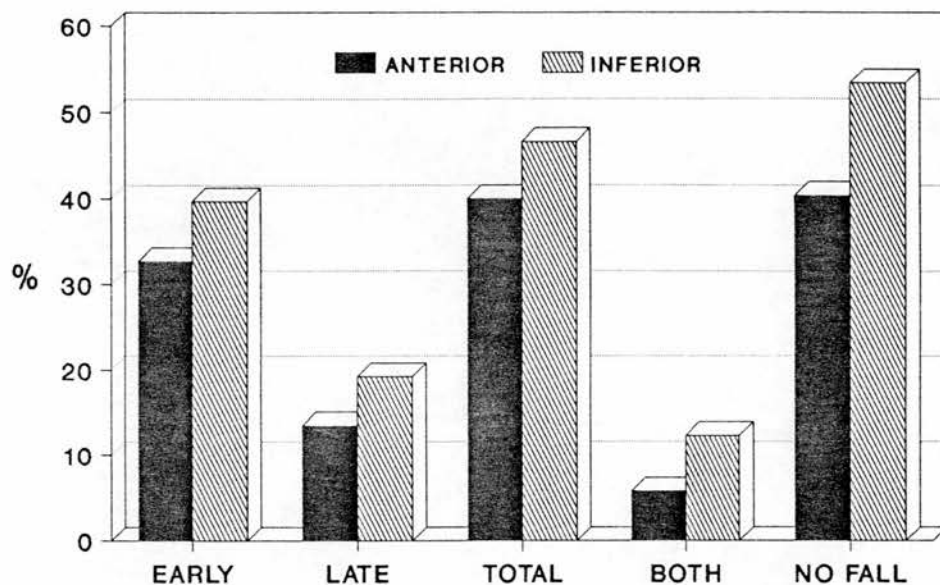
	<b>Early</b>	<b>Late</b>	<b>Total</b>	<b>Both</b>	<b>No</b>	<b>Fall</b>
Anterior	17	7	21	3	31	NS
Inferior	29	14	34	9	39	NS
SK	20	9	23	6	40	NS
Anistreplase	26	13	33	6	32	NS

maximum mean fall in diastolic blood pressure was 13.7 (95%CL 10.3 to 17.1, range -70 to +10) mmHg. The fall in both mean systolic and diastolic blood pressure commenced at 4 minutes after therapy, and had resolved by 34 minutes. Following anistreplase, there was a minor trend for the diastolic blood pressure to remain below baseline.

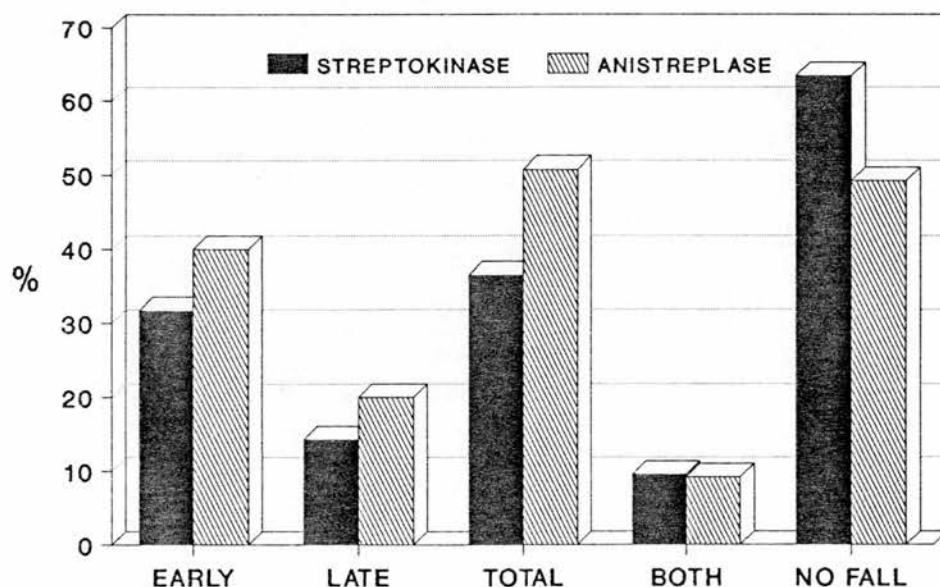
In the patients, the hypotension was in general well-tolerated, and in many was asymptomatic. In all patients, blood pressure was restored to acceptable levels by adoption of the Trendelenburg position and/or temporary discontinuation of the isosorbide dinitrate infusion, with only one patient requiring inotropic support at this time.

23 (37%) of the 63 patients treated with streptokinase had a hypotensive response, 20 occurring early, 9 late and 6 both early and late (Figure 14). Of the 65 patients treated with anistreplase, 33 (51%) had a hypotensive response, 26 early, 13 late and 6 both. There was a trend towards a higher frequency of hypotensive episodes in the anistreplase group, which did not reach statistical significance. There was no difference in the severity of hypotensive episodes between the two treatment groups, with only one requiring specific intervention. The streptokinase resistance titres and anti-SK IgG concentrations for the groups of blood pressure response were similar. The mean streptokinase resistance titre of those patients without a hypotensive response treated with

**INCIDENCE (%) OF HYPOTENSIVE EPISODES  
ACCORDING TO SITE OF INFARCT**



**INCIDENCE (%) OF HYPOTENSIVE EPISODES  
ACCORDING TO THROMBOLYTIC AGENT**



**Figure 14. HYPOTENSIVE EPISODES (PERCENTAGE OF TOTAL)  
IN RELATION TO SITE OF INFARCT AND THROMBOLYTIC  
AGENT**

SBP CHANGES - SK  
MEAN (95%CL)

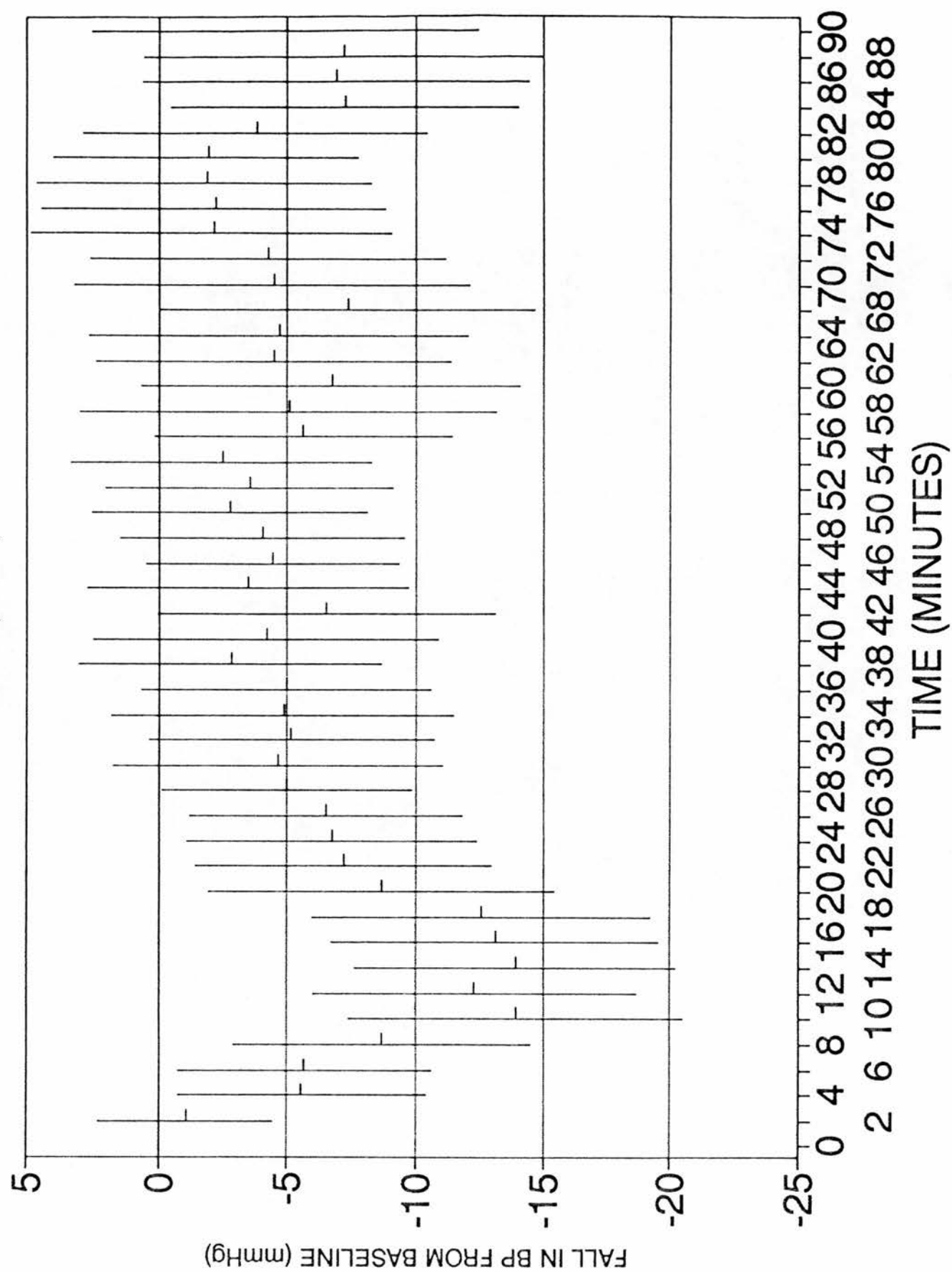


Figure 15. CHANGES IN SYSTOLIC BLOOD PRESSURE (MEAN, 95% CONFIDENCE LIMITS) FOLLOWING SK 1.5 MIU/60 MINUTES

DBP CHANGES - SK  
MEAN (95%CL)

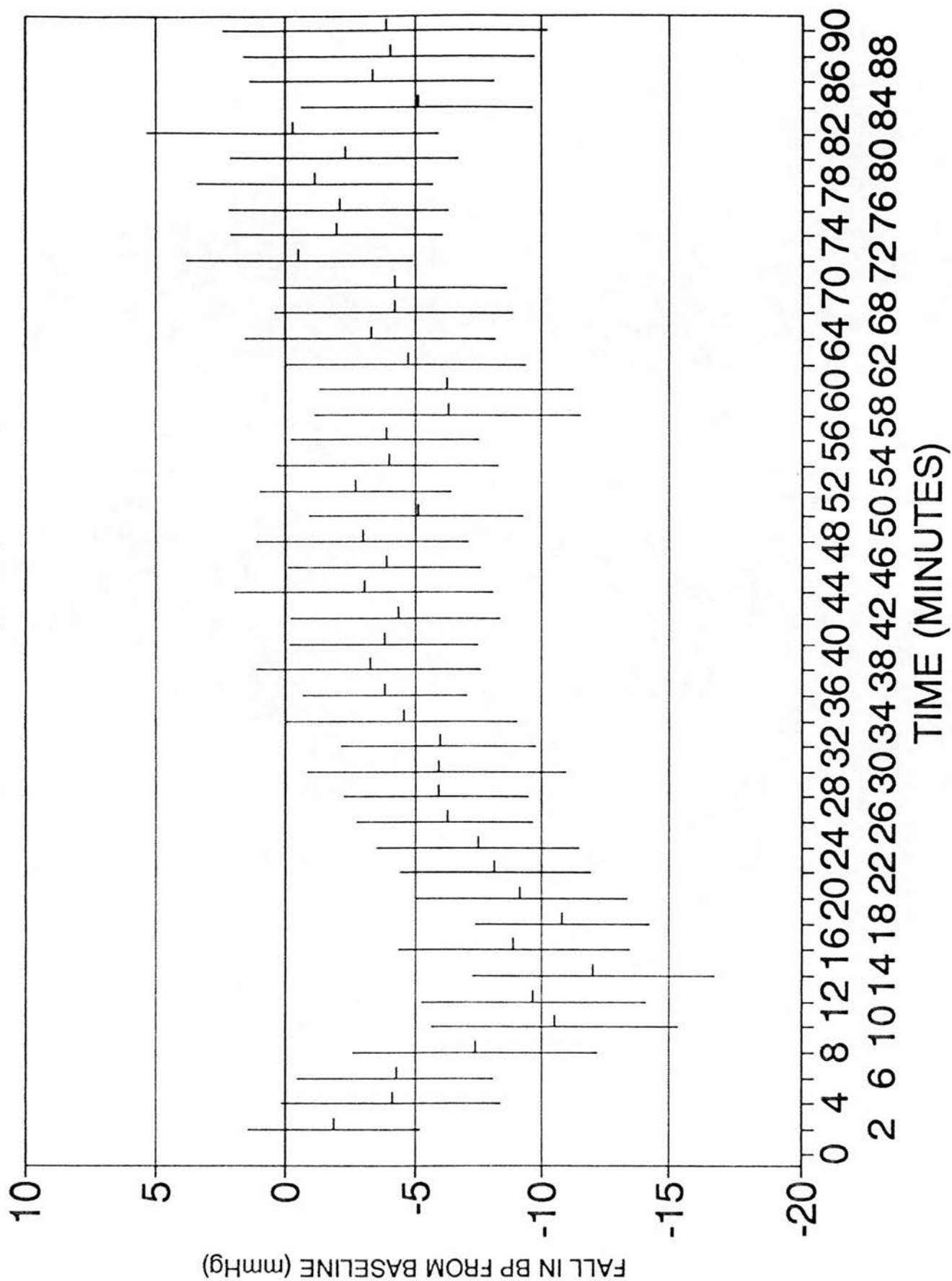


Figure 16. CHANGES IN DIASTOLIC BLOOD PRESSURE (MEAN, 95% CONFIDENCE LIMITS) FOLLOWING SK 1.5 MIU/60 MINUTES

SBP CHANGES - APSAC  
MEAN (95%CL)

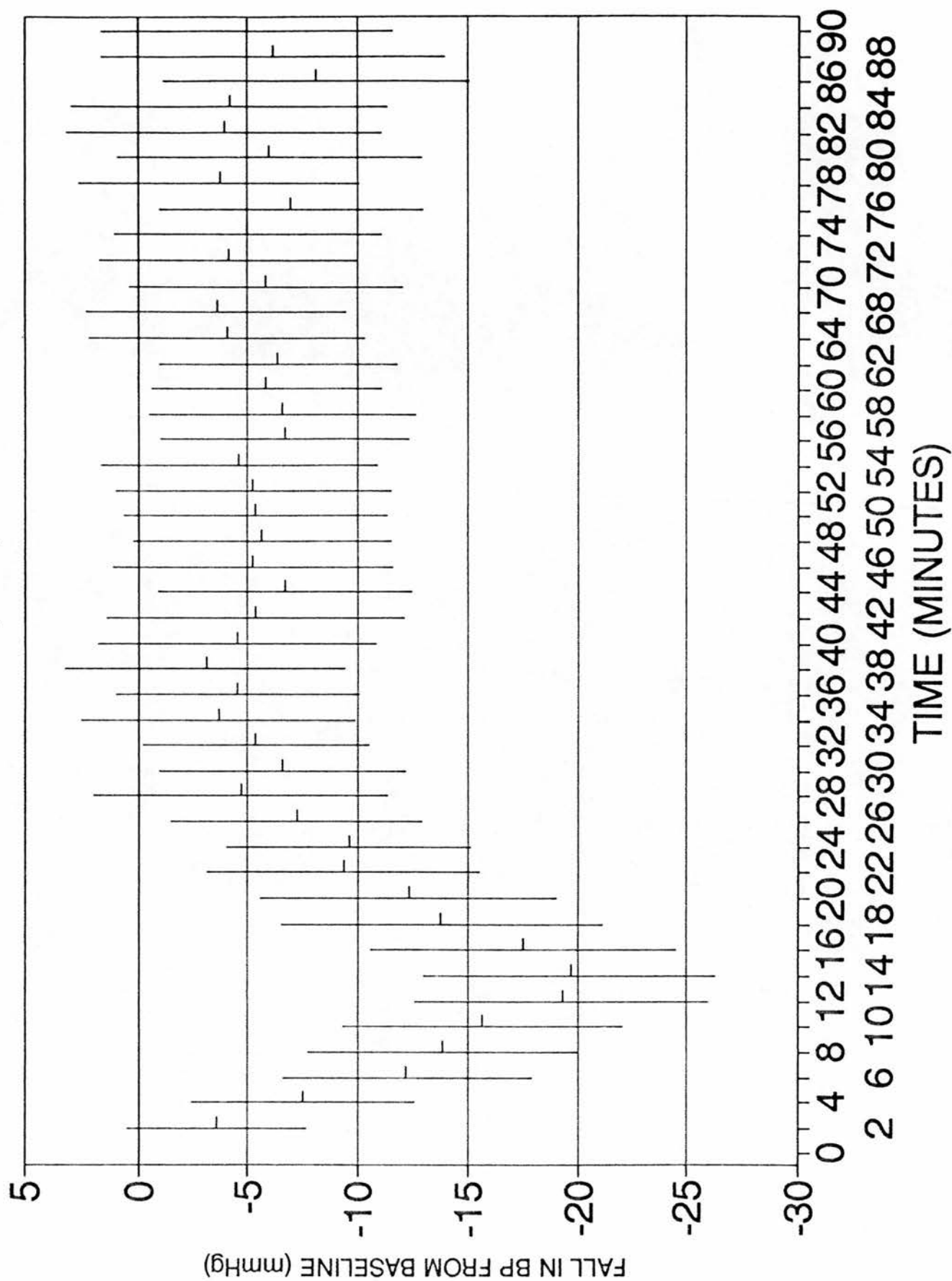


Figure 17. CHANGES IN SYSTOLIC BLOOD PRESSURE (MEAN, 95% CONFIDENCE LIMITS) FOLLOWING ANISTREPLASE 30 U/5 MINUTES

DBP CHANGES - APSAC  
MEAN (95%CL)

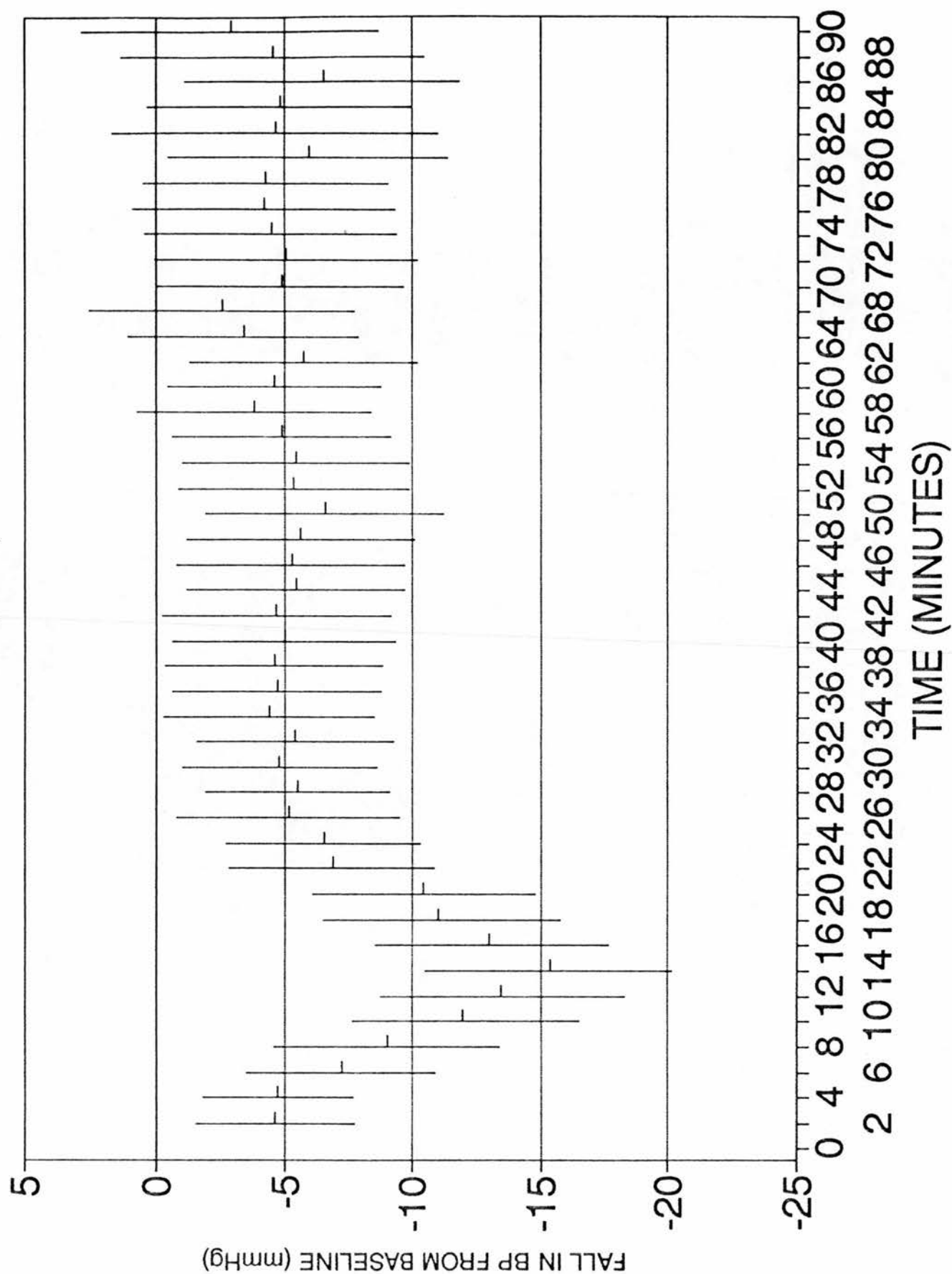


Figure 18. CHANGES IN DIASTOLIC BLOOD PRESSURE (MEAN, 95% CONFIDENCE LIMITS) FOLLOWING ANISTREPLASE 30 U/5 MINUTES

streptokinase was  $47.2 \pm 45.2$  compared to  $39.2 \pm 29.2$  SK IU.ml<sup>-1</sup> for all patients with a hypotensive response. Similarly for those patients treated with anistreplase, the streptokinase resistance titres were  $59.2 \pm 47.6$  vs  $75.9 \pm 86.4$  SK IU.ml<sup>-1</sup>. None of the differences were statistically significant, using non-parametric tests of location. The anti-SK IgG concentrations for the comparable groups were  $2.12 \pm 1.95$  vs  $1.19 \pm 1.10$  for the streptokinase-treated group, and  $1.45 \pm 1.38$  vs  $1.98 \pm 2.45$  µg SK binding.ml<sup>-1</sup>. None of the differences in anti-SK IgG were statistically significant. (Table 6 and Figure 19).

The areas under the concentration-time curves (AUCs) for B-β 15-42, D-dimer and the fall in fibrinogen and corrected plasma viscosity showed a moderate variance. The AUCs for all the parameters, for all the groups of blood pressure response were similar, with no significant differences identified. (Table 7 and Figure 20).

**Table 6**  
**Hypotensive episodes in relation to streptokinase**  
**resistance titres and anti-SK IgG concentrations. Also**  
**Figure 19**

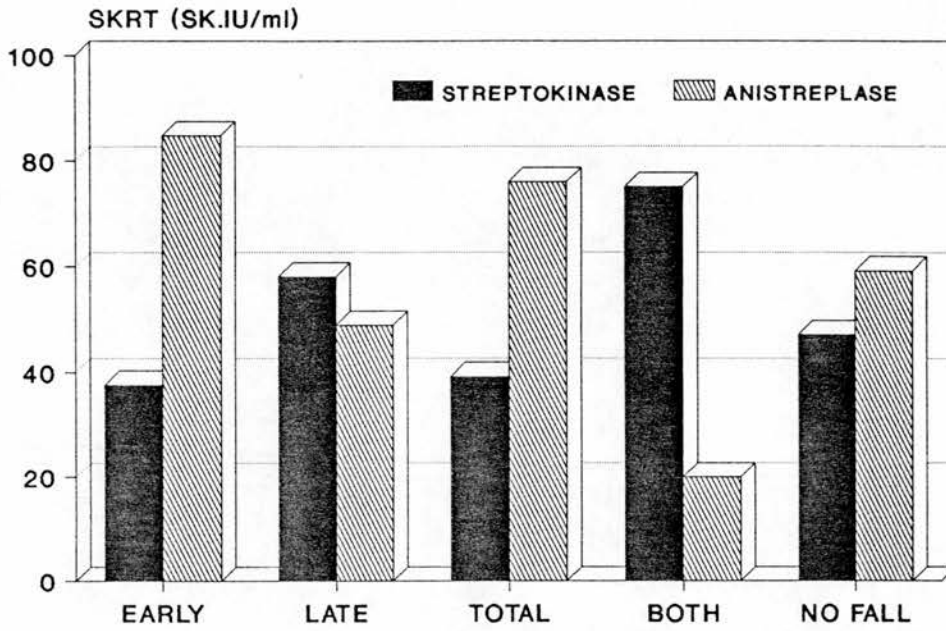
<b>SKRT</b> (SK IU ml <sup>-1</sup> )						
	Early	Late	Total	Both	No Fall	
<b>Streptokinase</b>						
Mean	37.6	58.0	39.2	75.0	47.2	NS
SD	29.2	40.2	31.0	35.4	45.2	
<b>Anistreplase</b>						
Mean	84.7	48.9	75.9	20	59.2	NS
SD	100.9	39.8	86.4	-	47.6	
<b>Anti-SK IgG</b> (µg SK binding ml <sup>-1</sup> )						
<b>Streptokinase</b>						
Mean	1.09	1.13	1.19	0.77	2.12	NS
SD	1.12	0.78	1.10	0.59	1.95	
<b>Anistreplase</b>						
Mean	2.03	1.41	1.98	0.95	1.45	NS
SD	2.65	1.26	2.45	0.86	1.38	

**Table 7.**

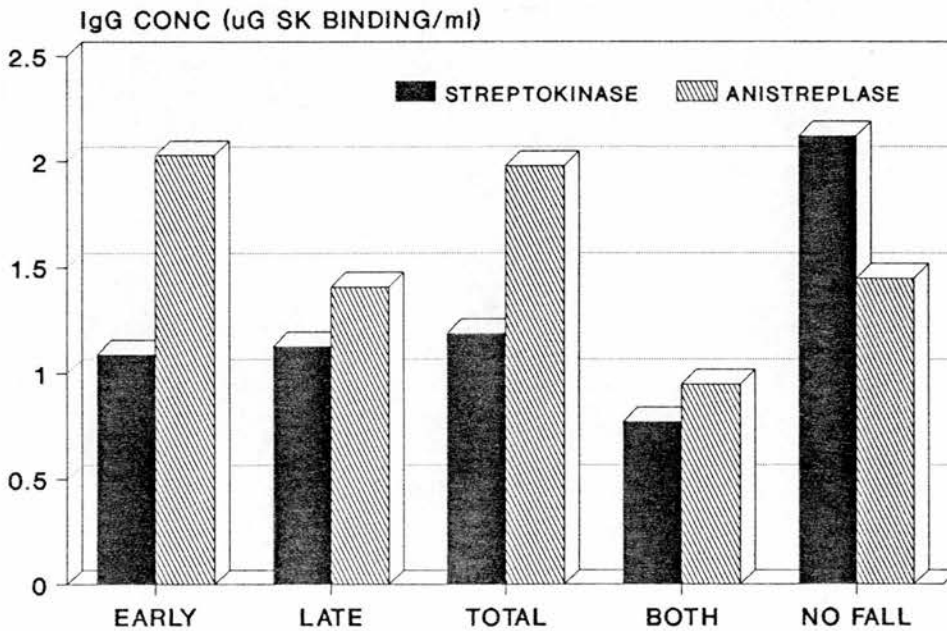
**Hypotensive episodes in relation to area under time-concentration curves of D-dimer (0-5 days), B-B 15-42 and fall in fibrinogen (0-90 minutes) (mean  $\pm$  SD). Also figure 20.**

	<b>D-dimer</b>	<b>B-B 15-42</b>	<b>Fibrinogen</b>
No fall	50019 (37084)	477 (239)	2.27 (1.04)
Early	53021 (37208)	482 (194)	2.20 (0.97)
Late	45338 (25381)	441 (164)	1.79 (1.08)
Both	45403 (30153)	452 (176)	1.96 (1.24)
p value	NS	NS	NS

**HYPOTENSIVE EPISODES RELATED  
TO MEAN STREPTOKINASE RESISTANCE TITRE**



**HYPOTENSIVE EPISODES RELATED  
TO MEAN IgG CONCENTRATIONS**



**Figure 19. HYPOTENSIVE EPISODES IN RELATION TO PRETREATMENT STREPTOKINASE RESISTANCE TITRES AND ANTI-SK IgG CONCENTRATIONS**

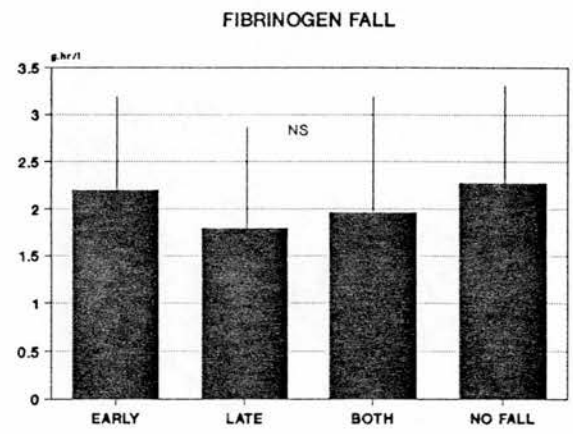
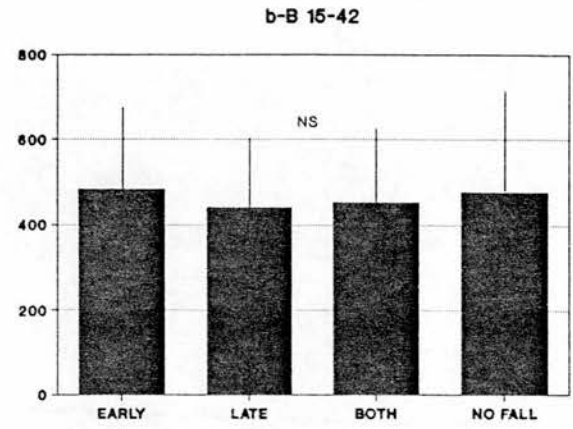
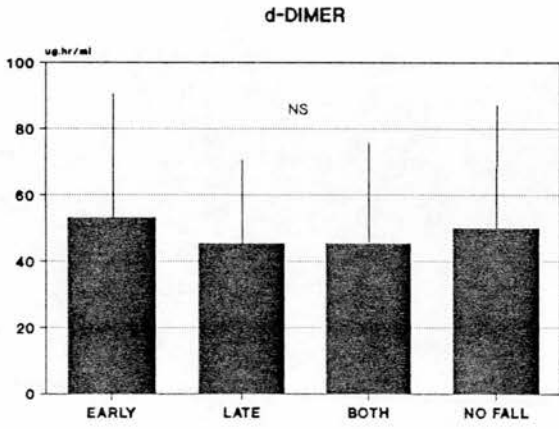


Figure 20. HYPOTENSIVE EPSISODES IN RELATION TO AREA UNDER TIME-CONCENTRATION CURVES OF D-DIMER (0-5 DAYS), B- $\beta$  15-42 AND FALL IN FIBRINOGEN (0-90 MINUTES) (MEAN  $\pm$  SD)

## DISCUSSION

Although the fall in blood pressure following thrombolytic therapy has been previously reported, this is the most detailed study examining the frequency, time of onset and duration of blood pressure response following streptokinase and anistreplase, using preset definitions of hypotension. In clinical experience, these falls in blood pressure are short-lived, often asymptomatic and rarely require therapeutic measures beyond the adoption of a head down position and the temporary discontinuation of co-administered vasodilator therapy, such as intravenous nitrates. Previous studies have reported similar findings. Valentine et al., (1985) commented on a 17% incidence of hypotension related to intravenous streptokinase infusion. In the GISSI-1 study (1986) there was a reported incidence of only 3% of 11806 patients, probably an underestimate reflecting the imprecision of the definition of hypotension, perhaps insufficiency of measurement, and the paucity of associated clinical events.

This study confirms the incidence of this side-effect as 23 of 63 (37%) patients following streptokinase, and 33 of 65 (51%) following anistreplase, in a population of patients with baseline systolic blood pressure exceeding 95 mmHg. The difference between the two agents is not statistically significant. The power of this study to detect the apparent difference in frequency of hypotensive response between the two agents, assuming a 5% chance of a Type I

error, is 62.7%, therefore a Type II error cannot be excluded. If such a difference is real, a study to demonstrate this difference with 90% power would require in excess of 500 patients.

Most of the hypotensive responses observed occurred within the first 30 minutes of therapy (46 of 55 hypotensive responses), be the treatment either a 5 minute intravenous bolus of anistreplase, or a 60 minute continuous intravenous infusion of streptokinase, and in most cases was short-lived, with only 12 of these cases showing hypotension persisting beyond 30 minutes after thrombolytic therapy.

We have also shown that the propensity for a hypotensive response is equally distributed between anterior and inferior infarcts. There are arguments for either site of myocardial infarct to be more prone to hypotension. Anterior infarcts are associated with a greater degree of left ventricular dysfunction, and they would therefore be more susceptible to hypotension, as has been described by Lew et al. (1985). However, one suggested mechanism for hypotension in this context is the Bezold-Jarisch vagal reflex, which is usually associated temporally with coronary reperfusion in inferior wall myocardial infarction (Wei et al., 1983, Koren et al., 1986), making inferior myocardial infarctions more prone to hypotension. In this study, 21 of 52 (40%) anterior myocardial infarcts had a

hypotensive response to thrombolytic therapy compared to 34 of 73 (46%) inferior infarcts (NS), not substantiating any difference between the two groups.

The degree of blood pressure fall in this study using conventional doses of streptokinase and anistreplase are less than those shown by Lew et al. (1985) who demonstrated a mean fall in systolic and diastolic blood pressure of 35 and 20 mmHg respectively, following a very rapid infusion of streptokinase of 750,000 IU over 7-78 minutes (mean 30 minutes), with systolic blood pressure falling below 90 mmHg in 38% of their patients. The comparable incidence for our patients was 33% for streptokinase, and 55% for anistreplase (NS, power 0.69, assuming Type I rate of 0.05). They too found the period of hypotension to be brief, lasting  $9 \pm 6$  minutes, and the incidence equally distributed between anterior and inferior myocardial infarcts. The magnitude of the hypotensive response is related to the rate of administration of streptokinase. White et al., (1987) noted the systolic blood pressure to fall below 80 mmHg in 9% of their patients receiving 1.5 MIU of streptokinase over only 30 minutes.

The streptokinase resistance titre is a well established functional parameter of global resistance to lysis of fibrin by streptokinase, and its level in man reflects previous exposure to streptococcal protein, probably in the form of previous streptococcal infections (Flute, 1973).

Streptokinase resistance titres in this study population were in general low, but with a marked skewing towards a few, very high titres. The anti-SK IgG concentrations measured are specific circulating antibodies of the IgG class raised against streptokinase, and can similarly be expected to reflect exposure to streptococci. In this study, no association has been demonstrated between these markers measured pretreatment, and the incidence of hypotensive responses to the streptokinase-containing thrombolytic agents. This would imply that these antibodies in these low concentrations are not directly implicated in an immunological mechanism responsible for the hypotensive responses observed. As markers of previous exposure to streptococcal protein and an associated immune response, these antibodies reflect other immunological mediators. The absence of an association suggests that the hypotensive response is not directly influenced by these immunological factors, at least in the low concentrations observed. It is difficult to extrapolate these results to the considerably higher streptokinase resistance titres seen up to one year following therapy with the streptokinase-containing thrombolytic agents (Jalihal et al., 1990), but the risks of retreatment may be less than has been suggested.

B- $\beta$  15-42 is a peptide degradation fragment of fibrin(ogen), and is a measure of plasmin activity due to plasminogen activation by the streptokinase-plasminogen

activator complex. D-dimer is a breakdown product of cross-linked fibrin, a measure of clot lysis. The systemic effects of the two thrombolytic agents are estimated by changes in circulating fibrinogen and plasma viscosity.

It has previously been demonstrated that the release curves of B- $\beta$  15-42 and D-dimer, and effects on plasma fibrinogen are identical in both timing and magnitude with both the streptokinase-containing thrombolytic agents (Douglas et al., 1988), and it is therefore justifiable to analyse data from both treatment groups. The time course of the release profiles of the two peptides, the reduction in plasma viscosity and circulating fibrinogen are very different. In this study, the changes in B- $\beta$  15-42, plasma viscosity and fibrinogen were calculated over the first 90 minutes, the period most relevant to the blood pressure changes. The D-dimer release has been measured over the period of 0-5 days in view of the persistence of this peptide in the circulation.

In this study no relation between these parameters of fibrin- and fibrinogen-olysis and the incidence of hypotensive response has been shown. The fibrinogen changes in particular have a large variance, and while it is not possible to entirely exclude a weak association, it is unlikely that any relation of clinical significance exists.

Previous workers (Lew et al., 1985) have discussed the possibility that the hypotensive response following streptokinase may be due to a reduction in peripheral vascular resistance reflecting reduced plasma viscosity caused by the fall in plasma fibrinogen. The present study excludes this hypothesis by demonstrating a lack of association between the fall in fibrinogen and corrected plasma viscosity and the blood pressure response.

It has also been suggested that fibrin degradation products can activate the prostaglandin-prostacyclin system causing vasodilatation and hypotension (Andersson, 1983). We were unable to show such a relationship between blood pressure and the fibrin product D-dimer, nor changes in fibrinogen. Similarly, it has been suggested that serious allergic-type reactions may be mediated by activation of the complement pathway, by complement lysis mediated by plasmin in response to plasminogen activators eg: rt-PA (Bennett et al., 1987). Again if this were the mechanism involved, it would be anticipated that there would be demonstrable differences in other parameters of plasmin activity.

The exact mechanism of the hypotension observed following the administration of streptokinase-containing thrombolytic agents remains unexplained. Other possible explanations include a direct negative inotropic or vasodilator effect

of the thrombolytic agents, an effect on the baroreceptors, endothelium mediated vasodilatation (for example due to endothelium derived relaxing factor) or neurohumoural mediation perhaps by atrial natriuretic peptide.

In this study, the hypotensive response to these agents is common, short-lived and generally well-tolerated. Nevertheless this side effect limits the rate of administration of the streptokinase-containing thrombolytic agents. It is therefore important to identify the mechanism of this response, possibly allowing the development of pharmacological means of modulating the blood pressure response to these agents.

**CHAPTER SIX**

**THE TIME COURSE OF ACQUIRED RESISTANCE TO  
STREPTOKINASE-CONTAINING THROMBOLYTIC AGENTS IN  
ACUTE MYOCARDIAL INFARCTION.**

## **INTRODUCTION**

The previous chapters have discussed the influence of pretreatment resistance to streptokinase on the efficacy of the streptokinase-containing thrombolytic agents, streptokinase and anistreplase, as judged by their ability to achieve coronary patency and their influence on hypotension following the administration of these agents. These studies did not demonstrate an obvious effect, but suggested that those patients with higher levels of resistance, reflected in high streptokinase resistance titre or anti-SK IgG concentration, have a poorer outcome in terms of grades of reperfusion.

As has been discussed above, the pretreatment resistance in the population was in general low, as would be expected in a population not previously exposed to streptokinase, and those with high levels were few in number. Previous studies have demonstrated that those patients with very high resistance to streptokinase, probably acquired by recent streptococcal infection, have a poor lytic response to streptokinase (Hirsh et al., 1970, James, 1973). With the increasing use of thrombolytic therapy for indications such as acute myocardial infarction, the major importance of acquired resistance to the streptokinase-containing thrombolytic agents lies in the area of those patients who

are re-exposed to these agents. In particular it is important to define the safe time period for retreatment with streptokinase, with regard to both efficacy and freedom from adverse events.

It was the purpose of this study to define the time course of induction and decline of resistance to streptokinase. It has already been shown that the streptokinase resistance titre reaches a peak value at two weeks following therapy, and is returning towards pretreatment levels by six months (Fletcher et al., 1959, Kostering et al., 1978, Massel et al., 1989, Jaliha and Morris, 1990).

In this study, the observation period was extended and includes observations on both the induction of specific antibodies as the anti-SK IgG concentration, in addition to the streptokinase resistance titre, a global reflection of both antibody-mediated resistance and the influence of components such as  $\alpha_2$ -antiplasmin, and proteins such as fibrinogen and plasminogen. The fibrinolytic response to streptokinase and anistreplase, in the time period following exposure to these agents is also examined in vitro.

## **METHODS**

128 consecutive eligible patients (101 male, 27 female, age range 31-70 years) were recruited, being the entire study population of the anistreplase / streptokinase comparative study, the details of which have already been recorded (Appendix I). All patients presented with acute myocardial infarction, and without contraindications to thrombolytic therapy, and received either a conventional dose of 1.5 MIU streptokinase infused intravenously over 60 minutes, or 30 U of anistreplase as a 5 minute continuous intravenous injection, in a double-blind double-dummy design. Patients with prior exposure to streptokinase-containing thrombolytic agents were excluded (Appendix IV).

Blood samples were collected from each patient prior to dosing and serially at 2 and 4 weeks, 3, 6, and 12 months. In a subgroup of 40, blood samples were taken daily for the first 14 days to determine the early rise in resistance following thrombolytic therapy. After one year, following interim analysis of the results, 67 of these patients were sampled on one further occasion, which was distributed over the period of 12 to 30 months following therapy.

Streptokinase resistance titre and specific anti-SK IgG concentrations were measured in individual plasma samples using methods which have already been described.

### **Clot lysis assay**

The clot assay described measures the release of soluble fibrin degradation products from radio-labelled plasma clots incubated in autologous plasma. [ $^{125}\text{I}$ ]-fibrinogen (3 $\mu\text{Ci}$ , 33 $\mu\text{g}/10\mu\text{l}$ ) was mixed with pooled citrated human plasma giving a final concentration of 6  $\mu\text{Ci}/\text{ml}$  plasma. This mixture was kept on ice until immediately prior to use when calcium chloride and bovine thrombin were added to give final concentrations of 23mM and 0.43U/ml respectively, and aliquots dispensed into 0.5 ml microtubes. The microtubes were incubated at 37 $^{\circ}\text{C}$  for 10 minutes then kept on ice for a further 20 minutes. The resulting clots were dislodged with a 200  $\mu\text{l}$  jet of human serum albumin (1% HSA) in phosphate buffered saline, pH 7.3 and transferred to a flat bottomed tube using a wide bore pipette. The HSA buffer was replaced by autologous plasma (300 $\mu\text{l}$ ) and incubated in a water bath at 37 $^{\circ}\text{C}$ .

The thrombolytic agents streptokinase and anistreplase were diluted into 0.05M sodium phosphate/0.1M sodium chloride/0.01% Tween 80 buffer pH 7.4 prior to use, and an aliquot of 30 $\mu\text{l}$  added to the plasma phase surrounding the clot and mixed. Aliquots of 20  $\mu\text{l}$  were removed at time intervals from 0.5 to 4 hours, and gamma-counted along with the remaining plasma containing the clot. A control lysis study using buffer alone was performed to calculate

spontaneous clot lysis. Thus clot lysis due to different concentrations of plasminogen activator over a range of incubation times was calculated and the spontaneous lysis subtracted. These assays were performed in quadruplicate.

#### **DATA ANALYSIS**

The data is markedly skewed toward the higher results, and for this reason a variety of stratagems have been used for the display of the data. The baseline pretreatment data can be transformed to a normal distribution by  $\log_{10}$  transformation for the anti-SK IgG concentration and the calculation of the fourth root for streptokinase resistance titre. Thus a proportion of each data set at each time point greater than one or two standard deviations above the mean can be calculated. In addition, the medians and means of the transformed data have been displayed, along with the proportion at each time point outwith the pretreatment range.

#### **RESULTS**

The pretreatment streptokinase resistance titre was measured in 97 patients, and a mean of 56 patients were studied at each time point to 12 months giving a follow-up rate of 57%. After the 12 month initial follow-up period, 67 patients attended for repeat measurement (68%), divided into three time points. The pretreatment anti-SK IgG concentration was measured in 125 patients, and a mean of 67 patients were available for follow-up specimens to 12

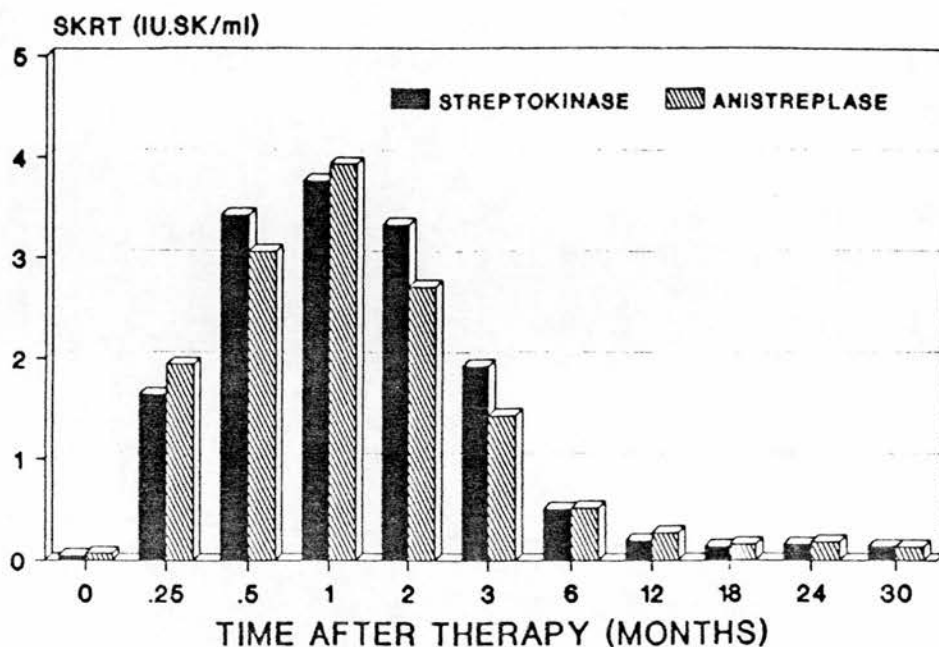
months (53%). The 67 patients in whom anti-SK IgG concentrations were measured in the period from 18-30 months represented 54% of the original population. Not all of the original cohort was available for follow-up over this period, due to death or disability.

### **Streptokinase Resistance Titre**

The time course of the streptokinase resistance titre was similar in both the streptokinase and anistreplase treatment groups (Figure 21). Pretreatment titres were exceeded by 5 days after dosing, and achieved peak values of 40-fold of median at 14 days after treatment. After 12 months, each patient was sampled on one more occasion, over the period of 12-30 months following therapy. The values were collated into groups at 18, 24 and 30 months, with different patients appearing at each time point.

Although the rate of rise in streptokinase resistance titre and time course of its fall were identical between the two treatment groups, there was a fortuitous higher pretreatment streptokinase resistance titre in the group allocated to anistreplase (median 50 vs 20 IU/ml,  $p < 0.05$ ). (Figure 9, Chapter 4).

### DEVELOPMENT OF SKRT ACCORDING TO THROMBOLYTIC AGENT



### DEVELOPMENT OF IgG ACCORDING TO THROMBOLYTIC AGENT

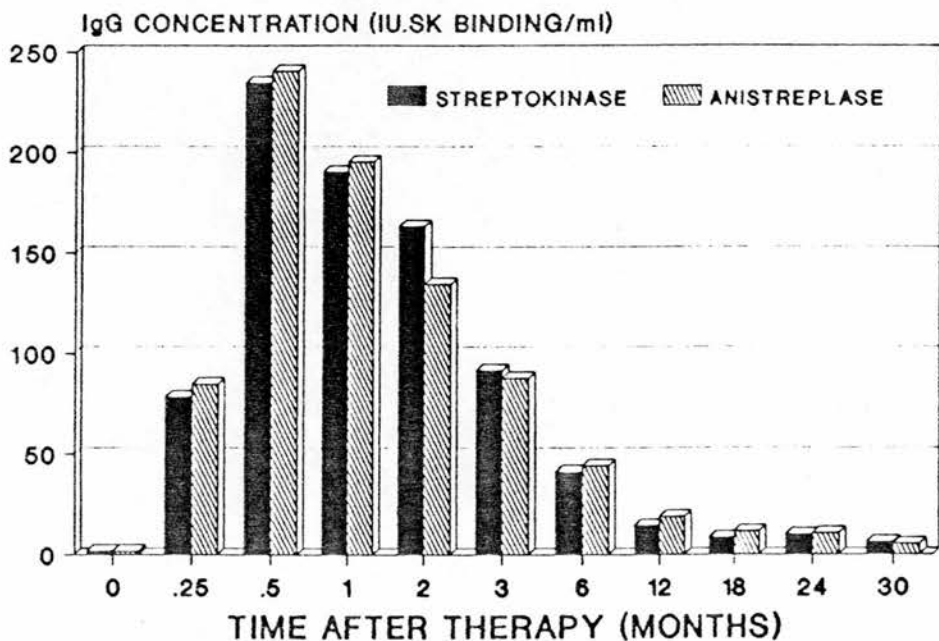


Figure 21. DEVELOPMENT OF STREPTOKINASE RESISTANCE TITRE FOLLOWING 1.5 MIU STREPTOKINASE AND 30 U ANISTREPLASE

Peak titres were attained 2 weeks after dosing (Figure 22), and started to decline by 3-6 months, however the elevation in individual pretreatment levels remained marked in those patients with a complete data set (Figure 23). For the whole population studied, 72% of the values had returned to within the pretreatment range by 6 months, and all were within the pretreatment range by eighteen months (Figure 24). However, only 49% of values were within two standard deviations of the pretreatment population at 12 months, and only 58% in the subgroup studied at 24 months (Figure 25). The decline in streptokinase resistance titre in the period from 12 to at least 24 months after treatment was very slow.

Using the median streptokinase resistance titre for each sampled group at each time point, and an estimated volume of distribution of plasma proteins of 6 l, it is possible to calculate the theoretical dose of streptokinase which would be neutralised by this degree of resistance. The results are displayed in Figure 26. Similar approaches have been used by other authors (Jalihal and Morris, 1990). Using this model, all of the standard dose of streptokinase is neutralised by the circulating resistance for at least 12 months, and approximately 50% of the dose remains inhibited to 30 months.

MEDIAN STREPTOKINASE  
RESISTANCE TITRE

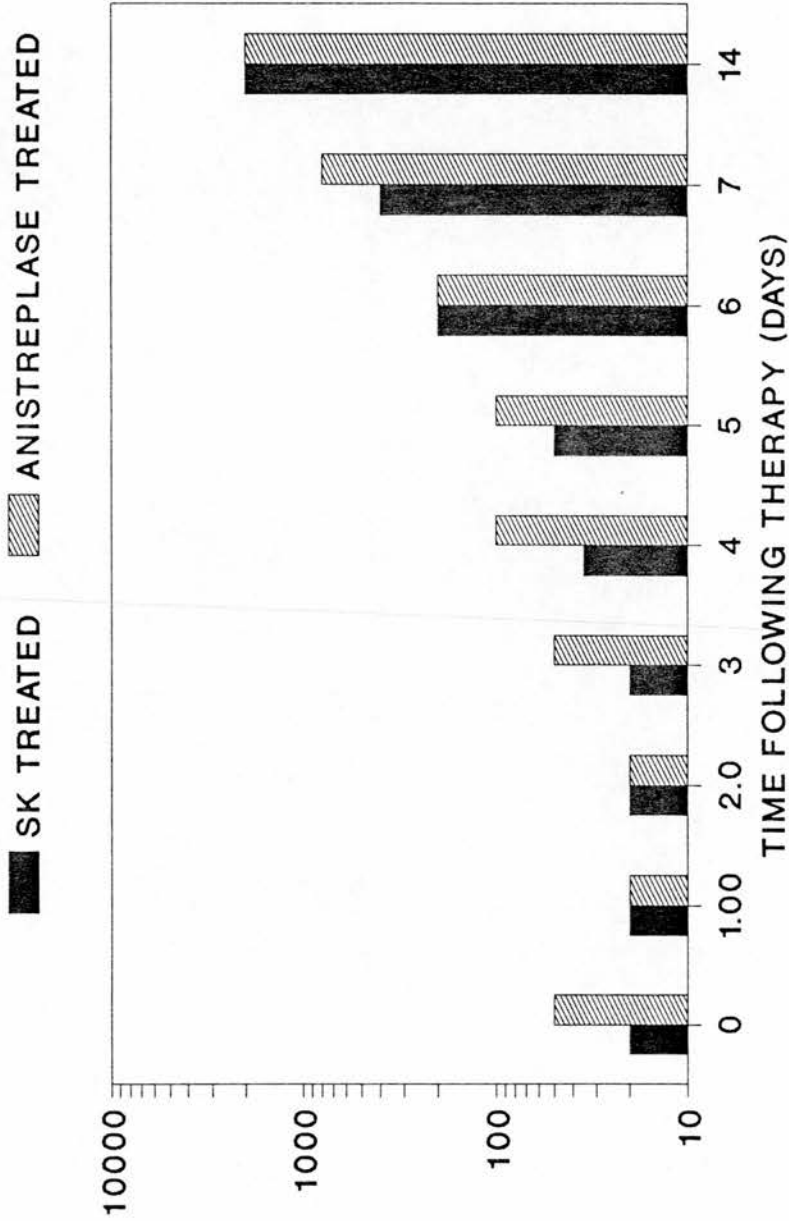
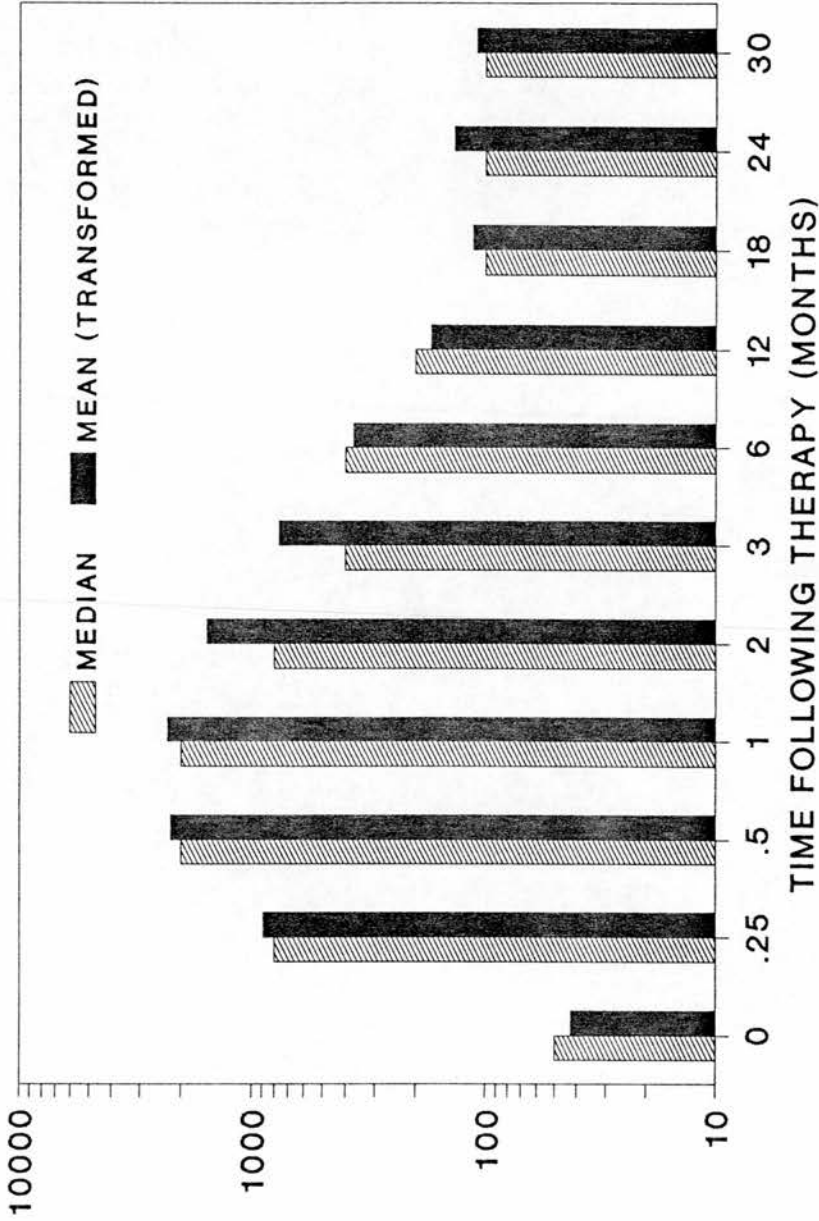


Figure 22. EARLY RISE IN STREPTOKINASE RESISTANCE TITRE OVER FIRST 14 DAYS FOLLOWING TREATMENT

**MEDIAN AND MEAN (TRANSFORMED)  
STREPTOKINASE RESISTANCE TITRE**



**Figure 23. TIME COURSE OF MEAN AND MEDIAN STREPTOKINASE RESISTANCE TITRE FOLLOWING STREPTOKINASE-CONTAINING THROMBOLYTIC AGENTS OVER 30 MONTH FOLLOW-UP PERIOD**

PROPORTION EXCEEDING PRETREATMENT RANGE OF STREPTOKINASE RESISTANCE TITRE

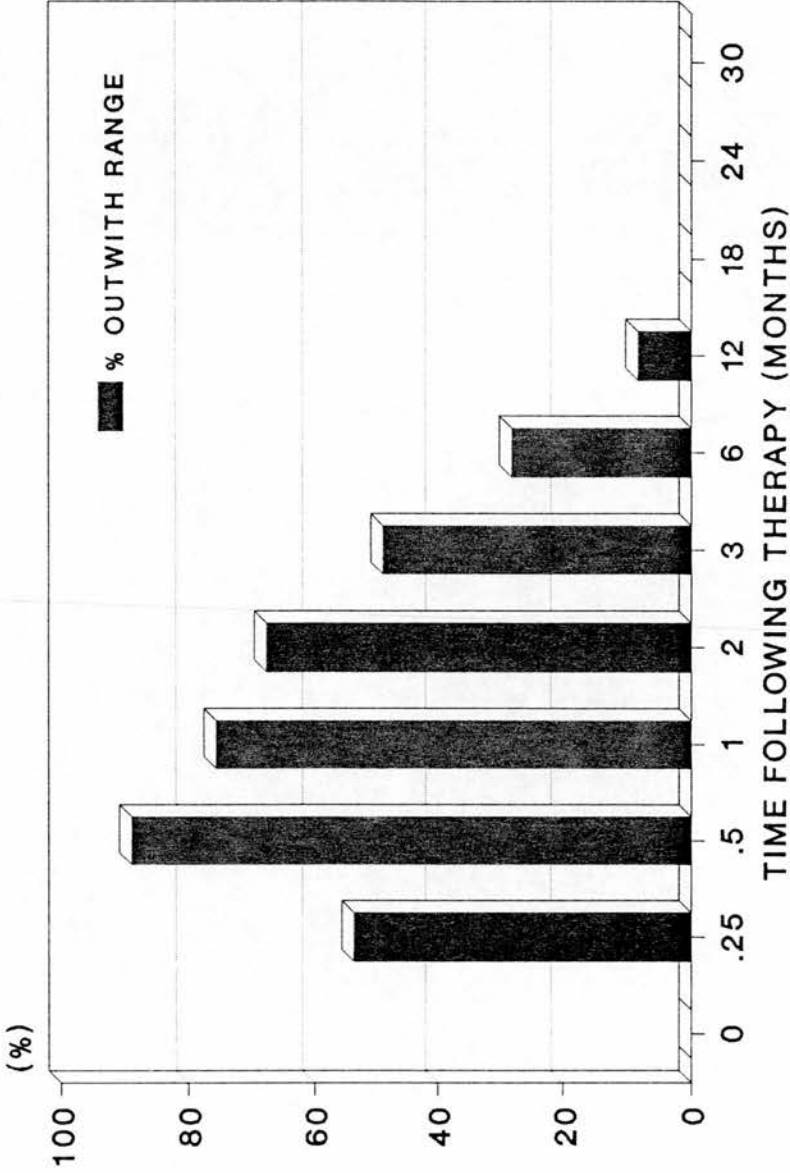


Figure 24. PROPORTION OF VALUES OF STREPTOKINASE RESISTANCE TITRE EXCEEDING THE PRETREATMENT RANGE OVER 30 MONTHS FOLLOWING TREATMENT

PROPORTION OF SKRT EXCEEDING  
1 OR 2 SDs OF PRETREATMENT VALUE

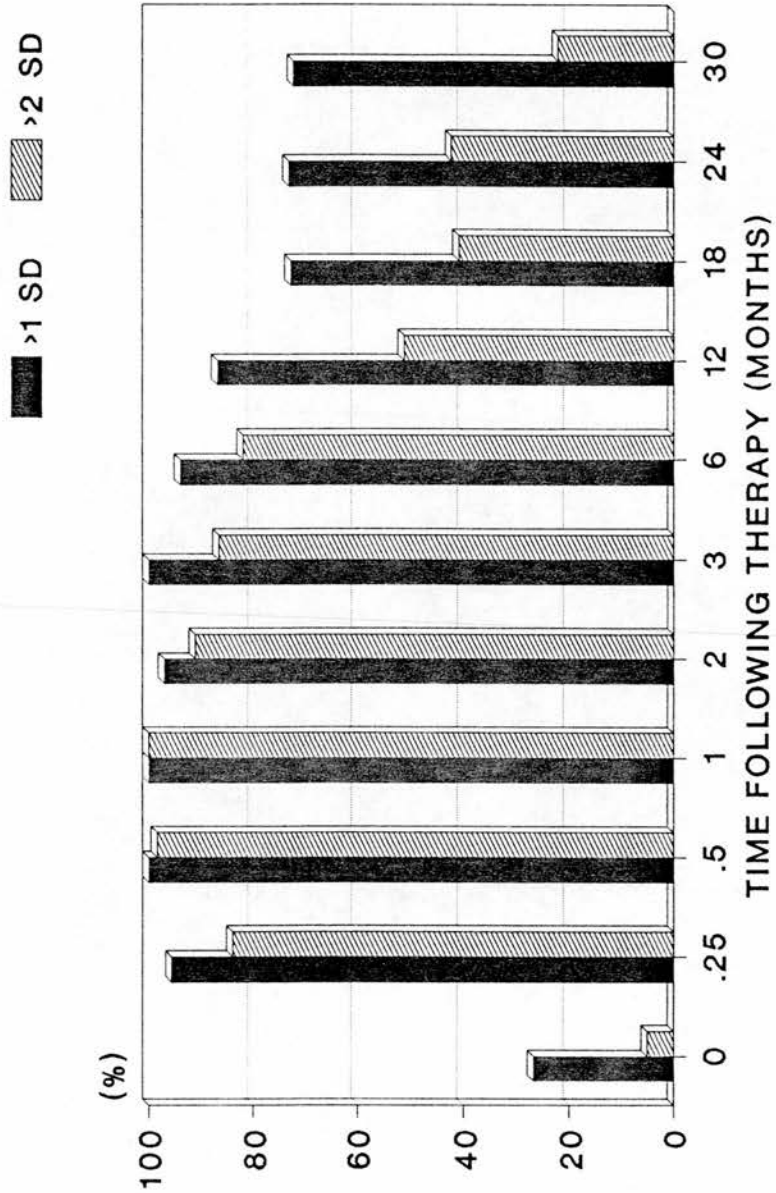


Figure 25. PROPORTION OF VALUES OF STREPTOKINASE RESISTANCE TITRE EXCEEDING PRETREATMENT MEAN PLUS 1 OR 2 STANDARD DEVIATIONS OVER 30 MONTHS FOLLOWING TREATMENT

# THEORETICAL DOSE OF SK NEUTRALISED WITH TIME AFTER THERAPY

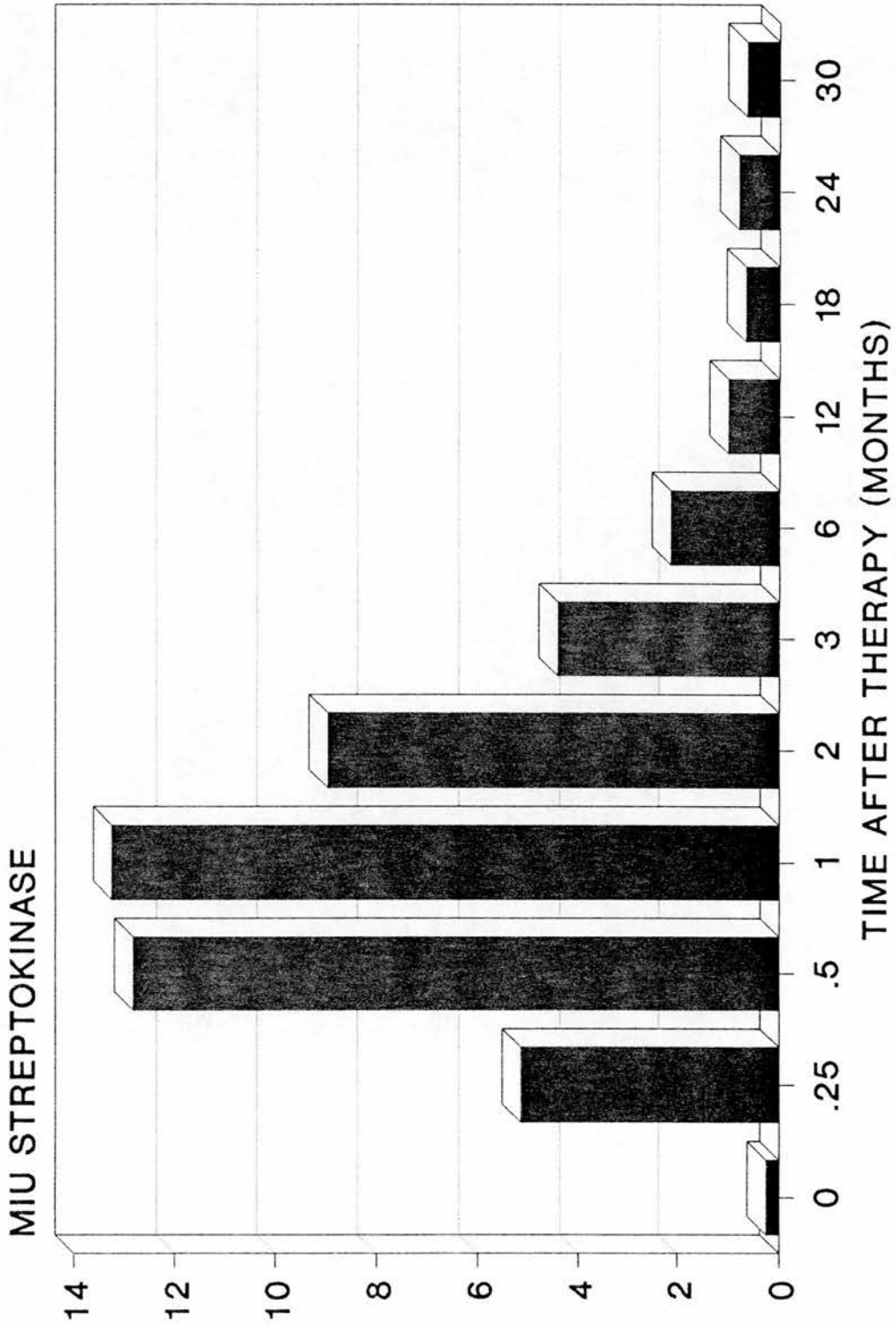


Figure 26. CALCULATED DOSE OF STREPTOKINASE NEUTRALISED

### **Anti-SK IgG Concentrations**

There were no differences in either the pretreatment anti-SK IgG concentrations, or the time course of changes in anti-SK IgG concentration between the two treatment groups. Following thrombolytic treatment there was a fall in anti-SK IgG concentration, attributable to antibody sequestration as antigen-antibody complexes, as has been described previously (Hoffmann et al., 1988). Subsequently, values returned to baseline within 5 days (Figure 27).

Peak values in anti-SK IgG concentration were achieved 14 days after therapy, with a peak level of 150-fold pretreatment level. The subsequent decline in antibody concentration was slow (Figure 28), with 48% of individual anti-SK IgG values returning to within the pretreatment range by 12 months after dosing, and 89% at 30 months (Figure 29). Only 39% of the population had returned to within two standard deviations of the pretreatment distribution at one year, and 50% at two years (Figure 30). At the latter time point there remained a nine-fold rise in median anti-SK IgG concentration relative to baseline.

MEDIAN ANTI-SK IgG  
CONCENTRATION

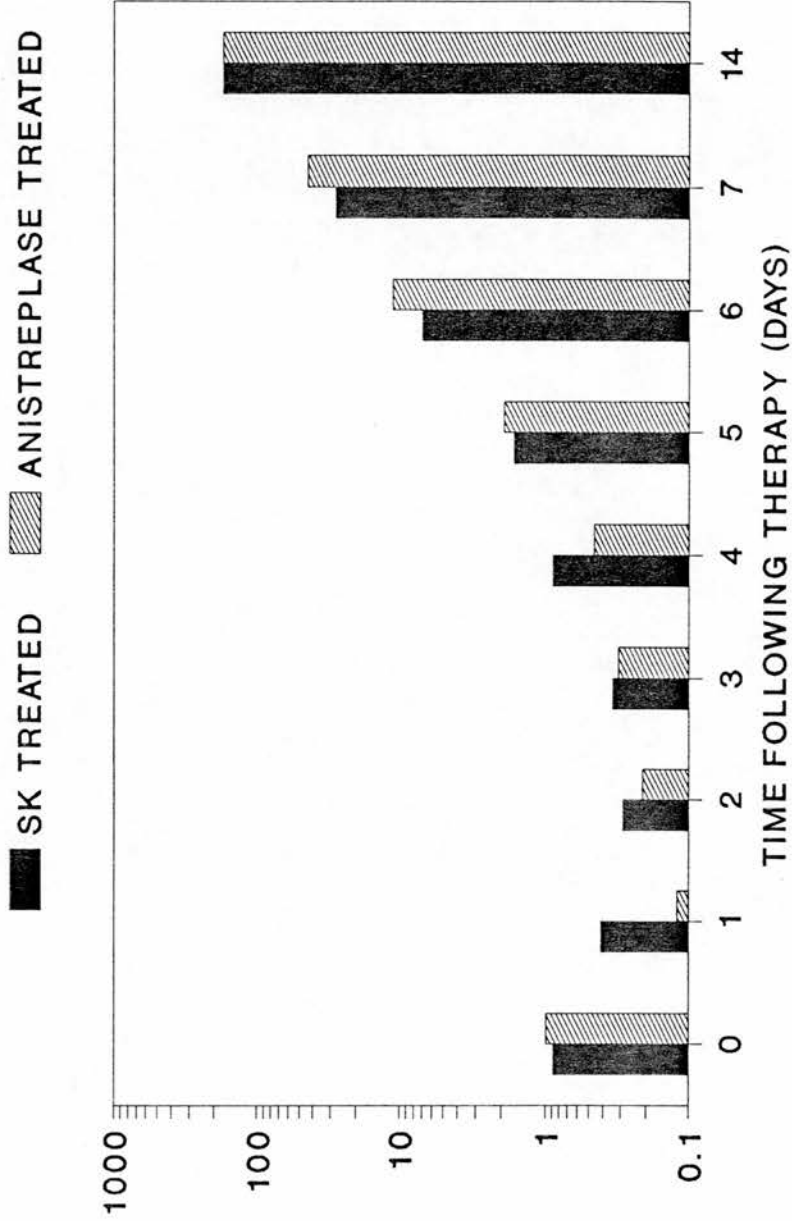


Figure 27. EARLY RISE IN ANTI-SK IgG CONCENTRATIONS OVER FIRST 14 DAYS FOLLOWING TREATMENT

MEDIAN AND MEAN (TRANSFORMED)  
IgG CONCENTRATIONS

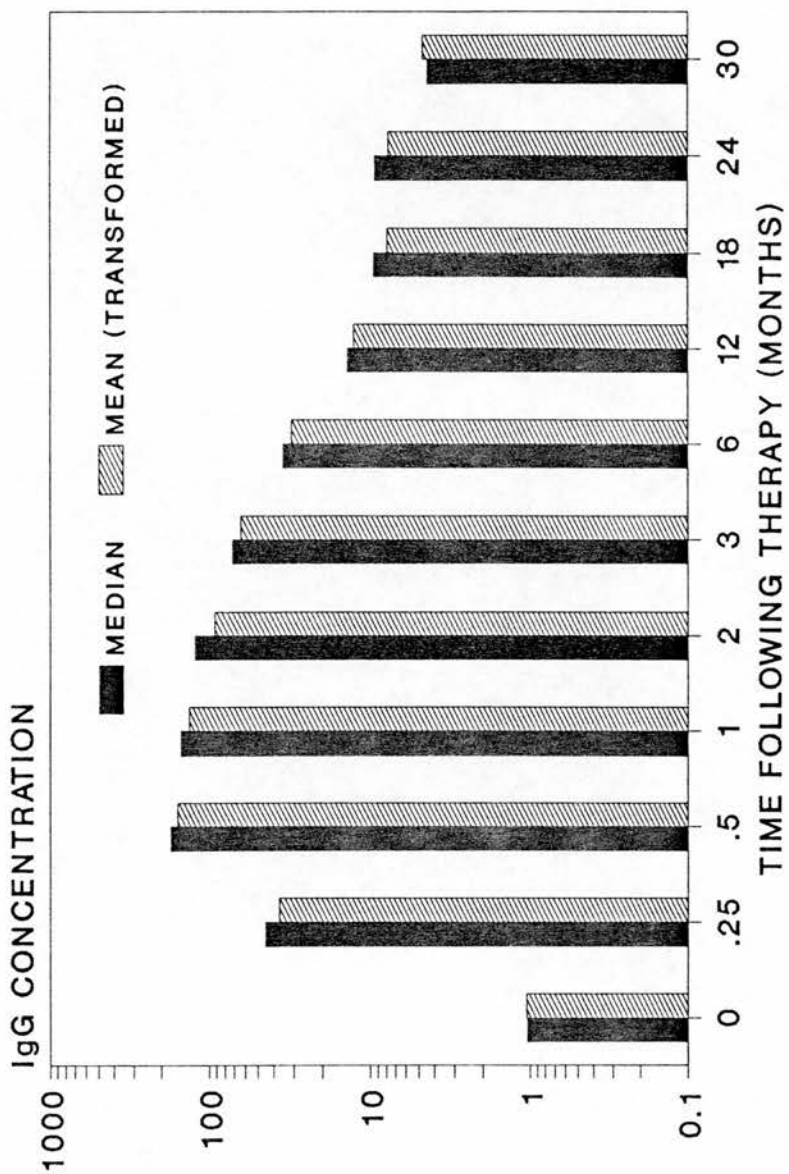


Figure 28. TIME COURSE OF MEAN AND MEDIAN ANTI-SK IgG CONCENTRATIONS FOLLOWING STREPTOKINASE-CONTAINING THROMBOLYTIC AGENTS OVER 30 MONTH FOLLOW-UP PERIOD

PROPORTION EXCEEDING PRETREATMENT RANGE OF IgG

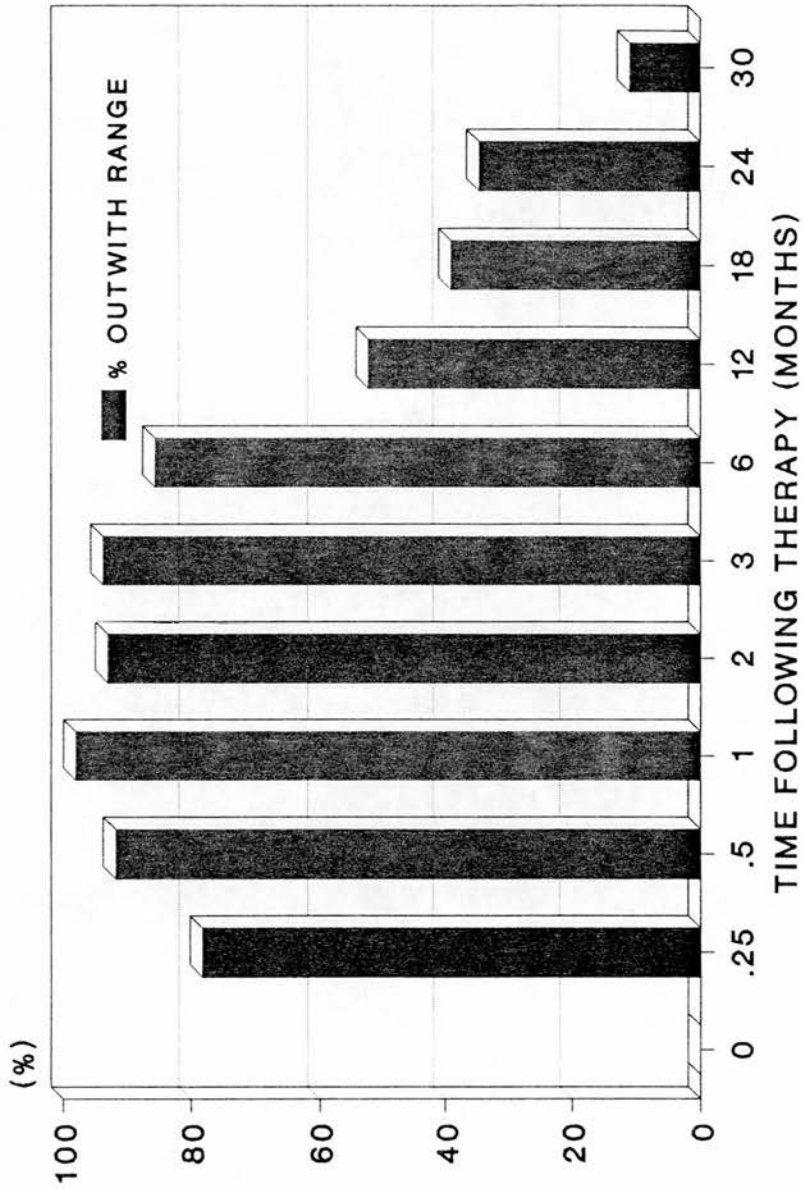


Figure 29. PROPORTION OF VALUES OF ANTI-SK IgG CONCENTRATION EXCEEDING THE PRETREATMENT RANGE OVER 30 MONTHS FOLLOWING TREATMENT

PROPORTION EXCEEDING 1 AND 2 SD  
OF PRETREATMENT IgG

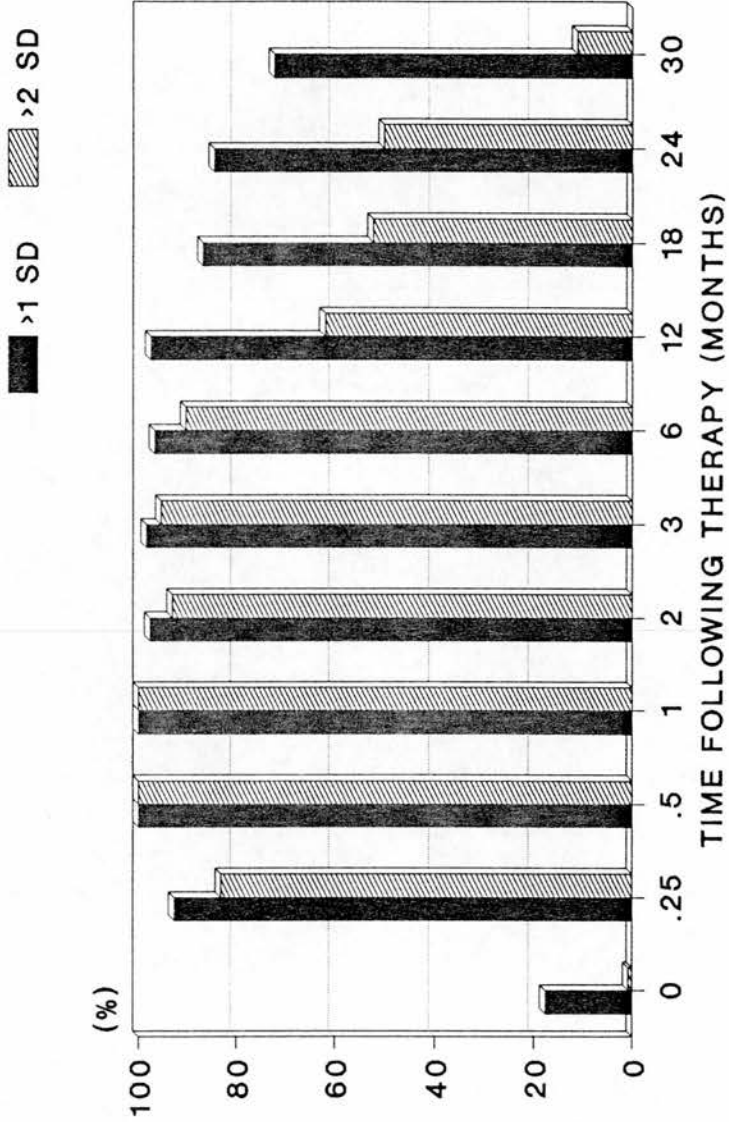


Figure 30. PROPORTION OF VALUES OF ANTI-SK IgG CONCENTRATION EXCEEDING PRETREATMENT MEAN PLUS 1 OR 2 STANDARD DEVIATION OVER 30 MONTHS FOLLOWING TREATMENT

### **Clot Lysis Assay on Pooled Plasma Samples**

It is difficult to extrapolate from the anti-SK IgG concentrations and streptokinase resistance titres to their possible functional (neutralising) effects, especially in view of the fact that the only relationship discussed in previous chapters is based on low levels of circulating resistance, in a population not previously exposed to streptokinase. The purpose of the pooled clot lysis assay was therefore to further investigate possible effects of the two measures of streptokinase resistance on the thrombolytic response. The limited sample volume available from individual patients necessitated pooling aliquots of plasma at each sampling point from pretreatment to 30 months, with samples from patients treated with anistreplase or streptokinase being pooled separately.

Fibrinogen, plasminogen,  $\alpha_2$ -antiplasmin, and  $\alpha_2$ -macroglobulin were measured in each pool, and there were no major differences in these proteins between the pretreatment pool and any of the pools for the time points to 30 months (Table 8). Differences in fibrinolytic responses to the two thrombolytic agents can therefore be ascribed to changes in antibody.

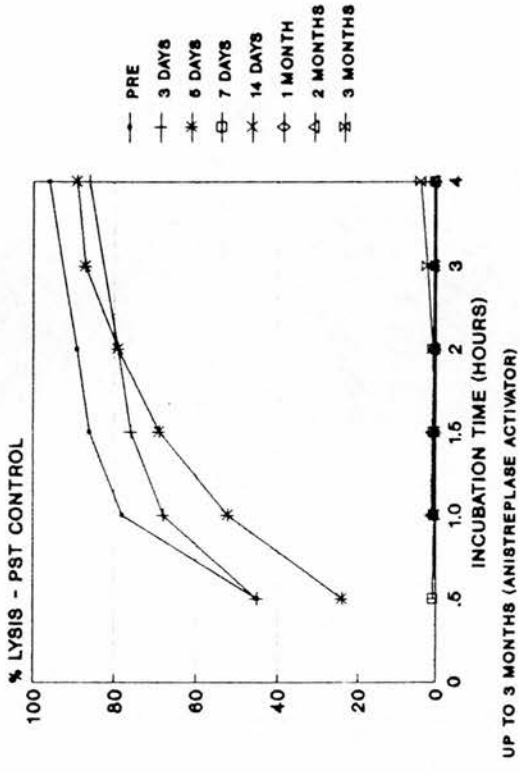
The concentration-effect curves for the period of 0-30 months are shown in Figures 31 & 32. The fibrinolytic

Table 8

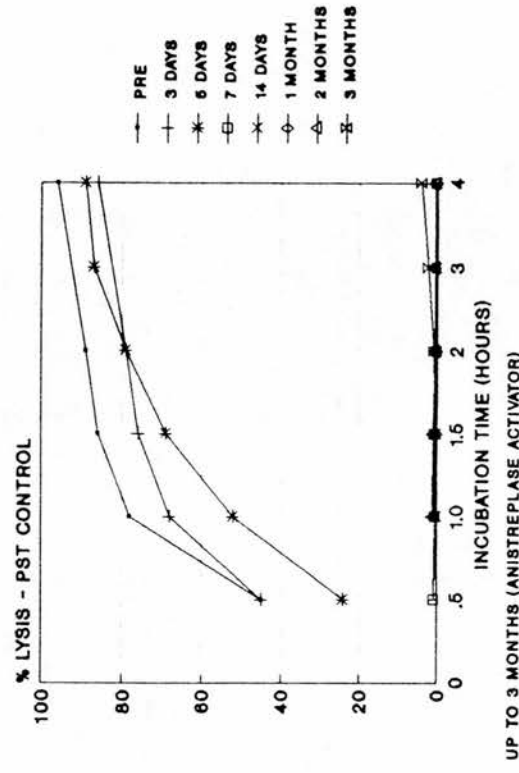
Pooled plasma samples from streptokinase treated patients used for clot lysis

Time	SKRT IU/ml	anti- SK IgG $\mu\text{g/ml}$	Number	Plasm- inogen $\mu\text{M}$	Fibrin- ogen mg/ml	$\alpha_2$ -macro globulin g/l	$\alpha_2$ -anti- plasmin $\mu\text{M}$
Pre	50	2.6	31	2.1	3.3	1.7	0.74
3 d	20	1.2	15	1.9	4.3	1.4	0.66
5 d	100	3.2	20	2.5	4.8	0.9	0.7
7 d	>2000	140	29	2.7	4.5	1.3	0.68
14 d	>2000	518	26	2.9	4.2	1.6	0.64
1 m	>2000	231	17	3.4	3.6	1.8	0.62
2 m	>2000	151	16	3.2	3.4	1.8	0.62
3 m	1200	121	21	2.6	3.3	1.8	0.54
6 m	800	53	21	2.7	3.4	1.7	0.72
12 m	200	14.5	33	2.3	3.2	1.8	0.72
12 m	400	31	9	2.4	3.3	2.1	0.76
18 m	200	11.7	12	2.2	3.3	1.5	0.70
24 m	100	13	13	2.3	3.4	1.8	0.81
30 m	100	6.6	10	2.2	3.7	2.3	0.74

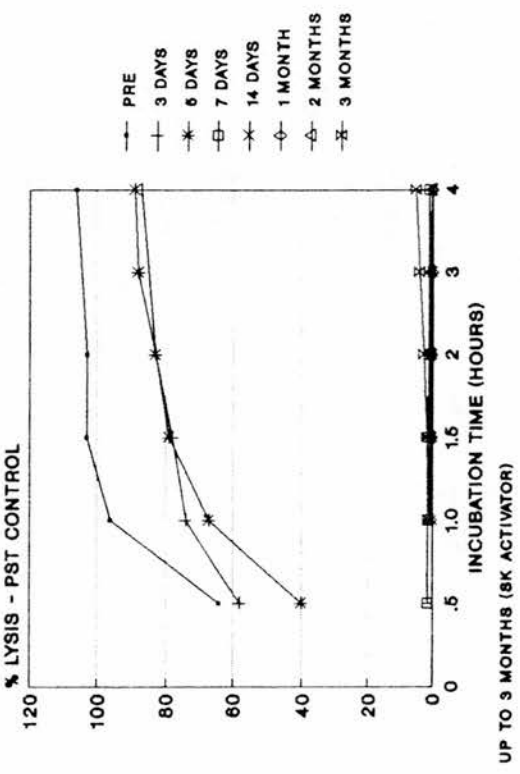
IN VITRO CLOT LYSIS  
SK-TREATED PATIENTS



IN VITRO CLOT LYSIS  
SK-TREATED PATIENTS



IN VITRO CLOT LYSIS  
SK-TREATED PATIENTS



IN VITRO CLOT LYSIS  
ANISTREPLASE-TREATED PATIENTS

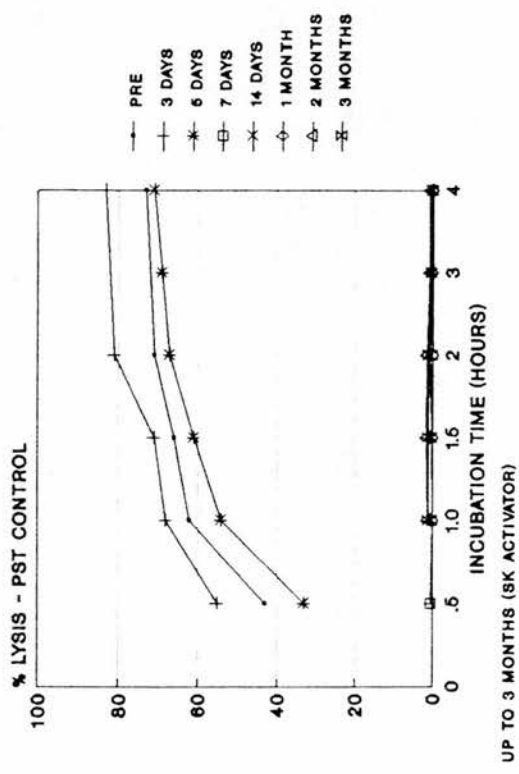
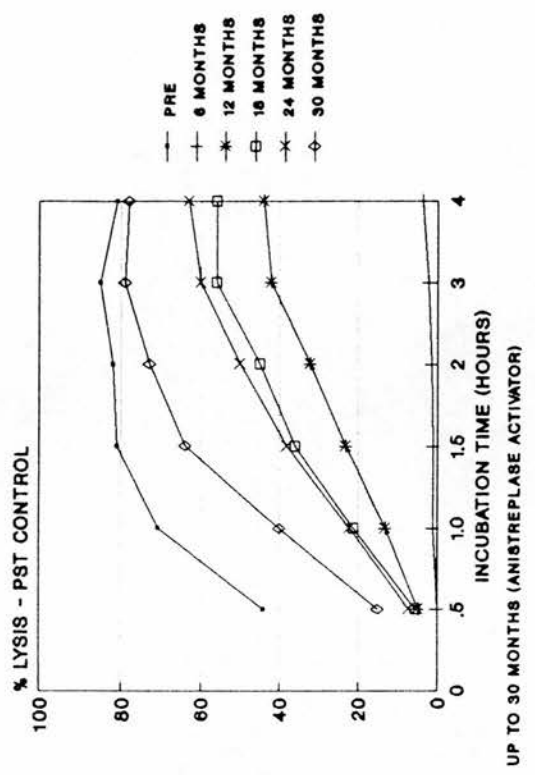
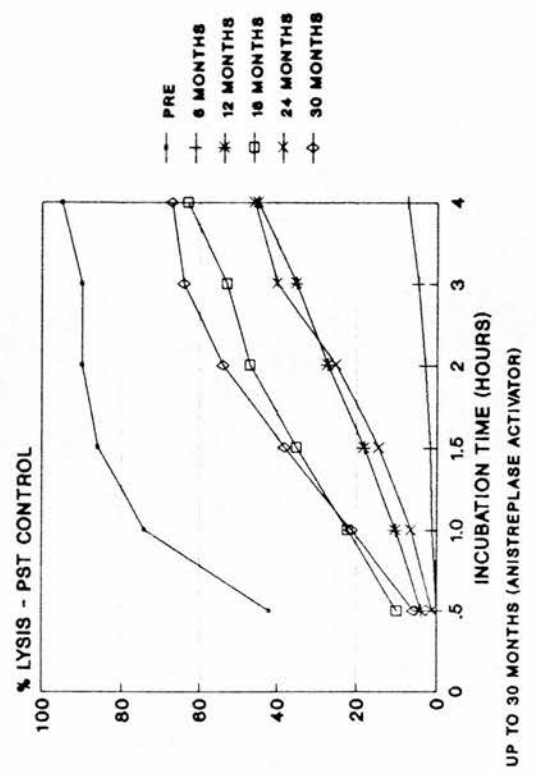


Figure 31. CONCENTRATION-EFFECT CURVES OF IN VITRO LYSIS ACHIEVED OVER THE 3 MONTH PERIOD FOLLOWING TREATMENT WITH STREPTOKINASE-CONTAINING THROMBOLYTIC AGENTS

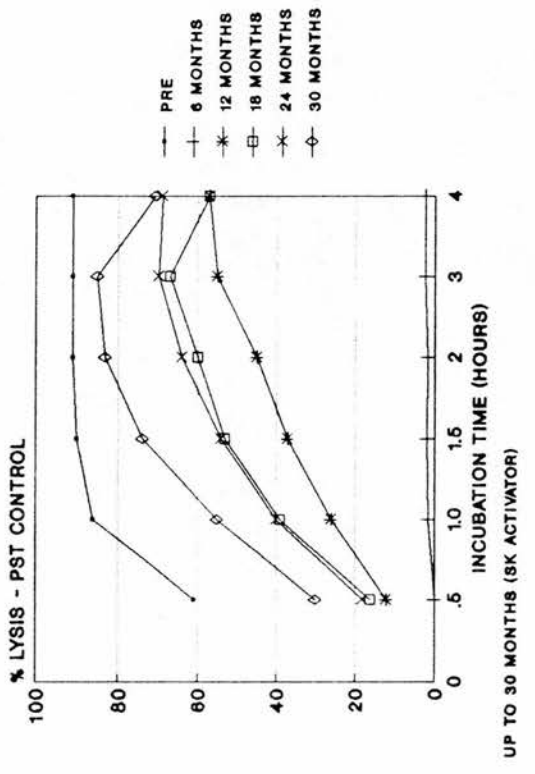
IN VITRO CLOT LYSIS  
ANISTREPLASE-TREATED PATIENTS



IN VITRO CLOT LYSIS  
SK-TREATED PATIENTS



IN VITRO CLOT LYSIS  
ANISTREPLASE-TREATED PATIENTS



IN VITRO CLOT LYSIS  
SK-TREATED PATIENTS

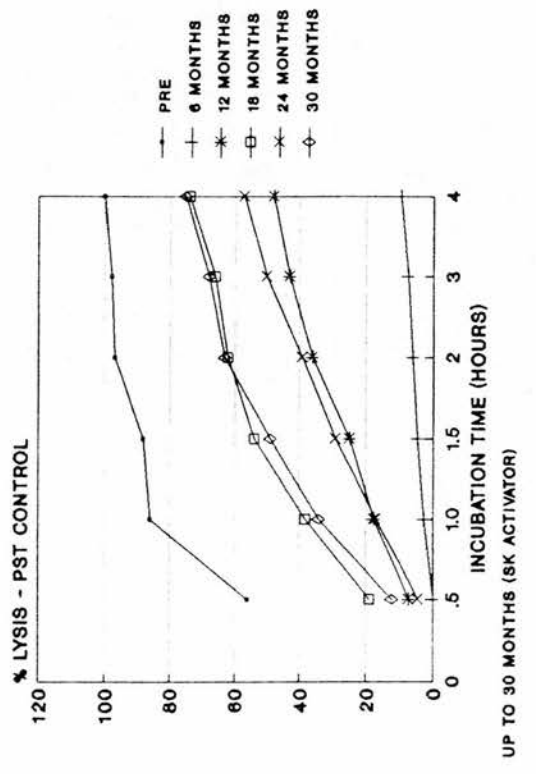


Figure 32. CONCENTRATION-EFFECT CURVES OF IN VITRO LYSIS ACHIEVED OVER THE 3-30 MONTH PERIOD FOLLOWING TREATMENT WITH STREPTOKINASE-CONTAINING THROMBOLYTIC AGENTS

response measured up to 5 days was unchanged, but the pooled results from 7 days to 6 months showed near complete inhibition of fibrinolysis in vivo. There was some recovery in response observed at 18-30 months, for example approximately 25% of the fibrinolytic response was present at 12 months, increasing to 75% at 30 months.

However, these pooled results reflect the arithmetic mean of antibody concentrations and resistance within the samples. In order to reduce the bias due to the influence of the relatively few very high values within the population, I constructed a model relating streptokinase resistance titre and anti-SK IgG concentration to the proportion of lysis achieved within the assay at 90 minutes and 4 hours. It is possible to derive the following linear regression equations:

$$\% \text{ Lysis at 90 minutes} = 108 - 17.3 \times \sqrt[4]{\text{SKRT}}$$
$$R^2 = 77\% \quad p < 0.0001$$

$$\% \text{ Lysis at 4 hours} = 134 - 20.7 \times \sqrt[4]{\text{SKRT}}$$
$$R^2 = 88\% \quad p < 0.0001$$

$$\% \text{ Lysis at 90 minutes} =$$
$$92.6 - 44.0 \times \log_{10}(\text{IgG concentration})$$
$$R^2 = 87\% \quad p < 0.0001$$

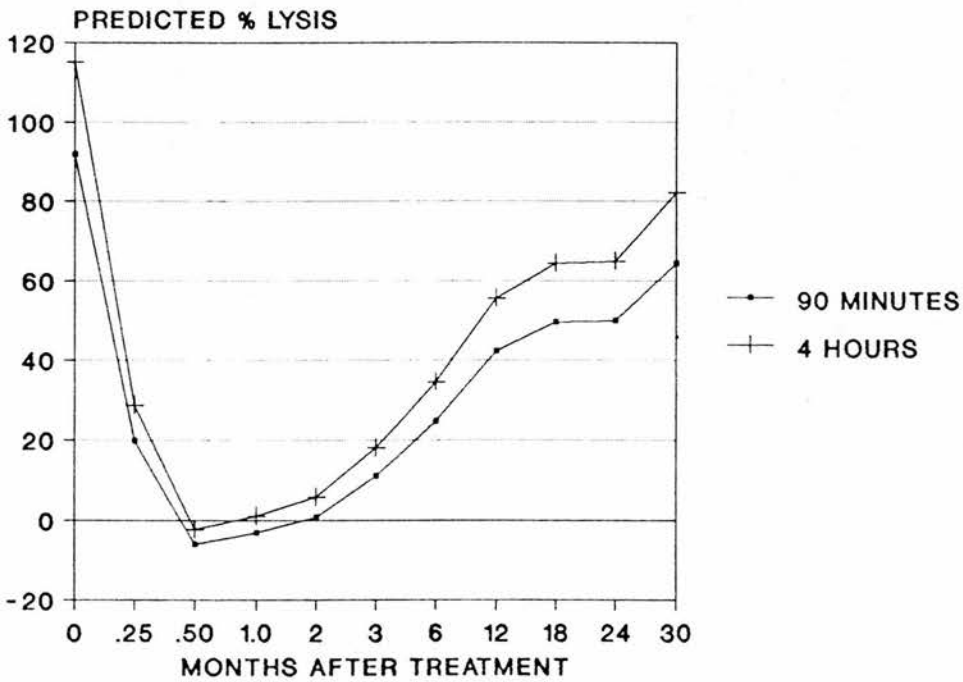
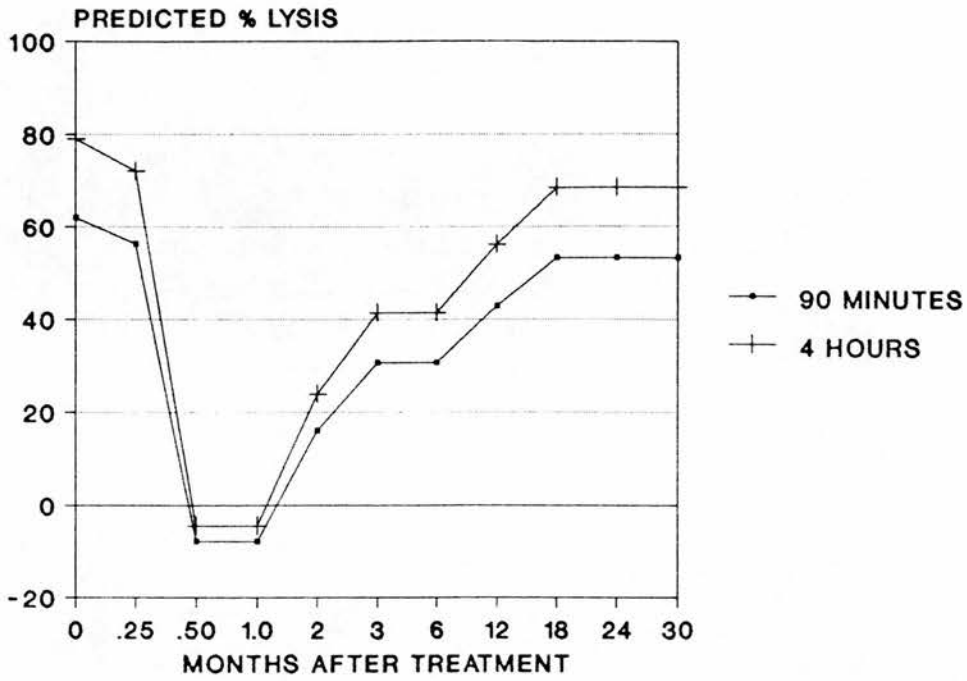
% Lysis at 4 hours =

$$116 - 52.8 \times \log_{10}(\text{IgG concentration})$$

$$R^2 = 95\% \quad p < 0.0001$$

Using these relationships, it is possible to titrate the more representative median values of anti-SK IgG concentration and streptokinase resistance titre for the time points studied and predict the proportion of in vitro lysis which would be achieved for these time points. As can be seen (Figure 33) it would appear that in vitro fibrinolysis is very rapidly inhibited, and remains less than 50% of baseline until 12 months. Following this there is recovery of in vitro lysis, but baseline lysis is not achieved within the 30 months of the study period.

As has been mentioned above, the time points after 12 months consisted of fewer patients with each pool comprising a mean of 22 patients compared to a mean of 56 for streptokinase resistance titre and 67 for anti-SK IgG patients for the time points up to 12 months. It cannot be excluded that the relatively small numbers of patients available may have introduced a sampling bias.



**Figure 33. TIME COURSE OF IN VITRO CLOT LYSIS AT 90 MINUTES AND 4 HOURS CALCULATED FROM THE MEDIAN STREPTOKINASE RESISTANCE TITRE AND ANTI-SK IgG CONCENTRATION USING POOLED CLOT LYSIS DATA**

## **DISCUSSION**

In the light of increasing use of thrombolytic therapy for acute myocardial infarction, there is an increasingly urgent need to establish a safe interval for repeat administration of streptokinase-containing thrombolytic agents. The data presented here show that high levels of neutralising antibody and streptokinase resistance are rapidly induced following treatment with streptokinase and anistreplase, and that the response to these two agents is identical.

Previous studies have demonstrated similar rises and time course in streptokinase resistance titre, with return of the titre towards baseline at 6-12 months (Fletcher et al., 1959, Kostering et al., 1978, Massel et al., 1989, Jalihal and Morris, 1990). Based on these studies, estimates of a recommended time to the possibility of re-dosing with streptokinase have varied from 3 to more than 6 months. Data published recently by Elliott (1991) suggest that the resistance to streptokinase may persist for at least 4 years before showing significant decline from the value of 12 months. Our streptokinase resistance titre data suggest that a large proportion of a standard repeat dose at 6 months would still be neutralised, and possibly a significant proportion of a similar dose at 12 months.

The induction of specific antibodies to streptokinase of the IgG class broadly parallels the induction of streptokinase resistance titre. The induction of anti-SK IgG over the first two months is similar to results published for an enzyme-linked immunosorbent assay (ELISA) method of analysis of anti-SK IgG (Davies et al., 1990). However, the induction of IgG antibodies appears to be more rapid when measured by ELISA than in this study or in other studies using the same microradioimmunoassay method (Hoffmann et al., 1988).

Although high levels of anti-SK IgG constitute a major contribution to streptokinase resistance, the initial fall in anti-SK IgG concentration in the first 4 days after treatment with streptokinase or anistreplase is not accompanied by a concomitant fall in streptokinase resistance titre. This could be explained by changes in the fibrinolytic assay reflecting changes in fibrinogen, plasminogen and  $\alpha_2$ -antiplasmin in the early phase of myocardial infarction treated with thrombolytic therapy. In addition, the difference in pretreatment streptokinase resistance titre between the two treatment groups despite similar anti-SK IgG concentration, and the poor correlation

between changes in anti-SK IgG and streptokinase resistance titre after 3 months must reflect the contributions of other inhibitors, or antibodies of other immunoglobulin classes.

As well as the question of efficacy with repeat dosing with streptokinase-containing thrombolytic agents, there is the important concern regarding whether high concentrations of anti-SK IgG and other classes of antibodies are a risk factor for allergic-type reactions. Anti-SK IgG antibodies have occasionally been implicated in adverse events following streptokinase therapy (Davies et al., 1990). In the large mortality studies of streptokinase (ISIS-2, 1988, GISSI, 1986) and anistreplase (AIMS, 1990), the incidence of severe allergic-type reactions was very low. Those few patients in the current study who showed an allergic-type response, were not obviously associated with different anti-SK IgG concentration or streptokinase resistance titre than those who did not (Hogg et al., 1990), and a correlation between anti-SK IgG concentration or streptokinase resistance titre and hypotensive responses has not been demonstrated as has been discussed in Chapter 6. However, in these studies, the levels of resistance was in a non-exposed population, and it is difficult to

extrapolate these results to the situation of patients being retreated with streptokinase-containing thrombolytic agents in the presence of higher, or possibly considerably higher, levels of resistance.

Interpretation of the pooled clot lysis data is complicated by several factors. Firstly, although as has been discussed, the distribution of anti-SK IgG concentration within the population was markedly skewed towards the higher values, by performing the assay on pooled samples an arithmetic mean is necessarily constructed, which would overestimate the influence of the higher values. To diminish these effects, the median streptokinase resistance titres, in conjunction with a linear regression model relating the SKRT to the degree of inhibition of in vitro lysis after 90 minutes and 4 hours of incubation, have been used to calculate the median degree of inhibition of in vitro lysis predicted for each time point.

In addition, IgG antibodies are polyclonal, and it is possible that mixing of samples in pools may lead to synergy between different populations of antibodies. This particular in vitro assay may also exaggerate the influence

of antibody, as other work using a human fibrin plate technique suggests that anti-SK IgG may have less inhibitory effect than observed here (Hoffmann et al., 1988, Fears et al., 1987).

It is also difficult to translate in vitro fibrinolysis into in vivo efficacy as judged by coronary patency. In particular, 1.5 MIU of streptokinase infused intravenously over 60 minutes achieves a maximum plasma concentration of streptokinase of  $3.85 \text{ M} \times 10^{-8}$ , and is associated with a coronary patency rate of 53% at 90 minutes and 87.5% at 24 hours (Hogg et al., 1990) and a reduction in mortality of 18% (GISSI, 1986). However, a similar concentration of streptokinase in vitro would achieve less than 50% lysis of fibrin. Similar conclusions can be drawn from a standard dose of 30 units of anistreplase, which achieves a peak plasma concentration of  $5.59 \text{ M} \times 10^{-8}$ .

Possible explanations for the underestimation of the potential clot lysis by the in vitro system is the relatively large mass of fibrin produced in vivo relative to plasminogen activator present, and the lack of any potential contribution of pulsatile flow and local vascular dynamics in vitro.

Nevertheless, these results indicate that relative to the unexposed pretreatment state, the degree of lysis achieved following exposure to streptokinase-containing antibodies and streptokinase resistance titre is substantially diminished up to at least 12 months, with some recovery thereafter but with little further recovery up to 30 months. The relatively greater inhibition of early in vitro fibrinolysis would suggest a delaying effect on the initiation of fibrinolysis. In the absence of further data to clarify the situation or the interpretation of the pooled clot lysis data, it has to be tentatively concluded that the efficacy of retreatment with a standard dose of streptokinase or anistreplase would be substantially compromised at these time points.

However, unless high anti-SK IgG concentration and streptokinase resistance titres are shown in due course to be a risk factor for serious allergic reactions, it may be possible to devise alternative dosing schedules for retreatment with streptokinase-containing thrombolytic agents, possibly with a "front-loaded" regimen, perhaps guided by pretreatment streptokinase resistance titres, with a view to saturating circulating resistance.

Clearly the most appropriate study would be to perform an efficacy/patency study on patients who have previously been treated with streptokinase. However this study would be particularly difficult to perform because of ethical concerns regarding efficacy and safety, and also because of the difficulty in recruiting sufficient numbers of suitable patients.

It must be considered that streptokinase and anistreplase administered more than five days, and at least up to 12 months, after prior streptokinase-containing thrombolytic agents may not be effective, and may have an increased risk of allergic-type reactions. Further studies are required to delineate this latter risk. At the present time, if retreatment with thrombolytic agents is required during this time period, the recommended course of action would be to use a thrombolytic agent, which would not be influenced by resistance to streptokinase, such as rt-PA.

**CHAPTER SEVEN**

**THE EFFICACY AND PHARMACOKINETICS OF TWO 35 MG BOLUSES OF  
ALTEPLASE IN ACUTE MYOCARDIAL INFARCTION**

## **INTRODUCTION**

The profile of the ideal thrombolytic drug has been mentioned already, with regard to its desired pharmacokinetic properties. It should be easily and rapidly administered by the intravenous route, and should effectively and rapidly restore coronary patency without adverse side-effects. The ease of administration of the agent becomes increasingly important with the stress laid on immediate therapy on arrival in hospital and the possibility of initiation of therapy in the community.

Rt-PA has been shown to be an efficacious agent in restoring coronary patency (TIMI-1, 1985, Chesebro et al., 1987, Williams et al., 1986, Collen et al., 1984, Verstraete et al., 1985a, Verstraete et al., 1985b) and achieves a higher patency than a conventional dose of intravenous streptokinase (TIMI-1, 1985, Chesebro et al., 1987). At present, the recommended dosage regimen for alteplase is a 10 mg bolus, followed by an involved decremental infusion of 90 mg over 3 hours. This regimen was based on the apparent short half-life of alteplase in the circulation (Tanswell et al., 1989), but clearly this regimen is cumbersome to apply urgently.

This study was performed to assess the efficacy of two boluses of 35 mg alteplase administered intravenously thirty minutes apart, in achieving coronary patency assessed by angiography, and to observe the pharmacokinetic corollaries of this regimen.

#### **PATIENTS AND METHODS**

Thirty-three consecutive patients were admitted to Stobhill Coronary Care Unit and recruited into the study. Patients were eligible for inclusion if they presented with chest pain of at least 30 minutes duration, could be treated within 6 hours of symptom onset and had electrocardiographic evidence of acute myocardial infarction (ST elevation >1 mm in 2 or more limb leads or >2 mm in 2 or more praecordial leads). Patients were excluded if they were over 75 years of age, had a previous infarct in the same anatomical distribution, or had any of the recognised contraindications to thrombolytic therapy (TIMI-1, 1985) or coronary angiography. The study protocol was approved by the local Research & Ethical committee and is included in Appendix III.

The base-line characteristics of the patients recruited are summarised in Table 9 and detailed in Appendix I. Of the 33 patients recruited, 23 were male and 10 female, their mean age was  $56 \pm 11$  years, with a range of 40 to 74 years.

**TABLE 9****DETAILS OF STUDY PATIENT POPULATION**

Total No. of patients	33
Sex	
Male	23
Female	10
Mean age	56.3 ± 10.5 years
Infarct Related Artery	
LAD	12
RCA	15
LMCA	1
Cx	2
Angiography not performed	3
Mean time to therapy	208 ± 75 minutes
Mean time to first angiographic injection	49.4 ± 13.3 minutes
Mean weight	73.2 ± 12.8 kg
Dose of alteplase administered	70 mg
Mean Dose of alteplase administered	0.984 ± 0.173 mg/kg

The mean time from onset of symptoms to the administration of the first bolus was  $208 \pm 75$  min. The mean weight of the patients was  $73.2 \pm 12.8$  kg, deriving a mean dose of alteplase administered of  $0.984 \pm 0.173$  mg/kg.

Following written informed consent, and discussion with accompanying relatives, the patients received 35 mg alteplase intravenously over 30 seconds, which was repeated 30 minutes later. All patients received 150 mg aspirin orally immediately on entry to the study and daily thereafter. All patients received an intravenous infusion of glyceryl trinitrate starting at 0.25 mg/hour unless there was a specific contraindication.

Coronary angiography was performed in the Coronary Care Unit using an Image Intensifier (Siremobil 2N/2H) linked to a video tape recorder (JVC CR8200E). Details of this system have been previously described (Hillis et al., 1986). The Seldinger percutaneous approach was used and a femoral sheath left in situ. The coronary angiogram was commenced immediately after the second bolus of alteplase (30 minutes), and the infarct related coronary artery, as indicated by the admission ECG was visualised as soon as possible after 30 minutes, with serial selective injections at 60 and 90 minutes. The degree of perfusion was scored according to the TIMI scale (Appendix II) on the first

angiographic run of each series, and reviewed by an independent experienced cardiologist, a score of 0-1 taken to indicate non-patency and 2-3 patency (TIMI-1, 1985).

If the artery was occluded at 90 minutes, the study protocol allowed an option for additional therapy to be given to the total dose of 100 mg alteplase, as recommended by the product license. The other coronary arteries were visualised in standard angiographic projections. The femoral sheath was left in situ and coronary angiography repeated at  $24 \pm 8$  hours following treatment, to assess patency and reocclusion.

Intravenous heparin infusion was commenced in all patients 3-4 hours after onset of therapy, and the dose titrated against the thrombin time to a therapeutic ratio of two to three to one. No bolus of heparin was given at initiation of alteplase therapy. In all cases, heparin was discontinued for a short period at the time of the second angiogram to allow removal of the femoral sheath.

Venous blood samples were collected via an indwelling venous catheter from the first 24 patients pretreatment, then 10,20,30,40,50,60 minutes, 2,4,8,12,24 hours after the first bolus of alteplase, into sodium citrate, centrifuged immediately at  $0^{\circ}\text{C}$ , separated and frozen at  $-40^{\circ}\text{C}$ .

Subsequent analysis for t-PA antigen was performed by ELISA.

## **RESULTS**

### **ANGIOGRAPHIC PATENCY**

Coronary angiography was commenced at 30 minutes after the administration of the first bolus of alteplase, and the infarct related artery was first visualised at a mean time of  $49.4 \pm 13.3$  minutes (Table 10). The artery was visualised within 55 minutes in 26 of 30 (86%) patients. In three patients an acute angiogram was not obtained, one because of haemodynamic instability, one because the patient died before 90 minutes, and one because it was not possible to inject the right coronary artery.

In three subjects, although the entry criteria were satisfied, serial ECG changes of acute myocardial infarction did not occur and cardiac enzymes were normal. In one of these subjects the right coronary artery could not be visualised, and is not included in the angiographic data. In all other patients angiography was obtained within 90 minutes of start of therapy and the results presented are patency rates of all patients in whom coronary visualisation was achieved at each time point. At the first injection of the infarct-related artery ( $49.4 \pm 13.3$  minutes) 23 of 30 (77%, 95% confidence limits 61 to 92%) arteries were patent. At 90 minutes, this had increased to 26 of 30 (87%, confidence limits 74 to 99%) arteries.

**TABLE 10****ANGIOGRAPHIC DETAILS**

	TIMI Grade	Number of Patients		
		First injection (49.4 ± 13.3 minutes)	90 min	24hrs
Non-patent	0	5	3	2
	1	2	1	3
Patent	2	15	18	10
	3	8	8	14
Reoccluded		-	0	3
Angio not performed		3	3	4

Two subjects received a further intracoronary dose of 30 mg alteplase following the 90 minute angiogram. In one, there was intermittent reperfusion of the index artery during the period of angiographic observation. In the other, following left main coronary artery occlusion there was penetration only (Grade 1) of the circumflex artery at 90 minutes. In both patients, the arteries were patent at the 24 hour angiogram.

One subject did not undergo 24 hour angiogram because of haematoma formation following splitting of the femoral sheath. At 24 hours, 24 of 29 arteries (83%, confidence limits 69 to 97%) were patent. Seven patients improved their angiographic appearance by at least one grade, 3 gaining reperfusion. However 5 deteriorated at least one grade with 3 arteries reoccluding (3 of 29, 10%, 95% confidence limits 0 to 22%).

#### **ADVERSE EVENTS**

The only adverse events documented in this study group were bleeding complications. Five patients developed significant haematomata related to the site of arterial access for coronary angiography. Two patients suffered minor coffee ground haematemesis and one other bled from the gums. No patient required blood transfusion or specific intervention, and there were no sequelae.

One patient died during the study. This patient was known to have severe triple vessel disease and a previous myocardial infarction. Shortly following admission and treatment, he developed cardiogenic shock and died despite inotropic support.

#### **PLASMA t-PA CONCENTRATIONS**

Mean pretreatment plasma t-PA concentration was  $20.1 \pm 4.3$  ng/ml, significantly higher than the laboratory normal ( $3.6 \pm 1.3$  ng/ml, MacGregor et al., 1990). Mean concentration rose to  $4435.8 \pm 2117.8$  ng/ml within ten minutes of the first bolus of alteplase, falling to  $425.8 \pm 288.3$  ng/ml prior to the second bolus, rising again to a peak of  $4233.3 \pm 2217.5$  ng/ml within 10 minutes of the second bolus (Figure 34). Mean concentrations then fell rapidly to approximately twice the pretreatment values at 4 hours ( $40.6 \pm 28.6$  ng/ml). After this time period, the t-PA concentrations were low, variable and approximately at physiological levels.

These low variable t-PA concentrations introduced considerable random variation in the pharmacokinetic fitting, therefore the data fitted were the baseline-subtracted concentrations confined to the four hour period after the administration of alteplase. This approach has been adopted by previous authors (Tanswell et al., 1989, Seifried et al., 1989).

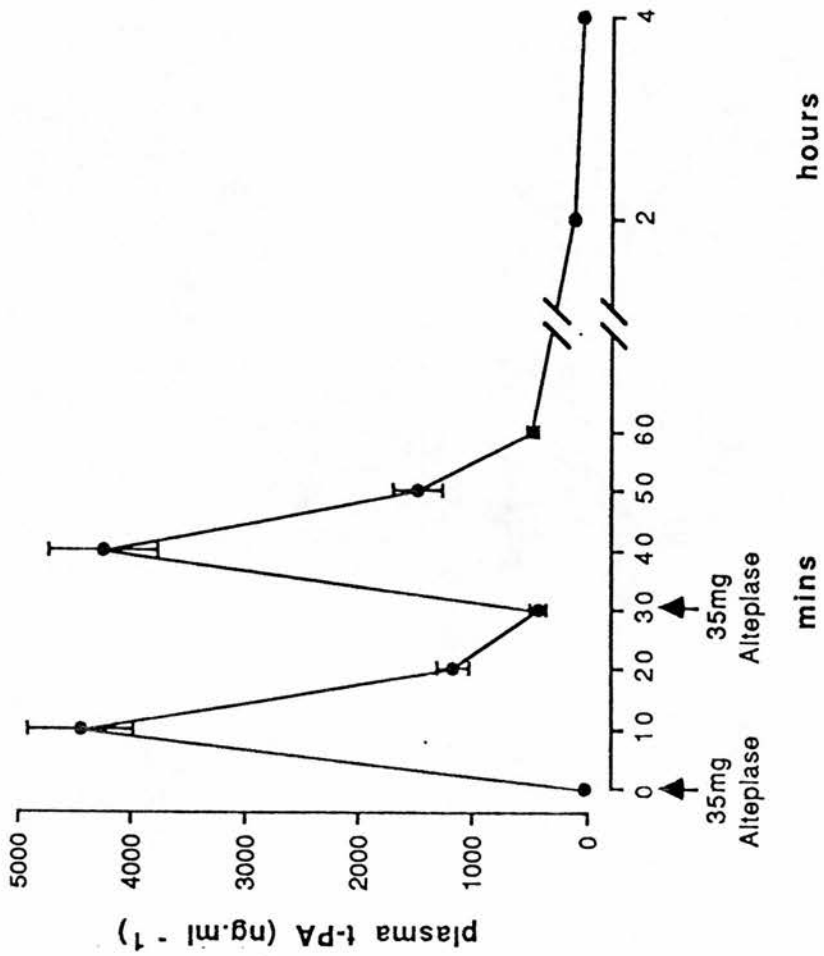


Figure 34. MEAN PLASMA t-PA CONCENTRATIONS FOLLOWING TWO INTRAVENOUS BOLUSES OF 35MG ALTEPLASE SEPARATED BY 30 MINUTES

The individual declines in t-PA concentration were fitted to one, two- and three-compartment pharmacokinetic models, but a satisfactory fit was obtained only with the one-compartment model. Pharmacokinetic parameters derived from this model were: volume of distribution  $3.11 \pm 1.89$  l (CV:  $35.6 \pm 26.7\%$ ); clearance  $21.3 \pm 9.3$  l/h (CV:  $14.1 \pm 11.7\%$ ) and half-life  $5.9 \pm 1.7$  min. The coefficients of variation (CV) expressed are means and standard deviations of the coefficients of variation of the individual fitted data and express the quality of fit of the model (Table 11). Using this model, the plasma t-PA concentrations were estimated at two minutes after the beginning of the intravenous boluses of alteplase, as a measure of the peak concentrations achieved using this administration regimen. The peak concentration was  $12389 \pm 8580$  ng/ml two minutes after the first bolus and  $10811 \pm 6802$  ng/ml after the second bolus of alteplase.

**TABLE 11****PHARMACOKINETIC PARAMETERS**

	<b>Mean</b>	<b>SD</b>
Clearance (l/h)	21.4	9.3
Coefficient of Variation of Estimates of Clearance (%)	14.1	11.7
Volume of Distribution (l)	3.11	1.89
Coefficient of Variation of Estimates of Volume (%)	35.6	26.7
Half-life (minutes)	5.9	1.7

## **DISCUSSION**

These results show that alteplase administered intravenously as two boluses of 35 mg separated by 30 minutes is effective in restoring coronary artery patency in acute myocardial infarction. To allow comparison with other patency studies, the primary end point of efficacy was patency at 90 minutes following therapy, but in addition the angiograms at earlier time points allow an assessment of the time to reperfusion. At the time of first injection, at a mean of 49 minutes post-therapy, 77% of the patients had patent index coronary arteries, improving to 87% within 90 minutes, showing that patency is achieved rapidly and effectively.

Direct comparison of this study with previous published studies using tissue-type tissue plasminogen activator (rt-PA) is difficult because of several confounding factors. Other studies have used differing administration regimens of widely varying doses of two different preparations of rt-PA, each with different specific activities (Garabedian et al., 1987, Muller et al., 1987). In addition, whereas some studies have observed patency, others have observed reperfusion, with a difference due to spontaneous reperfusion and sub-total coronary occlusion at presentation (deWood et al., 1980).

Verstraete and co-workers (Verstraete et al., 1985) have demonstrated patency rates of 61% at 75-90 minutes post-therapy using 0.75 mg/kg of double-chain rt-PA over 90 minutes. The same group using a 40mg infusion of double-chain rt-PA over 90 minutes, achieved a coronary patency rate of 66% at 90 minutes (Verstraete et al., 1987). Topol and the TAMI group (Topol et al., 1987a, 1987b, 1989) have achieved a patency rate of 68% patency using 70 mg of rt-PA over 90 minutes, and 79% using the high dose of 1.5 mg/kg of alteplase over 4 hours, in conjunction with a high dose of heparin. The TIMI group in TIMI-1 (TIMI-1, 1985, Chesebro, 1987) used 80 mg of double-chain rt-PA over 3 hours, finding a reperfusion rate of 56%. Williams et al. (1986) using the same dose of 80 mg over 3 hours found a similar reperfusion rate of 68%.

Published experience with bolus doses of alteplase is limited. Verstraete et al. (1989) using single boluses of alteplase, found that doses of 60 mg and 50 mg were associated with reperfusion rates at 90 minutes of 32% and 45% respectively, although a maximum dose of 70 mg achieved 72% reperfusion. However, these results could not be supported when repeated in larger numbers by the same authors (Tranchesi et al., 1991). Using the 70mg bolus dose, they found reperfusion rates of 32 and 45% at 60 and 90 minutes.

Khan et al., (1990) have evaluated 4 boluses of 25mg over 60 minutes of the double-chain t-PA and demonstrated recanalisation in 11 of 14 patients, and suggested that this regimen achieves coronary patency more rapidly. In this study, 70mg divided into two boluses, also achieves a high patency rate, and would be expected to be associated with fewer bleeding complications (TIMI-2, 1989).

A small study by Smalling et al. (1990) using rapid intravenous infusions of a weight-adjusted dose of alteplase, and a median dose of 145mg, reported a 90 minute patency rate of 84%, which was significantly higher than the control group who received a conventional 3 hour infusion of 100mg. The studies performed by Neuhaus and his group (Neuhaus et al., 1989, von Essen et al., 1991) looked at accelerated "front-loaded" intravenous infusion regimens of 100mg of alteplase over 150 and 90 minutes, and observed 90 minute patency rates of 91% and 84.4%. These results suggest that rapid administration of the thrombolytic agent achieves higher patency, and that it is the peak concentration of agent achieved which is important, an inference supported by the data in this study.

It is of note that the higher patency rates in the literature were achieved with higher weight adjusted doses of rt-PA, although the need for weight adjusted doses with alteplase has recently been questioned. In the recently

reported TAPS study, an accelerated front-loaded administration regimen of 100mg alteplase over 90 minutes, given with an initial 10mg bolus, there was little evidence to support higher patency in the lighter patients who received a higher weight-related dose. However, there was evidence that these patients achieved a more complete degree of reperfusion.

In the present study, the total of 70mg alteplase represents a mean dose of 0.98 mg/kg. This contrasts with 1.5 mg/kg administered by Topol et al. (1989) and the fixed dose of 150 mg used in the TIMI II study (1989) or 100 mg used in the Australian National Heart Foundation Study (1988), recommended by the manufacturers (Actilyse data Sheet, 1989), and now in regular use. Despite the lower dose used, the patency rate of 87% at 90 minutes compares favourably with the published data.

Pharmacokinetic analysis of the plasma t-PA concentration-time profile following this bolus administration regimen of alteplase shows that very high, short-lived concentrations of t-PA are achieved shortly after injection of the drug. The concentration of t-PA achieved at 10 minutes of  $4435.8 \pm 2117.8$  ng/ml is 34% higher than the peak concentration attained by Seifried et al., (1989) with 100mg of single chain rt-PA delivered as a 10mg bolus and 90 minute decremental infusion. We measured t-PA concentrations in excess of 2,300 ng/ml in all the patients, and predicted

concentrations at two minutes following the boluses of alteplase in excess of 10,000 ng/ml. As would be expected, the time period when t-PA concentration is in excess of 1000 ng/ml is shorter with bolus administration than with prolonged infusion.

Despite the short duration of high plasma t-PA concentrations, the reocclusion rate to 24 hours of 3 of 29 patients (10%) is comparable to previous experience (Verstraete et al., 1987).

The pretreatment t-PA concentrations were elevated, but 4-8 hours following alteplase, levels fell to physiological values. After 4 hours in all patients, t-PA levels were low and variable, making fitting to pharmacokinetic models difficult. To eradicate this variable, the data were fitted for the first 4 hours only, using baseline-subtracted values. Despite the frequent blood samples, multiple phases of elimination could not be defined, unlike in previous papers. The data fitted the chosen model satisfactorily, as reflected in the low standard deviations of each individually fitted parameter. These individual standard deviations have been expressed as coefficients of variation (CV) of their respective estimated parameter and the CV's expressed as the mean. Thus the mean CV of the estimate of mean clearance was 14% and that of the estimate of mean volume of distribution 36%, indicative of a reliable estimate of the mean parameters. Previous studies

have not published details of the quality of fit of their models, and therefore it is difficult to know the degree of reliance that can be placed on their estimations (Tanswell et al., 1989, Seifried et al., 1989).

The findings in this study confirm the rapid clearance of t-PA from the circulation with half-life of  $5.9 \pm 1.7$  minutes, and modest inter-patient variability, closely correlating with previous reports in patients, although somewhat slower than findings in normal volunteers with a lower total dose, possibly reflecting diminished cardiac output and hepatic blood flow in these patients with myocardial infarction. The estimate of volume of distribution of  $3.1 \pm 1.9$  l is very similar to previous estimates in both patients and volunteers.

Some workers have found a dose-related rise in bleeding complications, in particular intracranial bleeding (TIMI-2, 1989). In this study, using a relatively small dose of alteplase, bleeding complications were observed in 8 of 33 (24%), with 5 of these being related to arterial access for coronary angiography, leaving 3 minor gastrointestinal bleeding episodes (9%). No patients required transfusion or specific intervention. These results are similar to previously published data (TIMI-1, 1984, Collen et al., 1984, Verstraete et al., 1985), but the small numbers in this study do not allow firm conclusions to be drawn.

The data in this chapter show that alteplase administered as two intravenous boluses of 35 mg, 30 minutes apart, reliably produces very high concentrations of t-PA in the plasma, which is rapidly cleared from the circulation, and allows a high coronary patency rate in acute myocardial infarction. The total dose administered was less than that used in many studies reporting high patency, yet reocclusion rates were similar to those reported previously. The greater simplicity of administration and higher efficacy of this regimen may allow alteplase therapy to be safely initiated earlier in the course of acute myocardial infarction with the advantages that such earlier administration would give.

**CHAPTER EIGHT**

**LOW DOSE BOLUS ALTEPLASE IN ACUTE MYOCARDIAL INFARCTION.**

## **INTRODUCTION**

Previous studies have demonstrated that high doses of rt-PA, while associated with higher coronary patency than low dose regimens, are also associated with higher complication rates, in particular of intracranial haemorrhage (TIMI II, 1989). In addition, the high cost of some of the available thrombolytic agents makes the expense of therapy a significant consideration in the selection of the agent and dose used. Thus any administration regimen of thrombolytic agents is a compromise between efficacy, safety and cost.

Following the demonstration of the very high coronary patency achieved by a bolus dose regimen of two 35mg alteplase separated by 30 minutes, it was logical to assess the efficacy of a slightly smaller dose of alteplase to discern if this regimen would be associated with similar high efficacy, using less drug, possibly with further advantages with regard to safety, and less expense. The efficacy of three intravenous 20mg boluses of alteplase in achieving coronary patency was assessed in this study.

## **PATIENTS AND METHODS**

Twenty-one consecutive patients (13 male, 8 female, age range 40-72, mean 59 years) were recruited, using the same entry criteria as discussed in the previous chapter. The study protocol is included in Appendix III.

Following written, informed consent and discussion with accompanying relatives, the patients received 20 mg alteplase by the intravenous route over 30 seconds. This was repeated 20 and 40 minutes later. As in the previous study, all patients received 150 mg aspirin orally on admission and daily thereafter, and glyceryl trinitrate 0.25 mg/hour by continuous intravenous infusion.

The details of the methods and timing of coronary angiography were exactly as described in the previous chapter. All angiograms were successfully performed within 90 minutes of the start of therapy. The degree of perfusion was scored according to the TIMI grading (Chesebro et al., 1987, Appendix II).

If the infarct related artery was occluded at 90 minutes following therapy, "rescue" dosing of a further 40 mg was administered by the intracoronary route, to a total dose of 100mg. This allowed clarification that patency of the infarct related arteries was achievable even if not reperfused by initial therapy. To attempt to reopen arteries which had suffered early reocclusion, which was frequent in the early part of the study, it was decided that all patients should receive a further 40 mg following the 90 minute angiogram, by the intracoronary route over 20 minutes if the artery was occluded, or by the intravenous route over 60 minutes if the artery was patent at 90 minutes. Details of therapy administered are included in

Table 12. Overall, 13 of 21 patients received further alteplase after 90 minutes. Intravenous heparin infusion was commenced in all patients 3-4 hours after the start of therapy, and the infusion rate adjusted to maintain a thrombin time ratio of 2-3 to one. The heparin was discontinued for a short period prior to the second angiogram to allow removal of the femoral sheath.

In all patients blood was drawn from an in-dwelling venous catheter prior to treatment, then 10,20,30,40,50,60,90 minutes, 2,4,6,9,12 and 24 hours after administration of the first bolus of alteplase and plasma t-PA concentration measured using the same preparation and assay techniques as in the previous chapter (MacGregor et al., 1987).

## **RESULTS**

The characteristics of the patients recruited are summarised in Table 13 and detailed in Appendix I. 13 of the 21 patients recruited were male. The mean age of the population was  $59.0 \pm 10.2$  years, ranging from 40-75 years. The mean time from onset of chest pain to initiation of therapy was  $177 \pm 90$  minutes.

**TABLE 12**

**DETAILS OF THERAPY AND CORONARY ANGIOGRAPHY**

**TIMI GRADING**

<b>NAME</b>	<b>IRA</b>	<b>Rx</b>	<b>Time of first injection (mins)</b>	<b>First injection</b>	<b>60 mins</b>	<b>90 mins</b>	<b>24 hours</b>
DC	RCA	A	41	0	0	2	2
WG	LAD	A	52	2	2	2	2
JS	RCA	A	46	0	2	2	3
MH	RCA	A	53	2	2	2	3
WG	LAD	A	60	3	3	3	3
CS	RCA	A	60	2	2	3	3
JT	LAD	A	50	3	3	3	3
WM	RCA	A	55	2	2	2	0
MMF	RCA	B	60	2	2	0	0
AF	LAD	B	25	0	2	0	2
VC	LAD	B	43	0	1	0	3
TG	LAD	B	50	3	3	0	3
MM	RCA	B	50	2	2	2	3
JOD	LAD	B	60	0	0	0	2
JJ	RCA	B	49	0	2	1	0
JMK	LAD	B	47	2	3	1	2
MB	RCA	B	55	1	2	0	3
JJ'	RCA	C	60	3	3	3	ND
RG		C	ND				
WMK	RCA	D	41	0	0	0	0
AH	RCA	E	60	2	2	2	3

**Treatment Code (Rx)**

<b>CODE</b>	<b>TREATMENT</b>
A	20mg x 3 intravenous
B	20mg x 3 intravenous +40mg intracoronary at 90mins
C	20mg x 3 intravenous +40mg intravenous at 90mins
D	20mg x 3 intravenous +20mg intracoronary at 90mins
E	20mg x 3 intravenous +40mg intravenous at 120mins

IRA = infarct related artery

ND = not done

**TABLE 13****DETAILS OF STUDY PATIENT POPULATION**

Total No. of patients	21
Sex	
Male	13
Female	8
Mean age (SD)	59.0 ± 10.2
Infarct related artery	
LAD	8
RCA	11
Circumflex	1
Angiogram not performed	1
Mean time to therapy (SD)	177 ± 90 mins
Mean time to first angiographic injection (SD)	51 ± 9 mins

## **ANGIOGRAPHIC PATENCY**

Coronary angiography was commenced at 30 minutes after the first bolus of alteplase. In 20 of 21 patients, the infarct-related artery was injected within 60 minutes of the administration of the first bolus, at a mean time of  $51 \pm 9$  minutes. One patient in whom the right coronary artery was not injected, was excluded from analysis.

At the first injection of the coronary artery (51 minutes after onset of therapy), 12 of 20 coronary arteries were patent (60%, 95% confidence interval 37 to 83%). This patency rate increased to 16 of 20 arteries (80%, 95% confidence interval 61 to 97%) at 60 minutes, but then fell to 11 of 20 (55%, 95% confidence interval 32 to 78%, Table 14) at 90 minutes. In the time interval between the first coronary injection and the 90 minute injection, 7 arteries decreased their TIMI score by at least one grade, and 6 of these reoccluded. Thus the reocclusion rate to 90 minutes was 6 of 20 arteries (30%, 95% confidence limit 9 to 51%).

Twelve of the 20 patients undergoing coronary angiography received further doses of alteplase, 11 of these receiving a further 40 mg, to a total dose of 100mg, as currently recommended (Actilyse Data Sheet, 1989). Nineteen of the twenty patients had a patent coronary artery at the end of the procedure, and these arteries must therefore be considered as being capable of reperfusion.

**TABLE 14**  
**ANGIOGRAPHIC PATENCY**

	<b>TIMI GRADE</b>	<b>FIRST INJECTION (51 ± 9 MINS)</b>	<b>60 MINS</b>	<b>90 MINS</b>	<b>24 HOURS</b>
NON-PATENT	0	7	3	7	4
	1	1	1	2	0
PATENT	2	8	11	7	5
	3	4	5	4	10
PATENCY		12 of 20 (60%)	16 of 20 (80%)	11 of 20 (55%)	15 of 19 (79%)
95% CONFIDENCE INTERVAL		37-83%	61-97%	32-78%	59-98%

At repeat angiography at 24 hours, the coronary patency rate was 15 of 19 arteries (79%, 95% confidence limit 59 to 98%). One further patient did not undergo repeat angiography because of haematoma formation in relation to the femoral arterial sheath. Following rescue therapy, 4 of 19 arteries reoccluded before the 24 hour angiogram (21%, CL 2-40%), while one patient reperfused late.

#### **t-PA CONCENTRATIONS**

The pretreatment t-PA concentrations in the study population were elevated ( $12.2 \pm 4.3$  ng/ml) compared to the normal range ( $3.6 \pm 1.3$  ng/ml) as reported previously (Booth et al., 1990). Ten minutes following each of the three intravenous boluses of 20 mg alteplase, the mean plasma t-PA concentration rose to 1849-2138 ng/ml, falling to 523-731 ng/ml prior to the next bolus (Figure 35). Following the 90 minute sample, different dosing schedules were used on clinical judgment (Table 12).

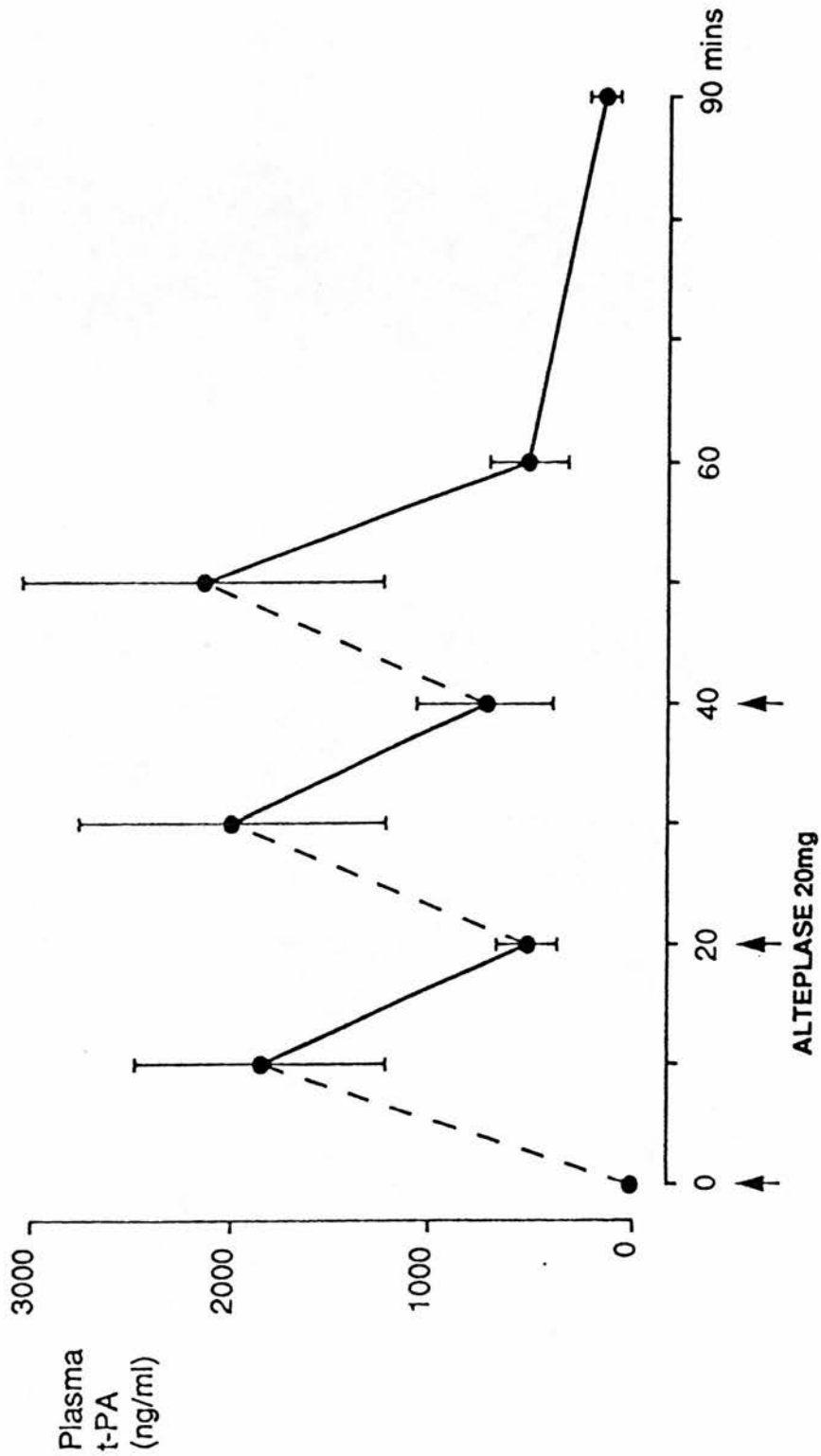


Figure 35. MEAN PLASMA t-PA CONCENTRATIONS FOLLOWING THREE INTRAVENOUS BOLUSES OF 20 MG ALTEPLASE EACH SEPARATED BY 20 MINUTES

## **ADVERSE EVENTS**

Four patients developed bleeding complications. Three of these were groin haematomata related to femoral artery puncture. One of these events occurred 36 hours after thrombolytic therapy, when the patient was excessively anticoagulated with heparin. One patient bled from an antecubital fossa venous cut-down site for insertion of Swan-Ganz catheter for haemodynamic monitoring. In none of the patients were specific measures or blood transfusion required, and none developed long term sequelae. No patients developed "spontaneous" bleeding in this series.

Of the first six patients given additional intracoronary alteplase after the 90 minute angiogram, at a rate of 40mg in 40 mls volume over approximately 8-10 minutes, three of these experienced ventricular fibrillation necessitating DC cardioversion, which was successful in all cases. The rate of subsequent intracoronary infusion of alteplase was reduced to 40 mg over 20 minutes.

## **DISCUSSION**

This study demonstrates that three 20mg boluses of alteplase over 60 minutes although effective at restoring coronary patency in 80% of arteries, is associated with a high reocclusion rate. Although the confidence limits are wide reflecting the relatively small study sample, this would suggest that this administration regimen is unsuitable for clinical use. However, further dosing with intracoronary alteplase to a total dose of 100mg, is able to restore

patency. The treatment regimen after the 90 minute angiogram varied between the patients according to clinical requirements, and it is therefore difficult to draw any conclusions.

High t-PA concentrations were achieved, but with the known short half-life of t-PA in the circulation, the high concentrations are predictably short-lived. The concentrations measured ten minutes following each bolus were 1849-2138 ng/ml, which is comparable to the steady-state concentration of approximately 2210 ng/ml found during the first infusion phase of 50 mg over 1 hour in the recommended decremental infusion regimen (Seifried et al., 1989). It can be concluded therefore that although the peak plasma levels achieved are high enough to achieve initial reperfusion, a longer duration of effect is required to allow more complete lysis of thrombus and prevention of reocclusion.

There is evidence that coronary patency following rt-PA is related to the weight-adjusted dose received (Smalling et al., 1990, von Essen et al., 1991, Topol et al., 1987). The mean (SD) initial dose of alteplase received in this study was 0.842 (0.144) mg/kg, which is less than that used in the studies quoted. In this study, the patients received different doses of alteplase after 90 minutes, and it is valid only to compare the t-PA concentrations and pharmacokinetics up to this point. Within these

limitations, and in the small numbers available, there was no significant relationship between  $AUC_{90}$  or peak t-PA concentration and coronary patency at the time points up to 90 minutes.

Previous studies with t-PA have shown various reocclusion rates, although few have assessed reocclusion at such an early time point. Early studies with t-PA on relatively small study populations found reocclusion rates of 20% and 45%, (Williams et al., 1986, Gold et al., 1986) the former using a three hour infusion of 80 mg of alteplase and repeating coronary angiography at an interval of 1-16 days, and the latter 0.4-0.75 mg/kg of rt-PA over 60-120 minutes, with reocclusion occurring despite heparin, in those with high grade residual stenoses, when the plasma concentration of t-PA fell below 700 ng/ml. Verstraete et al., (1987) gave 40 mg alteplase over 90 minutes, in a study specifically designed to examine reocclusion rates. Following initial angiography at 75-90 minutes, their patients were randomised to receive either a further 30mg of rt-PA or placebo over 6 hours, repeating angiography at 6-24 hours, and again prior to discharge. They found that reocclusion up to 6-24 hours was similar in both groups, namely 5 and 6%. The reocclusion rate at 1-4 weeks was 12%, showing no benefit of continued rt-PA infusion. A similar study using alteplase concluded that a continuing infusion of rt-PA over 4 hours prevented coronary reocclusion (Johns et al., 1988).

In this study, no initial bolus of heparin was given, but it was introduced by continuous intravenous infusion 2-3 hours after initial presentation, the rate of which was adjusted to maintain a strict state of anti-coagulation.

There has been much recent correspondence concerning the role of heparin as adjunctive therapy to rt-PA (White, 1990). Experimental studies have suggested the need for early administration of heparin at the time of initiation of thrombolytic therapy with rt-PA (Cercek et al., 1986). This finding is partially corroborated by clinical evidence, in that studies using a bolus dose of heparin coincident with the introduction of thrombolytic therapy were associated with a more favourable outcome (Chesebro et al., 1987, and TIMI II, 1989). However, bolus administration of heparin at the time of thrombolytic therapy did not improve coronary patency at 90 minutes with 1.5 mg/kg rt-PA infused over 3 hours, nor diminish reocclusion over the period of observation (Topol et al., 1989). Analysis of the role of heparin after the institution of thrombolytic therapy in the same study protocol as that used in GISSI-2 (International Study Group, 1990) did not reveal any benefit of introducing subcutaneous heparin 12 hours after thrombolytic treatment.

Some studies however have implied that early heparin may have a role in maintaining coronary patency. Ross et al., (1990) showed more beneficial effects of early heparin compared to a low dose of aspirin, with improved patency 18 hours after treatment with 100mg rt-PA, (82% vs 52%), and similarly, Bleich et al. (1989) showed a 71% patency at a

mean of 55-59 hours with heparin in addition to 100mg of rt-PA over 3 hours, as opposed to 44% without. The recently reported European Cooperative Study Group study (de Bono et al., 1992) suggested that early heparin had a beneficial role in the maintenance of patency measured at 48-120 hours after therapy with 100 mg alteplase by infusion, with the relative risk of an occluded vessel with heparin being 0.66 (95% confidence limits 0.47 to 0.93).

Rapold et al., (1989) have demonstrated that heparin can prevent the rise in Fibrinopeptide A after thrombolytic therapy, reflecting the suppression of on-going thrombotic activity. The role of thrombin in this phenomenon may be central, at least in part mediated by thrombin activation of platelets, which is prevented by heparin (Greenbaum et al., 1982) but not by aspirin (Chesebro et al., 1990).

Nevertheless, heparin administration does not appear to be absolutely essential for the maintenance of coronary patency following rt-PA in myocardial infarction. In the study reported in Chapter 7, two 35mg boluses of alteplase achieved a 90 minute coronary patency of 87%, with a reocclusion rate of 10% when coronary angiograms were repeated at 24 hours, when heparin was used in an identical fashion as in the present study.

It may be that less complete restoration of coronary patency with greater amounts of residual intraluminal thrombus after thrombolytic therapy, may predispose to propagation of thrombus and hence reocclusion. This suggestion is supported by previous studies which have suggested that the more severe the residual lesion, the greater the likelihood of reocclusion (Grines et al., 1988, Harrison et al., 1984, Gold et al., 1986, Williams et al., 1986). It is of note that in the patients in this study, 8 of the 12 patent arteries at first angiographic visualisation, 11 of 16 at 60 minutes, and 7 of 11 at 90 minutes, were TIMI grade 2. Thus a larger proportion of arteries had a less complete degree of reperfusion, probably indicating more residual intraluminal thrombus, acting as a persistent stimulus to thrombus formation and platelet activation leading to reocclusion.

Heparin administration in patients receiving thrombolytic therapy is associated with excess minor bleeding (GISSI-2, 1990), but so also are higher doses of t-PA (TIMI-2, 1989). Thus it would appear that the benefits of maintaining an anti-coagulated state following thrombolytic therapy of myocardial infarction, must be balanced against the risk of haemorrhagic complications. In this study, bleeding complications were relatively few, albeit in a small study group, and as has been found previously (Simoons et al., 1988), were largely related to invasive procedures. Of the four events in the series, at least one of them can be confidently attributed to over-anticoagulation with heparin,

emphasising the importance of close monitoring of anticoagulant therapy. None of the bleeding events were serious nor required specific intervention. Overall, this bleeding rate has to be contrasted with the high transfusion rates found in some thrombolytic studies (Simoons et al., 1985), although clearly firm conclusions cannot be drawn from such a small study.

Although the coronary patency rate at 90 minutes following therapy is disappointing, patency was effectively restored in 95% of patients at the end of the procedure, following administration of a further 40mg intracoronary alteplase, without local interventional procedures, confirming the efficacy of alteplase given by this route. The high incidence of ventricular fibrillation in the first patients receiving intracoronary alteplase, is most likely attributable to the physical properties of the agent and medium, and was not observed when a slower infusion rate was used.

Although three intravenous doses of 20mg of alteplase achieves plasma t-PA concentrations at least as high as conventional infusion doses, and achieves high initial coronary patency, the high concentrations are short-lived and there is an unacceptably high early reocclusion rate.

Further "rescue" doses of 40mg by the intracoronary route restores patency, and following this and in the presence of heparin, the reocclusion rate to 24 hours was low. In view of the high early patency, it may be appropriate to re-evaluate this regimen in conjunction with heparin initiated at the onset of thrombolytic therapy.

## **CHAPTER NINE**

### **CONCLUSIONS**

"The termination of angina pectoris is remarkable. For if no accident intervene, but the disease go on to its height, the patients all suddenly fall down and perish almost immediately"

- Heberden (1710-1801)

"Fashions in therapy may have some justification; fashions in diagnosis have none."

- Herrick and Tyson

"Some griefs are med'cinable."

- William Shakespeare, Cymbeline

The introduction of thrombolytic therapy in the treatment of acute myocardial infarction is probably the single most important development in the management of ischaemic heart disease in recent times. Although initial assessment suggested that the benefits of therapy were small, and its application limited by the practicability of intracoronary administration, studies comparing intravenous and intracoronary streptokinase, and then large scale studies assessing intravenous administration proved beyond doubt that thrombolysis in acute myocardial infarction could be widely applied and give considerable clinical benefits.

Although the principle of thrombolytic therapy in acute myocardial infarction is established, there remain many questions to be answered. These include which is the safest and most effective agent; when can dosing with streptokinase-containing thrombolytic agents be safely and reliably repeated; do different thrombolytic agents have specific indications and roles; and what is the optimal dose and administration regimen of the thrombolytic agents. The work in this thesis addresses these questions.

Streptokinase is the thrombolytic agent which has been available the longest time. The current standard recommended dose of streptokinase in acute myocardial infarction is 1.5 MIU as a continuous intravenous infusion over 60 minutes. This dosage regimen is empirical, and although this dose has been shown to achieve a lytic state in a large proportion of a normal population (Verstraete et al., 1966) there have been no studies adequately assessing the dose-response relationship of intravenous streptokinase. In practice, the rate of infusion of streptokinase is limited by haemodynamic responses, in particular systemic hypotension, yet details of the relationship between dose and blood pressure and the mechanism of these changes have never been fully investigated.

#### **Time to treatment: Pharmacokinetic considerations and early treatment**

As has been discussed above, the benefits of thrombolytic therapy for acute myocardial infarction are greater if the agent can be given earlier in the course of the infarct. Therefore attempts must be made to facilitate early referral, assessment and treatment of suitable patients. If the priority of treatment with a thrombolytic agent is to restore coronary patency, then the ideal thrombolytic agent would be easily administered, preferably as an intravenous bolus, without significant adverse effects. The ideal agent should rapidly achieve a high plasma concentration, and a high fibrinolytic activity, thereby maximising the

concentration gradient at the thrombus interface, and initiating early clot lysis. It would be desirable for the fibrinolytic effect to persist for a period of time to minimise early coronary reocclusion.

In chapter three, the pharmacokinetic properties of the two streptokinase-containing thrombolytic agents, streptokinase and anistreplase, have been compared. The higher and earlier peak plasma concentrations achieved (large  $C_{max}$  and short  $t_{max}$ ), and the longer half-life available with a bolus of 30 U of anistreplase, have been demonstrated, compared to a 60 minute continuous intravenous of 1.5 MIU of streptokinase. Therefore, in this context, anistreplase has pharmacokinetic advantages over streptokinase, at least when both agents are administered in their recommended standard dosage regimen.

**Limitations of the streptokinase-containing thrombolytic agents: the role of acquired resistance and haemodynamic responses**

Both streptokinase and anistreplase contain streptokinase which is derived from streptococcal protein. This exogenous protein both stimulates an immune response and reacts with circulating antibodies. However, understanding of the relevance of antibodies and acquired resistance to these agents is limited. Previous studies have testified to the loss of efficacy of streptokinase when administered to patients with high levels of circulating resistance (Hirsh et al., 1970, James, 1973), following antecedent

streptococcal infections, but these reports have been based on few patients with unusually high levels of resistance, and the relevance of lower levels of resistance has not been fully explored.

In chapter four, the relationships between both the streptokinase resistance titre and anti-SK IgG concentrations, and efficacy, assessed as angiographic coronary patency at 90 minutes and 24 hours after therapy with streptokinase and anistreplase, have been studied. The results must be interpreted in the context of the study population which had not been previously exposed to streptokinase, and therefore had generally low circulating resistance to streptokinase. While these factors did not grossly affect the ability of either of the thrombolytic agents to restore coronary patency, there were consistent trends for those patients with high levels of resistance to have a poorer result. When the results from the two agents were considered together, there was a significant difference in the streptokinase resistance titres between those patients with patent and occluded arteries, implying a small but possibly important role for streptokinase resistance titre in modifying the efficacy of the streptokinase-containing thrombolytic agents.

Hypotension following the administration of the streptokinase-containing thrombolytic agents is also a limitation to their use in acute myocardial infarction, in a population who may be susceptible to hypotension. In the fifth chapter, blood pressure changes following streptokinase and anistreplase have been documented in detail. Although there are significant falls in blood pressure, these falls are short-lived and well-tolerated in the great majority of patients.

The mechanisms of these blood pressure changes have not been elucidated. In this study an association was sought between hypotensive episodes and the markers of prior exposure to streptococcal antigen, the streptokinase resistance titre and anti-SK IgG concentrations; and also the release curves of D-dimer, B- $\beta$  15-42, and the fall in fibrinogen, as markers of thrombin activity; and corrected plasma viscosity. No relationship between any of these parameters was confirmed, refuting their implication in the hypotensive mechanism. Further research is required to establish this mechanism.

With the widespread use of streptokinase in the treatment of acute myocardial infarction, the question of the safe time interval for the readministration of the streptokinase-containing thrombolytic agents is going to be of increasing importance. The amplitude and duration of the antibody response following therapy is of central importance in this regard, and an area of particular current interest. The

data in this thesis show that median streptokinase resistance titre rises to a peak of a 40-fold rise at 14 days after therapy, with only 58% of the population returning to within two standard deviations of the pretreatment population within two years, and suggest that the further decline in the titres after this time is very slow. Calculations based on these data indicate that all of the standard dose of streptokinase would be neutralised at 12 months, and approximately 50% at 30 months. Similar results are presented for median anti-SK IgG concentration, which reaches a peak of a 150-fold at 14 days, with 39% of the population returning within two standard deviations at one year, and 50% at two years, at which point the median anti-SK IgG concentration was still nine-fold the pretreatment concentration.

Attempts to translate these concentration data and resistance titres to functional clot lysis were made using an in vitro clot lysis assay. Unfortunately, the necessity of using pooled plasma samples for this assay led to an unavoidable biasing of the pools towards the relatively few high antibody/resistance values. Although care must be taken in the interpretation of these studies and extrapolating to the in vivo situation, it would appear that there was near complete inhibition of lysis over the time period from 7 days to 9 months following thrombolytic treatment. Even at 12 months, only 25% of the baseline lytic activity was seen, increasing to 75% at 30 months. In

order to compensate for the bias mentioned above, the data have been examined using regression analysis techniques relating the streptokinase resistance titre and anti-SK IgG concentration to in vitro lytic activity, and the results used to construct a model of inhibition of in vitro lysis with time following thrombolytic therapy based on the median values of the population. Using this technique, it still appears that in vitro lysis remains less than 50% of baseline at 12 months, thereafter recovering partially.

The question of retreatment with streptokinase-containing thrombolytic agents depends on two pivotal questions. Firstly, will the induced immunological responses block the effect of the thrombolytic agent administered for the second time, and secondly will they influence the side effect profile, adversely affecting the risk:benefit ratio of these agents?

The data in this thesis relating to the former question are limited. Firstly there are the difficulties I have discussed in relation to the interpretation of the in vitro clot lysis, and secondly, in the population studied in Chapter Four, the pretreatment resistance is much lower than the values recorded following thrombolytic therapy, as would be expected in a previously unexposed population. Nevertheless, a negative effect can be demonstrated even

within this small distribution, and it must be considered that it is very likely that acquired resistance will affect the efficacy of standard doses of the streptokinase-containing thrombolytic agents.

The blood pressure changes following administration of streptokinase and anistreplase in acute myocardial infarction are short-lived and well-tolerated. In the same previously unexposed population, no relationship can be found between the likelihood of the occurrence of significant hypotension and the immunological markers, streptokinase resistance titre and anti-SK IgG concentration. Again it must be stressed that the pretreatment levels of resistance are low and it is difficult to extrapolate to the much higher levels found post-treatment, but the data in this thesis suggest that the hypotension observed is not obviously related to immunological factors. However, there are documented relatively rare anaphylactoid reactions, and it seems likely that at least some of these are related to antibody levels. Further clinical data is required relevant to this topic before hard conclusions can be drawn.

If the incidence of side effects is truly not related to acquired resistance to streptokinase, it may be possible to overcome the diminished efficacy by developing a front-loaded infusion regimen of streptokinase, such that the rapid infusion phase would be designed to saturate circulating resistance, possibly with the required dose

calculated from the patients' pretreatment streptokinase resistance titre, allowing the latter part of the infusion to be effective. Similarly, a larger dose of anistreplase as an intravenous injection may achieve the same end. However the limiting factor to such an approach is likely to be hypotension following the more rapid infusion of streptokinase. This argument stresses the need for further basic research to identify the mechanism of the blood pressure changes with streptokinase-containing thrombolytic agents, with the ultimate aim of developing pharmacological approaches to modifying this reaction.

In the meantime, repeat administration of streptokinase within a short time scale cannot be advised. It is currently recommended that streptokinase should not be readministered from 5 days to 6 months following prior treatment (Kabikinase data sheet, 1991), or possibly as long as 12 months (Streptase data sheet, 1991). The manufacturers of anistreplase also recommend a dosage interval of 5 days to 12 months (Eminase data sheet, 1991). Jalihal and Morris monitored streptokinase resistance titres for 36 weeks following streptokinase, and recommended that streptokinase should not be readministered for 8 months, and probably one year. The data in this thesis would suggest that there is persistence of increased streptokinase resistance titres and anti-SK IgG concentrations for considerably longer than this, and if this represents a contraindication to repeat therapy, then the recommended

interval should be at least two years. Recent studies by Elliott et al. (1991) have suggested that the antibody concentrations plateau following this and there is little further decline over a four year period.

In the light of present knowledge, it is recommended that if repeat thrombolytic therapy is indicated within these time points, that a non-allergenic drug such as rt-PA should be used.

### **Rt-PA bolus dosing**

There has been much research into the use and efficacy of rt-PA, both alteplase and duteplase, although the interpretation of these studies has been confused by differences in dosage regimens, preparations of rt-PA, methods of assessment and study populations. The currently recommended dose of alteplase is 100mg administered intravenously as a 10mg bolus followed by 50 mg over 1 hour, then 40mg over 2 hours. This regimen is relatively cumbersome to use and obviously requires the availability of equipment such as an infusion pump. The last chapters of this thesis address the use of alteplase in acute myocardial infarction administered as two different intravenous bolus regimens, assessing efficacy by coronary patency assessed angiographically, and studying the pharmacokinetics of these regimens.

The use of two 35mg boluses of alteplase 30 minutes apart achieved extremely high plasma concentrations of t-PA of  $4435.8 \pm 2117.8$  ng/ml within ten minutes of the first bolus of alteplase, falling to  $425.8 \pm 288.3$  ng/ml prior to the second bolus, rising again to a peak of  $4233.3 \pm 2217.5$  ng/ml within 10 minutes of the second bolus. The coronary patency at 90 minutes associated with this regimen was also very high, being 26 of 30 (87%, 95% confidence limits 74 to 99%) arteries, comparable to the best published results. The coronary reocclusion rate to 24 hours was relatively low, being 3 of 29 (10%, 95% confidence limits 0 to 22%) arteries, even although heparin was not introduced until 2-3 hours after therapy. In the small numbers involved in this pilot study, this regimen is most promising both in terms of efficacy and convenience of administration, and therefore ease and rapidity of use. This dose is worthy of further evaluation in a larger scale study.

In view of the high patency achieved with the total dose of 70mg alteplase, the last study assessed the efficacy of a total dose of 60mg, given as three intravenous boluses of 20mg each separated in time by 20 minutes. Although very high plasma t-PA concentrations were again achieved, with peaks of 1849-2138 ng/ml, the patency results were disappointing. A high coronary patency of 16 of 20 arteries (80%, 95% confidence interval 61 to 97%) was produced at 60 minutes, but early reocclusion caused the overall patency rate to fall to 11 of 20 (55%, 95% confidence interval 32 to 78%) at 90 minutes. High coronary patency was however

restored following "rescue" therapy, indicating that a high proportion of the arteries were able to be reperfused. Again this study was performed without heparin introduced at the initiation of thrombolytic therapy, and it would be interesting to repeat the study in the presence of heparin. The current study provides a model for reocclusion in the context of acute myocardial infarction, but the low 90 minute patency rate in the admittedly small numbers studied must suggest that this regimen is unlikely to become widely used in clinical practice.

### **Future developments**

There is no doubt that thrombolytic therapy has revolutionised the modern management of acute myocardial infarction, and that it will continue to have a continued important role in the future. All the available plasminogen activators have been unequivocally demonstrated to restore coronary patency, preserve left ventricular function, and reduce mortality.

Although this class of drugs has arguably been more extensively researched than any other innovation in modern medicine, the development of these drugs remains incomplete. Many of the recommended administration regimens for these drugs have been formulated empirically, and may not be optimal. Further assessment of the pharmacokinetic and pharmacodynamic properties of these drugs will allow a more logical and critical approach to administration regimens.

To date, the standard approach to recommended drug doses is to provide a dose suitable for all patients. It is likely that this is an over-simplification, and it may be necessary to develop pharmacokinetic models allowing the tailoring of thrombolytic regimens to an individual patient, in order to maximise efficacy, ease, and therefore speed, of administration and minimise side effects.

One other major outstanding question with regard to the current and future use of thrombolytic therapy is when is it safe to repeat therapy with the streptokinase-containing thrombolytic agents? To provide clues to this answer, further basic mechanistic research is required into the haemodynamic changes following streptokinase and anistreplase, and the possible development of antagonists to these pathways; and further pursuit of the immune responses to streptokinase and their clinical relevance. It may be possible to identify those patients prone to the development of higher and more persistent resistance, and to develop a rapid bedside test of streptokinase resistance allowing the tailoring of thrombolytic therapy regimens to the individual patient, in order to overcome circulating resistance.

The story of thrombolytic therapy in acute myocardial infarction has been a long and fascinating one, dating from the first administration of streptokinase in the 1950s to the recent development of new and exciting agents. Despite initial skepticism and discouraging results, it is now accepted as a standard part of modern coronary care.

There can be little doubt that in the short period of its widespread use, thrombolytic therapy has beneficially influenced the prognosis of the most important cause of death in our era. Nevertheless, there remain limitations to its use, and further work in this fascinating area will continue to refine this weapon, allowing more widespread, safer application and greater clinical benefits.

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**APPENDIX I**

**PATIENT DEMOGRAPHY**

PATIENTS INCLUDED IN STREPTOKINASE/ANISTREPLASE COMPARISON

PATIENT	SEX	AGE	IRA	TIME	TIMI GRADE AT		DRUG	SKRT	IgG
				TO Rx	90MIN	24HR			
JM	M	68	RCA	156	1	1	1	-1	4
JMcG	M	66	RCA	250	2	2	1	-1	1.48
DC	M	50	RCA	138	2	-1	1	-1	1.8
JC	M	51	LAD	270	2	2	1	-1	1.27
NMcD	F	57	RCA	222	1	2	2	-1	0.8
WN	M	42	RCA	112	2	2	2	-1	1.46
JO'C	M	42	RCA	313	1	2	2	-1	0.51
WS	M	55	-1	105	-1	-1	1	-1	0.8
JH	M	61	LAD	120	1	1	2	-1	1.6
CMcN	M	59	RCA	180	2	1	1	-1	0.3
TR	M	67	RCA	210	1	2	1	-1	0.21
PW	M	49	RCA	155	2	2	2	-1	-1
EMcN	M	60	LAD	76	1	-1	1	-1	-1
IA	F	64	LAD	210	1	1	2	-1	4.1
TMcQ	M	64	LAD	240	2	2	2	-1	1.06
WW	M	55	RCA	260	2	2	2	-1	0.6
RM	M	59	LAD	130	0	3	1	-1	5.3
BR	M	49	LAD	200	2	2	2	-1	0.91
MH	F	52	-1	260	-1	-1	2	-1	0.94
JF	M	45	LAD	150	1	2	1	-1	1.43
SA	M	47	LAD	335	2	2	2	-1	0.83
TG	M	45	RCA	185	1	2	1	-1	4.3
RMcC	M	42	RCA	278	2	2	2	-1	0.93
PK	M	44	RCA	71	2	2	1	-1	0.42
DU	M	49	Cx	295	2	2	2	-1	0.3
JH	M	45	RCA	155	2	2	1	-1	6.8
WC	M	55	RCA	350	1	1	2	-1	2
RR	M	55	RCA	315	2	2	2	-1	3.9
KA	M	59	LAD	280	0	2	1	-1	1.04
MK	F	52	LAD	200	2	2	2	-1	0.2
GMcM	F	58	RCA	160	1	-1	2	50	2.57
NG	F	58	RCA	235	2	2	1	20	0.18
WT	M	61	LAD	270	2	2	1	20	2.32
DD	M	50	LAD	125	2	2	1	20	3.61
AB	M	48	LAD	180	1	1	1	20	1.57
WMcL	M	58	RCA	150	1	-1	2	50	0.54
AP	M	68	RCA	330	2	2	1	20	0.49
WM	M	53	LAD	175	1	-1	2	20	0.4
CM	M	55	RCA	165	1	3	2	100	3.2
EH	F	68	RCA	195	1	-1	1	100	4.74
JC	M	65	LAD	295	1	2	2	20	0.83
WD	M	52	RCA	360	2	2	1	20	1.84
JA	F	52	RCA	375	1	1	1	100	1.87
SB	F	66	RCA	90	2	2	2	50	1.6
FC	M	44	LAD	235	2	2	2	50	0.76
JL	M	70	LAD	270	2	2	2	50	0.78
GC	M	47	LAD	195	1	2	1	50	4.34
RMcD	M	67	RCA	105	2	2	1	50	1.22
MH	F	64	Cx	320	-1	2	2	20	0.4
RE	M	60	RCA	135	-1	-1	2	50	1.16
PL	M	58	LAD	180	0	1	2	100	2.13
JD	M	43	RCA	190	3	3	1	50	1.17

JF	M	68	LAD	340	0	-1	2	50	0.66
NMcL	F	59	RCA	150	0	3	2	50	0.36
RD	M	40	RCA	210	1	2	1	100	5.45
JH	M	54	-1	140	-1	-1	1	20	0.55
PM	M	59	LAD	80	1	2	1	20	1.73
JG	M	48	LAD	365	3	3	1	20	0.39
AF	M	44	RCA	215	1	2	1	20	1.86
SW	F	65	Cx	305	0	3	1	20	0.98
AW	F	68	LAD	357	1	2	1	50	0.07
MMcG	M	53	RCA	165	-1	0	2	50	1.7
AO'N	F	58	Cx	165	3	3	2	20	2.59
EMcG	M	57	RCA	300	0	2	1	50	0.44
JB	M	67	LAD	275	3	3	2	20	1.72
WL	M	61	LAD	215	3	2	1	5	0.27
JS	M	53	LAD	175	1	2	2	20	1.9
JE	M	40	LAD	185	0	2	2	50	4.54
JC	M	31	Cx	260	2	2	2	20	2.35
AM	M	56	-1	380	-1	-1	1	20	0.8
JM	M	62	LAD	210	1	2	2	50	5.1
WMcC	M	59	RCA	95	2	2	2	50	0.76
DC	M	46	LAD	55	2	2	2	50	1.17
JS	M	57	LAD	300	2	2	1	50	0.24
DMcC	F	59	LAD	180	2	2	2	100	0.36
WMcL	M	64	LAD	330	2	2	2	50	0.22
CA	F	65	RCA	230	2	2	1	100	0.92
ED	M	55	LAD	235	2	2	1	100	3.48
FH	M	54	RCA	270	1	2	2	100	1.46
WA	M	47	RCA	245	2	2	1	100	1.04
DP	M	56	Cx	195	1	2	1	20	72
WMcI	M	51	RCA	175	2	2	1	20	2.1
JO	M	59	LAD	330	1	2	1	20	0.013
NMcN	M	61	Cx	220	1	2	2	100	0.6
CG	F	60	RCA	90	1	2	2	200	3.24
WS	M	50	RCA	55	2	1	2	50	0.99
GH	M	67	LAD	250	1	2	1	20	0.5
RN	M	56	LAD	275	1	1	2	200	0.14
JB	M	55	LAD	165	2	2	2	100	1.13
GT	M	67	LAD	175	2	2	1	100	0.51
GR	M	51	Cx	120	2	2	2	20	0.17
RS	M	58	-1	135	-1	-1	2	-1	-1
JN	M	64	RCA	155	2	2	1	20	0.23
WD	M	54	RCA	85	1	1	1	20	1.49
JH	M	55	LAD	215	2	2	1	5	0.3
JA	F	57	LAD	180	1	2	1	100	2.7
MD	F	59	LAD	220	2	2	2	20	0.56
NU	M	42	LAD	140	1	1	1	100	1.04
RK	M	41	LAD	185	-1	2	1	100	2.81
TM	M	46	LAD	80	2	2	1	20	0.68
MM	F	62	LAD	230	2	2	2	20	0.8
JL	M	62	LAD	320	1	2	2	50	1.03
EH	M	45	RCA	170	2	2	2	100	1.57
HH	M	59	Cx	95	2	2	2	20	0.14
JW	M	56	RCA	135	1	1	2	50	1.14
EC	F	52	RCA	125	-1	0	2	400	12.6
TS	M	54	LAD	135	2	2	1	20	1.35
JMcG	M	42	RCA	130	3	3	2	200	7.011
AB	F	47	RCA	270	0	2	1	50	5.1

MT	F	55	RCA	142	3	3	1	50	2.67
MS	F	55	LAD	185	0	2	1	20	2.39
RH	M	55	RCA	125	2	3	1	20	0.54
PM	M	52	Cx	215	3	3	1	20	0.59
AR	M	64	RCA	55	3	3	2	20	0.82
JJ	F	70	-1	350	-1	-1	1	5	0.32
PR	M	61	-1	210	-1	-1	2	20	0.46
DMcG	M	43	Cx	140	0	0	2	100	2.88
JS	M	61	Cx	210	0	0	2	20	0.79
PC	M	51	LAD	160	1	2	2	20	0.56
TJ	M	41	LAD	290	0	0	1	200	3.79
TM	M	63	RCA	285	2	3	2	100	2.75
CM	F	41	RCA	185	0	3	2	50	3.69
GMcD	M	65	LAD	250	3	3	1	20	1.07
CC	F	66	RCA	165	2	2	2	100	4.13
DC	M	65	LAD	170	2	3	1	5	0.63
RK	M	69	LAD	90	3	3	1	100	5.98
HT	M	67	LAD	150	1	2	1	20	0.05
RH	M	57	LAD	170	3	3	2	20	0.78

LAD = Left anterior descending coronary artery

RCA = Right coronary artery

Cx = circumflex coronary artery

TimeRx = time from onset of pain to treatment

Drug code: 1 = Streptokinase

2 = Anistreplase

-1 = missing data

**PATIENTS INCLUDED IN ANISTREPLASE/STREPTOKINASE  
PHARMACOKINETIC STUDY**

<b>PATIENT</b>	<b>SEX</b>	<b>AGE</b>	<b>DRUG</b>
JM	M	68	Streptokinase
JMcG	M	66	Streptokinase
DC	M	50	Streptokinase
JC	M	51	Streptokinase
NMcD	F	57	Anistreplase
WN	M	42	Anistreplase
JO'C	M	42	Anistreplase
WS	M	55	Streptokinase
JH	M	61	Anistreplase
CMcN	M	59	Streptokinase
TR	M	67	Streptokinase
PW	M	49	Anistreplase
EMcN	M	60	Streptokinase
IA	F	64	Anistreplase
TMcQ	M	64	Anistreplase
WW	M	55	Anistreplase
RM	M	59	Streptokinase
BR	M	49	Anistreplase
MH	F	52	Anistreplase
JF	M	45	Streptokinase
SA	M	47	Anistreplase
TG	M	45	Streptokinase
RMcC	M	42	Anistreplase
PK	M	44	Streptokinase

**PATIENTS INCLUDED IN 2x35mg BOLUS ALTEPLASE STUDY**

PATIENT	SEX	AGE	IRA	TIME TO Rx	---TIMI GRADES AT---					24 HR
					1st INJECTION	1st	45	60 MINUTES	90	
CB	F	68	RCA	220	55	0	-1	0	0	0
JMcI	M	40	LAD	340	38	0	0	0	0	0
DS	F	74	LAD	250	51	1	-1	2	2	2
EL	F	69	RCA	255	41	2	2	2	2	3
KS	M	52	LAD	170	46	2	-1	2	2	2
MG	F	48	LAD	370	35	2	2	2	2	3
JD	F	57	LAD	185	37	2	2	2	2	3
JL	M	53	RCA	165	60	2	-1	2	2	2
RMcM	M	66	LAD	232	47	2	-1	2	2	3
DG	M	68	RCA	48	41	2	2	2	2	2
HMcB	F	58	RCA	187	50	2	-1	2	2	2
JG	M	40	LAD	180	41	2	2	2	2	2
AC	M	70	LAD	330	53	2	-1	0	3	3
MS	M	62	RCA	355	47	2	-1	2	2	3
GS	M	55	RCA	260	35	2	2	2	2	2
JC	M	72	LAD	135	42	2	2	2	2	2
AO'H	M	45	Cx	140	45	3	2	2	2	3
IL	M	52	LAD	143	63	3	-1	-1	3	2
AW	M	40	LAD	238	44	3	3	3	3	3
RMcB	M	43	RCA	75	40	3	3	3	3	3
TP	M	59	Cx	180	90	3	-1	-1	2	3
RN	M	30	RCA	95	47	1	-1	2	2	2
AB	M	52	RCA	185	47	2	-1	2	2	3
DD	M	69	LAD	240	36	2	2	2	2	1
RN	M	47	RCA	200	51	0	-1	2	2	1
MMcL	F	64	-1	270	-1	-1	-1	-1	-1	-1
JD	M	68	RCA	240	50	3	-1	3	3	3
GB	M	45	RCA	140	47	2	-1	2	2	1
JM	M	62	LMCA	185	60	0	-1	0	1	3
AH	M	51	-1	255	-1	-1	-1	-1	-1	-1
JD	F	46	RCA	247	55	3	-1	3	3	3
IB	F	59	RCA	213	45	0	0	1	1	3
BMcC	F	43	RCA	165	90	3	-1	-1	3	3

IRA = infarct related artery  
 LAD = Left anterior descending coronary artery  
 RCA = Right coronary artery  
 Cx = circumflex coronary artery  
 -1 = missing data

**PATIENTS INCLUDED IN 3x20mg ALTEPLASE STUDY**

PATIENT	SEX	AGE	IRA	Rx	TIME		TIMI GRADES AT-----			
					TO Rx	1st INJECTION	1st	60 MINUTES	90	24 HR
DC	M	59	RCA	A	135	41	0	0	2	2
WG	M	52	LAD	A	110	52	2	2	2	2
MMCF	F	67	RCA	B	280	60	2	2	0	0
WMK	M	68	RCA	D	85	41	0	0	0	0
JS	M	69	RCA	A	235	46	0	2	2	3
AF	F	60	LAD	B	195	25	0	2	0	2
VC	M	52	LAD	B	85	43	0	1	0	3
TG	M	53	LAD	B	60	50	3	3	0	3
MM	F	53	RCA	B	200	50	2	2	2	3
MH	F	69	RCA	A	275	53	2	2	2	3
RG	M	40	-1	C	195	-1	-1	-1	-1	-1
WG	M	52	LAD	A	110	60	3	3	3	3
CS	M	66	RCA	A	115	60	2	2	2	3
JT	M	71	LAD	A	350	50	3	3	3	3
JOD	M	41	LAD	B	65	60	0	0	0	2
JJ	F	51	RCA	B	325	49	0	2	1	0
JMK	M	42	LAD	B	185	47	2	3	1	2
WM	M	62	RCA	A	120	55	2	2	2	0
MB	M	59	RCA	B	128	55	1	2	0	3
JJ'	F	71	RCA	C	340	60	3	3	3	-1
AH	F	72	RCA	E	150	60	2	2	2	2

**Treatment Code (Rx)**

**CODE TREATMENT**

- A 20mg x 3 intravenous**
- B 20mg x 3 intravenous +40mg intracoronary at 90mins**
- C 20mg x 3 intravenous +40mg intravenous at 90mins**
- D 20mg x 3 intravenous +20mg intracoronary at 90mins**
- E 20mg x 3 intravenous +40mg intravenous at 120mins**

**IRA = infarct related artery**

**LAD = Left anterior descending coronary artery**

**RCA = Right coronary artery**

**-1 = missing data**

**APPENDIX II**

**DEFINITION OF TIMI SCORES**

**DEFINITION OF TIMI SCORES**  
(Williams et al., 1986)

- GRADE 0 NO REPERFUSION:** There is no antegrade flow beyond the point of occlusion.
- GRADE 1 PENETRATION WITH MINIMAL PERFUSION:** The contrast material passes beyond the area of obstruction, but "hangs up" and fails to opacify the entire coronary bed distal to the obstruction for duration of the cine run.
- GRADE 2 PARTIAL PERFUSION:** The contrast material passes across the obstruction and opacifies the coronary bed distal to the obstruction. However, the rate of entry into the vessel distal to the obstruction or its rate of clearance from the distal bed (or both) are perceptibly slower than its entry or clearance from comparable areas not perfused by the previously occluded vessel, e.g. the opposite coronary artery or the coronary bed proximal to the obstruction.
- GRADE 3 COMPLETE PERFUSION:** Antegrade flow into the bed distal to the obstruction occurs as promptly as antegrade flow into the bed proximal to the obstruction, and clearance of contrast material from the involved bed is as rapid as clearance from an uninvolved bed as the same vessel or the opposite artery.

**APPENDIX III**

**STUDY PROTOCOLS**

**ANISTREPLASE IN THE TREATMENT  
OF ACUTE MYOCARDIAL INFARCTION.**

**1. STUDY OBJECTIVES**

- 1.1. To measure and compare angiographically documented coronary artery patency at 90 minutes following intravenous APSAC 30 Units or intravenous streptokinase 1.5 million units.
- 1.2. To compare reocclusion rates at 24 hours after dosing.
- 1.3. To compare the safety of the different compounds with special reference to effects on blood pressure in the first 90 minutes after dosing.
- 1.4. To document the immunological changes following administration of the streptokinase-containing thrombolytic agents, measuring streptokinase resistance titre and anti-SK IgG concentrations prior to therapy and over the following 12 months.
- 1.5. To investigate the role of pretreatment streptokinase resistance titre and anti-SK IgG concentration in the efficacy of and haemodynamic responses to the streptokinase-containing thrombolytic agents.

- 1.6. To seek a relationship between markers of fibrin- and fibrinogen-olysis, and plasma viscosity and haemodynamic changes.
- 1.7. To investigate the pharmacokinetic properties of the pharmacological agents.

## 2. **STUDY MEDICATION**

### 2.1. **Intravenous APSAC**

APSAC is the active site p-anisoylated derivative of the primary (human) lys-plasminogen- streptokinase complex prepared by immediate acylation of the serine residue in the active centre of that complex as it is formed. The molecular weight is close to 131,000 Daltons.

APSAC is formulated in a mixture of clinical grade human albumin, D-mannitol and L-lysine. It is presented in vials, each containing 30 Units APSAC as a sterile, white lyophilized powder.

### 2.2. **Storage**

APSAC 30U vials have a shelf-life of 2 years when stored at or below 5°C.

### 2.3. **Streptokinase**

Streptokinase is presented as a freeze-dried powder in vials containing 600,000 units of streptokinase. Streptokinase is stable for at least 3 years when stored at room temperature (<25°C). Solutions prepared for infusion but left over or not used should be discarded.

## 3. **STUDY DESIGN**

A double blind, randomised, angiographically controlled study of intravenous APSAC or intravenous streptokinase in acute myocardial infarction with stratification according to infarct site.

### 3.1. **Number of patients**

A minimum of 80 patients will complete the study.

### 3.2. **Patient Entry**

Patients with clinical evidence of acute myocardial infarction who satisfy the inclusion/exclusion criteria will be eligible for randomisation.

### 3.3. **Stratification and Randomisation**

Patients will be stratified according to the site of infarction. Each stratum has been separately pre-randomised to receive APSAC 30 Units intravenous or streptokinase 1.5 million Units intravenous

3.4. **Coronary Angiography**

Angiography will be performed at 90 minutes and at 24 hours after dosing.

3.5. **Blood pressure and heart rate monitoring**

Blood pressure and heart rate will be monitored immediately before drug and continuously throughout the 90 minutes post-treatment period. Blood pressure and heart rate will be recorded on the case report forms every 2 minutes until the end of the 90 minute angiogram.

The blood pressure will be observed for at least 10 minutes prior to the start of thrombolytic therapy, on a stable dose of intravenous nitrates (2 mg/hr of isosorbide dinitrate), and the final recording taken as the baseline recording. A hypotensive response is arbitrarily defined as a fall in systolic blood pressure of at least 20mmHg lasting for at least three cycles of the automatic cuff (6 minutes), and will be classified as early (within 30 minutes of start of thrombolytic therapy), late (30 - 90 minutes) or both.

### 3.6. **Primary Data End Points**

#### 3.6.1. Angiographically documented patency

(occlusion/perfusion grade 2,3) or occlusion (occlusion/perfusion grade 0.1) of the presumed infarct related vessel at 90 minutes after dosing.

#### 3.6.2. Angiographically documented reocclusion

(occlusion/perfusion grade 0,1) or sustained patency (occlusion/perfusion grade 2,3) at 24 hours after dosing in those who had patent infarct related vessels at 90 minutes.

### 3.7. **Blood sampling**

#### 3.7.1. Venous blood samples will be collected prior to

thrombolytic therapy then at 6, 10, 15, 30, 45, 60, 90 minutes, 2,4,6,9,12 hours, 1, 2, 3, 4, and 5 days after thrombolytic therapy and subsequently assayed for plasma fibrinogen, B- $\beta$  15-42 peptide, D-dimer and plasma viscosity corrected for haematocrit.

#### 3.7.2. Venous blood samples will be collected prior to

therapy and then 2 and 4 weeks, 3, 6, and 12 months and the streptokinase resistance titre and anti-SK IgG concentrations measured. In the first 40

patients, samples will be taken daily for the first 14 days to document the early rise in antibody titres.

3.7.3. In twenty-four (12 streptokinase, 12 APSAC) patients an intensive blood sampling protocol will be undertaken for pharmacokinetic studies. Venous blood samples will be taken at 0, 6, 10, 20, 30, 45, 60, 75, 90 minutes, 2, 4, 6, 9, 12 and 24 hours and frozen immediately for subsequent analysis of total fibrinolytic activity.

#### 4. **PATIENTS AND METHODS**

All patients with suspected acute myocardial infarction who satisfy the inclusion/exclusion criteria will be admitted.

#### 5. **INCLUSION CRITERIA**

Patients admitted to hospital;

5.1. Aged 70 years or under.

5.2. With chest pain or other symptoms of acute myocardial infarction of at least 30 minutes duration who can be treated within 6 hours of symptom onset.

5.3. With ECG evidence of ST segment elevation of at least 0.1mV in two or more standard leads and/or 0.2mV in two or more praecordial leads.

5.4. In whom appropriate consent is obtained for participation in the study.

6. **EXCLUSION CRITERIA**

6.1. Patients with systolic blood pressure below 95 mmHg.

6.2. Patients on anticoagulant therapy.

6.3. Patients with a known history of haemorrhagic diatheses or significant recent bleeding from another site.

6.4. Patients with documented or suspected active peptic ulceration with 1 year.

6.5. Patients with a history of cerebrovascular accident.

6.6. Patients who have had surgery, major trauma or head injury within the previous 4 months.

- 6.7. Patients who have received streptokinase or APSAC therapy within the previous 6 months.
- 6.8. Patients with severe hypertension, blood pressure above 200/120 mmHg.
- 6.9. Patients who have received prolonged chest compression prior to randomisation.
- 6.10. Pregnant females or those in whom pregnancy cannot be ruled out. Females who are menstruating or who are of child bearing potential.
- 6.11. Patients with diabetic proliferative retinopathy.
- 6.12. Patients in whom coronary angiography is contra-indicated.
- 6.13. Patients who have had coronary angioplasty within 1 month of presentation or those with a history of CABG or prosthetic valve insertion.
- 6.14. Any condition requiring immediate surgical intervention.
- 6.15. Any clinical suspicion of dissecting aneurysm.
- 6.16. Transmural myocardial infarction within 3 months.

6.17. Patients with serious or life-threatening disease unrelated to the circulatory system.

7. **STRATIFICATION AND RANDOMISATION**

Patients will be stratified according to the site of infarction on the admission ECG. Those with anterior or lateral infarction will be assigned to group A and inferior or posterior infarction to group I. Each of the 2 groups (A and I) has a separate randomisation sequence for 30 Units APSAC or 1.5 million units of streptokinase. Consecutive numbers starting from 1 will be used within each group.

Two identification, self-adhesive labels will be detached from study drug pack, one to be placed in the record form and the other attached to a postcard which should be posted within 12 hours of patient entry. The label identifies the precise batch of study drug.

The double blind nature of the study will be ensured by a "double dummy" technique. Patients will receive "placebo APSAC" and "active streptokinase" or "active APSAC" and "placebo streptokinase".

8. **TREATMENT**

The treatment regimen will be as follows:

The vial labelled APSAC will be dissolved in 5 mls of water for injection or physiological saline to be

administered intravenously over 5 minutes. To minimise foaming, the solution must be gently swirled AND NOT SHAKEN.

Two and one half of the three ampoules marked streptokinase will be dissolved in 500ml of 5% Dextrose to be infused over 60 minutes.

Injection and infusion will begin at the same time. Solutions must be administered immediately after reconstitution.

## 9. **CORONARY ANGIOGRAPHY**

9.1. Coronary angiography will be via a brachial or femoral artery approach. At least three views (LCA) or two views (RCA) of the infarct related vessel will be taken during each procedure. One of these will be the optimal view for maximising the percent residual stenosis.

9.2. An angiogram will be performed at 90 minutes from the start of dosing. The catheter will then be withdrawn, but the sheath will be left in place for

up to 48 hours.

**Perfusion is defined as a grade 2 or 3 (occlusion/perfusion grade) at 90 minutes.** Patients with grade 0-1 occlusion at 90 minutes will not require further angiographic study and will be given the benefit of the best available treatment at the discretion of the attending physician. They will be recorded as "persistent occlusions" for the purpose of the analysis.

9.3. Only patients with patency (occlusion/perfusion grade 2-3) at 90 minutes will be required to undergo coronary angiography at 24 hours after dosing, but patients with non-patent arteries at 90 minutes may undergo further angiography at the discretion of the physician in charge.

10. **BLOOD PRESSURE MONITORING**

Arterial pressure and heart rate will be recorded immediately before and continuously for 90 minutes after dosing. Copies of all tracings will be reported in the relevant section of the case record form prior to their being placed in the inside

cover.

11. **HEPARIN ADMINISTRATION**

Based on the result of the 90 minute post treatment angiogram, patients with Grade 0-1 occlusion may be given heparin and/or other therapy at the discretion of the attending physician. Patients with Grade 2-3 perfusion should receive heparin in a dose of 1000-1500 units per hour from between 4 and 6 hours after APSAC therapy or when the thrombin time has decreased to less than twice the control value.

Heparin treatment will be continued for 24 hours and further anticoagulation is at the physicians discretion.

12. **ECG RECORDINGS**

All patients will have single lead continuous ECG rhythm recordings for the first 24 hours of the study. Rhythm disturbances will be reported in the appropriate section of the case report form.

Five 12 lead ECG's are required, these include one on admission, at 2, 4, 16, 18 and 24 hours. If the patients clinical condition changes substantially within the first 24 hours, further ECG's may be required.

13. **CLINICAL ASSESSMENTS**

- 13.1. Any symptoms thought to be associated with the procedure, the treatment or the disease will be noted.
- 13.2. Heart rate and blood pressure will be measured before dosing and continually for the first 90 minutes after dosing. There will be further non-invasive measurements at 6, 12 and 24 hours after dosing or more frequently if indicated by a change in the patient's condition.
- 13.3. Temperature will be monitored at 6, 12 and 24 hours after dosing. Measurements will be taken more frequently if indicated by changes in the patient's condition.

14. **LABORATORY OBSERVATIONS**

Blood samples will be drawn to determine Hepatitis B status, cardiac enzyme concentrations, clinical chemistry, haematology.

14.1. **Hepatitis B status**

A blood sample will be taken before dosing and

tested for HBsAg.

**14.2. Cardiac Enzymes Concentrations**

Blood samples must be taken before dosing, at 90 minutes and 24 hours after dosing for the estimation of CPK and routine cardiac enzymes.

**14.3. Clinical Chemistry and Haematology**

Blood samples will be taken before dosing, at 90 minutes and 24 hours after dosing for a routine screen.

**14.4. Urinalysis**

A urine sample will be collected at 24 hours after dosing for the estimation of pH, blood, ketones, protein and bilirubin by means of "Dipstix".

**15. PRECAUTIONS**

**15.1. Ancillary medications**

No anti-platelet medication for 24 hours after thrombolytic therapy.

Additional drugs, such as analgesics,

antiarrhythmics etc., should be administered in accordance with normal hospital practice. All patients will receive a continuous infusion of intravenous nitrates.

#### 15.2. Dealing with excessive production of plasmin

For the treatment of severe uncontrolled bleeding, it is suggested that the following steps be taken:

- a) discontinue thrombolytic agent
- b) application of local pressure where possible
- c) reversal of the lytic state: Administer TRANEXAMIC ACID in a standard dose of 500 mg intravenously over 2 minutes (other agents may be used). A further 500 mg tranexamic acid may be administered if the bleeding is not controlled after the replacement of clotting factors.
- d) replace clotting factors with cryoprecipitate,

fresh frozen plasma, or whole blood.

e) replace blood loss.

16. **STATISTICAL CONSIDERATION**

The major efficacy variables in this protocol will be patency at 90 minutes and reocclusion after patency.

The main objectives of this study are the measurements of patency and reocclusion after APSAC and streptokinase and the effect of these agents on blood pressure. Results will be compared using relevant statistical procedures.

Information regarding adverse reactions and abnormal laboratory tests will be presented in the form of listings and tabulations.

17. **DRUG ACCOUNTABILITY**

17.1 **On-site Storage and Distribution**

All investigational drug supplies will be stored in a refrigerator, maintained at 5°C at the study site. Shelf life for APSAC is 2 years. Access to the

study medication must be limited to the principal investigator and other authorised members of his staff.

During periodic monitoring by Beecham staff, the drug supplies and case records will be reviewed for accuracy and completeness.

18. **INFORMED CONSENT AND PATIENT PRIVACY**

It is the investigator's responsibility that each subject or subject's legal representative signs an informed consent statement prior to participation in this study. Copies of the consent form with the patient's initials will be retained by the investigator.

The patients will be informed of their rights to privacy but will be made aware that study data will be submitted to Beecham and to drug regulatory authorities for review and evaluation. They will also be informed that both Beecham and the

regulatory authorities have the right to inspect the patient's medical records to verify the accuracy and completeness of the study records and results.

19. **REPORTING OF ADVERSE EVENTS**

The date, time of onset, duration and severity of any adverse reaction will be recorded. In the event of a persistent severe adverse event, e.g. hemiplegia resulting from CVA, the outcome should be determined at intervals up to 1 year.

## **A PILOT STUDY OF BOLUS TPA IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION**

### **INTRODUCTION**

Thrombolytic therapy is now established as standard therapy for the treatment of acute myocardial infarction. Tissue plasminogen activator has been under clinical study since the early 1980's and is now available for routine clinical use. Until the present time, rt-PA has been used given as a complicated decremental infusion regime. The rationale behind this was that the half-life of the single chain preparation of rt-PA (Actilyse) is about 5 minutes. Recent clinical studies including observations by our own group have indicated that the pharmacodynamic effects of rt-PA exist for longer than the pharmacokinetic effects, i.e. continued thrombolysis may occur up to 7 hours after dosing. This has been suggested by animal studies, and has recently been confirmed clinically. In animal studies, Cercek (1987) and Eisert (1987) have suggested that bolus t-PA increased the rate of lysis by a factor of two-fold or more within the first 15 minutes after injection in a dose-dependent manner. Clinical studies have also been performed using bolus dosing of rt-PA in patients with acute myocardial infarction (Eisert et al., 1988). Initial bolus dosing has been used with 15-30 mg being given, with follow on dosage schedules up to 100-150 mg. Initial clinical experience with bolus dosing suggests that

reperfusion is seen earlier, and that the minimal luminal stenosis left after thrombolysis is lower in such groups. The aim of the present study is to determine an efficacious and safe bolus regimen of rt-PA to be administered in patients admitted with acute myocardial infarction.

### **OBJECTIVES**

1. To assess the effect of two boluses of rt-PA on the patency of coronary vessels at 30, 60 and 90 minutes in patients with acute myocardial infarction by invasive and non invasive methods.
2. To assess the pharmacodynamic profile of rt-PA when given as a bolus.
3. To assess the effects of bolus rt-PA on blood rheology.

### **STUDY DESIGN**

This study has an open dose-ranging design to evaluate bolus rt-PA. Initially 20 patients will be treated with two boluses, the initial bolus being 35 mg rt-PA. This would be repeated at 30 minutes, giving a total dose of 70 mg. Following the first 20 patient's treatment, the results will be analysed, and a further 20 patients will be treated at a further dosing level of 50 mg, repeated after

30 minutes. This dose increment will be dependent on the results obtained. The maximum total dose of rt-PA used in this study will be 100 mg, as is the recommended dose schedule at present used in clinical practice.

## **STUDY POPULATION**

### **Inclusion Criteria**

(i) Patients with acute myocardial infarction defined as:

a) Cardiac pain at rest lasting from thirty minutes up to six hours.

b) ECG changes at presentation consistent with a diagnosis of acute myocardial infarction.

Normally this will include ST segment elevation of at least 2 mm in two praecordial leads or ST segment elevation of 1 mm in two inferior leads. However, patients with ECG diagnosis of posterior myocardial infarction and right ventricular myocardial infarction will also be included.

(ii) Males and females.

(iii) Age 18 to 75 inclusive.

(iv) Patients who have no contra-indications to

angiography.

- (v) Patients able to give informed consent to participate.
  
- (vi) Patients with no history or ECG changes of previous myocardial infarction in same distribution.
  
- (vii) Patients weighing over 67 kg.

**Exclusion Criteria**

- (i) Patients with ECG abnormalities that make it impossible to diagnose acute myocardial infarction. This will include patients with left bundle branch block and other severe conduction defect abnormalities.
  
- (ii) Patients with any contra-indications to thrombolytic therapy:
  - Any bleeding diathesis
  - Major trauma or surgery within three months
  - Puncture of a non-compressible vessel within 10 days
  - Any major haemorrhage
  - Any patient on Warfarin or Coumarin anticoagulants
  - Any previous cerebrovascular accident including TIA
  - Any previous history of peptic ulceration

Any proliferative retinopathy

Women who are pregnant, lactating or menstruating

History of severe poorly controlled hypertension

Any additional contraindication that is felt by the clinician to be relevant at that time to bolus rt-PA therapy.

## **METHODS**

The patients will be assessed pre-study within the Coronary Care Unit of Stobhill General Hospital. Thrombolytic therapy has been administered as standard therapy since 1982, and this study will be conducted within the Unit using standard monitoring facilities and general nursing medical care as appropriate. Written informed consent will be obtained from patients, both for administration of thrombolytic therapy, and also permission for performance of coronary arteriography. Where appropriate, and when available, the protocol and procedure will be discussed with any accompanying relatives.

The first bolus dose of rt-PA will be given as soon as possible following diagnosis. In addition, all patients will receive 150 mg of aspirin orally on admission. Analgesia will be given as per standard practice, and at no stage will any routine antiarrhythmic, inotropic nor vasodilator therapy be withheld. A second bolus dose of rt-PA will be given 30 minutes after the first bolus. At 3 hours following dosing, a bolus of heparin 5,000 units and

an infusion of 1,000 units per hour will then be commenced with the rate of infusion being controlled using standard coagulation screens. The second bolus of therapy will not be administered if there are any new contra-indications such as the occurrence of bleeding, cerebrovascular accident or if any surgical intervention is required.

Coronary arteriography will be performed 30 minutes after the infusion of rt-PA. An angiogram of the culprit vessel, as judged by the admission ECG, will be performed, and a second bolus dose will be administered thereafter. An arterial sheath will be left in situ, and the coronary arteriogram continued with further angiograms performed at 45, 60 and 90 minutes. The arterial sheath will be left in situ, and coronary angiography will be repeated at 24 hours.

At all times the performance of coronary angiography will be undertaken if the patient's clinical condition is stable, and the investigation appropriate. Any form of medical or intervention therapy can be performed at any time at the discretion of the Consultant Cardiologist managing the patient's overall condition.

Standard twelve lead ECGs will be performed at 0 time, time 2 hours, 4 hours, 8 hours, 12 hours and 24 hours following dosing. Serial cardiac enzymes will be performed, including CPK, AST, ALT and LDH at 4, 12 and 24 hours

following admission to confirm diagnosis of acute

myocardial infarction. Routine haematology and biochemistry will be performed as required for patient management, and a coagulation status will be performed to allow anticoagulation.

#### **PHARMACOKINETIC ASSESSMENT**

Serial blood samples will be taken to measure whole blood viscosity and blood samples to determine measurements of factions of thrombolysis and fibrinogenolysis, including measurement of d-Dimer and b-Beta 15-42 fragments. In addition to routine measurements of fibrinogen, plasminogen and alpha<sub>2</sub> anti-plasmin. Blood samples will be taken pre-bolus and at 10, 20, 30 minutes post and at 10, 20, 30 minutes post second bolus, and at 2, 4, 8 and 24 hours. The total volume of blood will be 175-230 mls.

Standard electrocardiographic monitoring with Holter monitor will be performed for 24 hours, and this data will be analysed by Reynolds Pathfinder to correlate ST segment changes with the reperfusion data obtained from coronary angiography.

All clinical events will be monitored and the patient's follow up therapy will be undertaken as deemed appropriate

by the Cardiologist.

The coronary arteriograms will be analysed blindly to assess the achievement of reperfusion, and to assess the relative degree. In addition, the morphological appearance of the coronary artery following reperfusion will also be assessed using standard techniques, as previously performed in the European Collaborative Study for Tissue Plasminogen Activator.

#### **REFERENCES**

1. Cercek B. Thrombolytic effect of rt-PA administered as a bolus. *Cl.Research* 1987;**35**:55102 Abstract.
2. Eisert et al. Bolus treatment regimen of rt-PA expedites rate of lysis without increasing risk of bleeding. *Blood* 1987;**70**(Suppl.1):370a Abstract 1335.
3. Eisert et al. Data on file: data presented at European Cardiac Conference Vienna 1988. *Eur.Heart J.* 1988;**Suppl.1**:1035 Abstract.

**CONSENT FORM**

I .....of .....  
hereby consent to receive the new thrombolytic agent called  
rt-PA and to undergo the procedure of coronary  
arteriography, the nature and effect of which have been  
explained to me by Dr. .... I consent to the  
administration of a local anaesthetic for these purposes.  
I also consent to such further or alternative procedures  
which may be found to be necessary during the course of the  
arteriogram.

Date ..... Signed .....

I confirm that I have explained to the patient the proposed  
nature and effect of this therapy and the procedure of  
coronary arteriography.

Date ..... (Doctor's Signature) .....

## **PATIENT INFORMATION SHEET: THROMBOLYTIC THERAPY**

A heart attack or a coronary thrombosis is the term used for a condition in which the flow of blood to the heart muscle is reduced to a degree that damage occurs. The reduction in blood flow is usually due to a blood clot or thrombus obstructing the arteries leading to the heart muscle. After a period of time, damage is irreversible and the course of the illness will follow the normal healing process. If a patient is admitted to hospital early enough, then treatment may be introduced which can dissolve the blood clot and can restore flow of blood to the area of muscle under threat of permanent damage.

The standard treatment for heart attack within the Coronary Care Unit of Stobhill Hospital is to give this therapy to dissolve the blood clot - that is thrombolytic treatment, to those patients admitted in the early stage of coronary thrombosis to obtain coronary artery flow, and to reduce the degree of damage. This form of treatment cannot be given to patients who have a predisposition to bleeding, such as those with stomach or duodenal ulcers, or to patients who have had strokes or operations in the recent past.

Two standard drugs are available at present for thrombolytic therapy called Streptokinase and Tissue Plasminogen Activator. This latter drug has been shown to

be very successful in dissolving blood clots, and has been used in a widespread manner in both United States and mainland Europe, as well as in the U.K. We use thrombolytic treatment only in those cases who should benefit, and who have no contra- indication. This treatment is in addition to all normal forms of treatment. Although we are studying Tissue Plasminogen Activator, its use will not prevent the introduction of any other treatment which is necessary for the underlying condition. To maximise the dose of Tissue Plasminogen Activator to be given to any patient, we are studying its effects on blood clotting, and therefore frequent blood sampling is taken from a small plastic tube inserted in the arm.

As a follow up to thrombolytic therapy to assess its success and to determine if any other procedures or treatment should be given, an x-ray test, called a coronary arteriogram, is often helpful and we are performing this as part of the present study. This requires the insertion of a little plastic tube into an artery, and therefore signed consent is required before the performance of this test. The actual method of the test will be explained separately by the Doctor who will perform the procedure at the time.

## **FURTHER STUDIES OF BOLUS rt-PA IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION**

### **INTRODUCTION**

Thrombolytic therapy is now established as standard therapy for the treatment of acute myocardial infarction. Tissue plasminogen activator has been under clinical study since the early 1990's and is now available for routine clinical use. Until the present time, rt-PA has been used given as a complicated decremental infusion regime. The rationale behind this was that the half-life of the single chain preparation of rt-PA (Actilyse) is about 5 minutes. Recent clinical studies, including observations by our own group have indicated that the pharmacodynamic effects of rt-PA exist for longer than the pharmacokinetic effects, i.e. continued thrombolysis may occur up to 7 hours after dosing. This has been suggested by animal studies, and has recently been confirmed clinically. In animal studies, Cercek (1987) and Eisert (1987) have suggested that bolus TPA increased the rate of lysis by a factor of two-fold or more within the first 15 minutes after injection in a dose-dependent manner.

Clinical studies have also been performed using bolus dosing of rt-PA in patients with acute myocardial infarction (Eisert et al., 1988). Initial bolus dosing has been used with 15-30mg being given, with follow on dosage schedules up to 100-150mg. Initial clinical experience with bolus dosing suggests that reperfusion is seen earlier, and that the minimal luminal stenosis left after

thrombolysis is lower in such groups. Our initial bolus studies have been completed using an infusion regime of 35 mg followed by a further 35 mg in 30 minutes. This has shown a high reperfusion rate and confirms that the principle of bolus dose administration is worthy of further study. The aim of the present study is to determine the efficacy of an alternative regime of 20 mg, followed by two bolus doses of 20 mg given at 20 minute intervals to a total dose of 60 mg. This is an attempt to reduce the initial loading dose and to reduce the total dose given to try to reduce possible side-effects.

#### **OBJECTIVES**

1. To assess the effect of three boluses of rt-PA on the patency of coronary vessels at 30, 60 and 90 minutes in patients with acute myocardial infarction by invasive and non invasive methods.
2. To assess the pharmacodynamic profile of rt-PA when given in this dosage schedule.
3. To assess the effects of bolus rt-PA on blood rheology.

Twenty patients will be treated with three boluses of 20 mg, 20 mg and 20 mg. The maximum total dose of rt-PA for generalised use as an infusion is 100 mg.

## **STUDY POPULATION**

### **Inclusion Criteria**

(i) Patients with acute myocardial infarction defined as:

a) cardiac pain at rest lasting from 30 minutes up to 6 hours

b) ECG changes at presentation consistent with a diagnosis of acute myocardial infarction.

Normally this will include ST segment elevation of at least 2 mms in two precordial leads or ST segment elevation of 1 mm in two inferior leads. However, patients with ECG diagnosis of posterior myocardial infarction and right ventricular myocardial infarction will also be included.

(ii) Males and females.

(iii) Age 18 to 75 inclusive.

(iv) Patients who have no contra-indications for angiography.

- (v) Informed consent to participate.
- (vi) No previous history on ECG of acute myocardial infarction in same distribution.
- (vii) Patients weighing over 67 kg.

### **Exclusion Criteria**

- (i) Patients with ECG abnormalities that make it impossible to diagnose acute myocardial infarction. This will include patients with left bundle branch block and severe conduction defect abnormalities.
- (ii) Patients with any contra-indications to thrombolytic therapy:
  - Any bleeding diathesis
  - Major trauma or surgery within three months
  - Puncture of a non-compressible vessel within 10 days
  - Any major haemorrhage
  - Any patient on Warfarin or Coumarin anticoagulants
  - Any previous cerebrovascular accident including TIA
  - Any previous history of peptic ulceration
  - Any proliferative retinopathy
  - Women who are pregnant, lactating or menstruating
  - History of severe poorly controlled hypertension
  - Any additional contraindication that is felt by the clinician to be relevant at that time to bolus rt-PA

therapy.

#### **METHOD**

The patients will be assessed pre-study within the Coronary Care Unit of Stobhill General Hospital. Thrombolytic therapy has been administered as standard therapy since 1982, and this study will be conducted within the Unit using standard monitoring facilities and general nursing medical care as appropriate. Written informed consent will be obtained from patients, both in terms of administration of thrombolytic therapy, and also in terms of permission for performance of coronary arteriography. Where appropriate, and when available, the protocol and procedure will be discussed with any accompanying relatives. The first bolus dose of rt-PA will be given as soon as possible following diagnosis. Analgesia will be given as per standard practice, and at no stage will any routine antiarrhythmic, inotropic nor vasodilator therapy be withheld. A second bolus dose of rt-PA will be given 20 minutes after the first bolus, and a third bolus will be given in a further 20 minutes. Aspirin 150 mg will be given orally after the third bolus, and at 3 hours following dosing a bolus of heparin 5,000 units and an infusion of 1,000 units per hour will then be commenced with the rate of infusion being controlled using standard coagulation screens. The second or third bolus of therapy will not be administered if there are any contraindications such as the occurrence of bleeding,

cerebrovascular accident of if any surgical intervention is required.

Coronary arteriography will be performed as close as possible to 30 minutes after the commencement of rt-PA. An angiogram of the culprit vessel will be performed and angiography will be repeated at 60 and 90 minutes to determine coronary artery reperfusion. If the artery remains occluded, the responsible Consultant may give a further 40 mg, either by the intravenous or intracoronary route, according to what is felt best and appropriate management for the patient. An arterial sheath will be left in situ and coronary angiography will be repeated at 24 hours.

At all times the performance of angiography will be undertaken only if the patient's clinical condition is stable, and the investigation appropriate. Any formal medical or interventional therapy can be performed at any time at the discretion of the Consultant Cardiologist managing the patient's overall condition.

Standard 12 lead ECGs will be performed at time zero, 2, 4, 8 12 and 24 hours following dosing. Serial cardiac enzymes will be performed, including CPK, AST, ALT and LDH at 4, 12 and 24 hours following admission to confirm diagnosis of acute myocardial infarction. Routine haematology and biochemistry will be performed as required for patient

management, and a coagulation status will be performed to allow anticoagulation.

#### **PHARMACOKINETIC ASSESSMENT**

Serial blood samples will be taken to measure whole blood viscosity and blood samples to determine measurements of factions of thrombolysis and fibrinogenolysis, including measurement of d-Dimer and b-Beta 15-42 fragments. In addition to routine measurements of fibrinogen, plasminogen and alpha<sub>2</sub> anti-plasmin. Blood samples will be taken pre-bolus and at 10 and 20 minutes following the first and second boluses, then at 10, 20, 30, 60 minutes and at 2, 4, 8 and 24 hours following the final bolus. The total volume of blood will be 175-230 mls.

Standard electrocardiographic monitoring with Holter monitor will be performed for 24 hours, and this data will be analysed by Reynolds Pathfinder to correlate ST segment changes with the reperfusion data obtained from coronary angiography.

All clinical events will be monitored and the patient's follow up therapy will be undertaken as deemed appropriate by the Cardiologist.

The coronary arteriograms will be analysed blindly to assess the achievement of reperfusion, and to assess the relative degree. In addition, the morphological appearance of the coronary artery following reperfusion

will also be assessed using standard techniques, as previously performed in the European Collaborative Study for Tissue plasminogen Activator.

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**CONSENT FORM**

I .....of .....  
hereby consent to receive the new thrombolytic agent called  
rt-PA and to undergo the procedure of coronary  
arteriography, the nature and effect of which have been  
explained to me by Dr. .... I consent to the  
administration of a local anaesthetic for these purposes.  
I also consent to such further or alternative procedures  
which may be found to be necessary during the course of the  
arteriogram.

Date ..... Signed .....

I confirm that I have explained to the patient the proposed  
nature and effect of this therapy and the procedure of  
coronary arteriography.

Date ..... (Doctor's  
Signature) .....pa

**PATIENT INFORMATION SHEET: THROMBOLYTIC THERAPY**

A heart attack or a coronary thrombosis is the term used  
for a condition in which the flow of blood to the heart  
muscle is reduced to a degree that damage occurs. The  
reduction in blood flow is usually due to a blood clot or  
thrombus obstructing the arteries leading to the heart  
muscle. After a period of time, damage is irreversible and  
the course of the illness will follow the normal healing  
process. If a patient is admitted to hospital early  
enough, then treatment may be introduced which can dissolve  
the blood clot and can restore flow of blood to the area of  
muscle under threat of permanent damage.

The standard treatment for heart attack within the Coronary  
Care Unit of Stobhill Hospital is to give this therapy to  
dissolve the blood clot - that is thrombolytic treatment,

to those patients admitted in the early stage of coronary thrombosis to obtain coronary artery flow, and to reduce the degree of damage. This form of treatment cannot be given to patients who have a predisposition to bleeding, such as those with stomach or duodenal ulcers, or to patients who have had strokes or operations in the recent past.

Two standard drugs are available at present for thrombolytic therapy called Streptokinase and Tissue Plasminogen Activator. This latter drug has been shown to be very successful in dissolving blood clots, and has been used in a widespread manner in both United States and mainland Europe, as well as in the U.K. We use thrombolytic treatment only in those cases who should benefit, and who have no contra- indication. This treatment is in addition to all normal forms of treatment. Although we are studying Tissue Plasminogen Activator, its use will not prevent the introduction of any other treatment which is necessary for the underlying condition. To maximise the dose of Tissue Plasminogen Activator to be given to any patient, we are studying its effects on blood clotting, and therefore frequent blood sampling is taken from a small plastic tube inserted in the arm.

As a follow up to thrombolytic therapy to assess its success and to determine if any other procedures or treatment should be given, an x-ray test, called a coronary arteriogram, is often helpful and we are performing this as part of the present study. This requires the insertion of a little plastic tube into an artery, and therefore signed consent is required before the performance of this test. The actual method of the test will be explained separately by the Doctor who will perform the procedure at the time.

**APPENDIX IV**

**PUBLICATIONS ARISING FROM WORK OF THESIS**

Gemmill JD, Hogg KJ, Burns JMA, Rae AP, Dunn FG, Fears R, Ferres H, Standring R, Greenwood H, Pierce D, Hillis WS. Comparison of the pharmacokinetic properties of streptokinase and anistreplase in the treatment of acute myocardial infarction. *British Journal of Clinical Pharmacology*, 1991;**31**:143-47.

Gemmill JD, Hogg KJ, MacIntyre PD, Rae AP, Dunn FG, Hillis WS. A pilot study of the efficacy and safety of bolus administration of alteplase in acute myocardial infarction. *Brit.Heart J.* 1991;**66**:134-8.

Fears R, Ferres H, Glasgow E, Greenwood HC, Standring R, Gemmill JD, Hogg KJ, Burns JMA, Rae AP, Dunn FG, Hillis WS. Monitoring of streptokinase resistance titre in acute myocardial infarction patients up to 30 months after administering streptokinase or anistreplase, and related studies to measure specific anti-streptokinase IgG and fibrinolysis in vitro. *British Heart Journal* 1992 (in press).

Formal permission has been granted by the co-authors and publishers of these publications for the inclusion of data in this thesis.

# A pilot study of the efficacy and safety of bolus administration of alteplase in acute myocardial infarction

J D Gemmill, K J Hogg, P D MacIntyre, N Booth, A P Rae, F G Dunn, W S Hillis

## Abstract

**Objective**—To examine the efficacy, safety, and the pharmacokinetic profile of a bolus dose administration regimen of alteplase in the treatment of acute myocardial infarction.

**Design**—An open pilot study.

**Setting**—District general hospital.

**Patients**—33 suitable consecutive patients presenting within six hours of the onset of symptoms who satisfied the electrocardiographic criteria for acute myocardial infarction.

**Interventions**—Two intravenous boluses of 35 mg alteplase, 30 minutes apart.

**Main outcome measures**—Angiographic coronary patency at 90 minutes and 24 hours. Plasma alteplase concentration-time profile and pharmacokinetic analysis.

**Results**—Coronary patency at 90 minutes: 26 of 30 arteries (87%, 95% confidence interval (CI) 74–99%). Coronary patency at 24 hours: 24 of 29 arteries (83%, CI 69–97%). Mean (SD) plasma tissue plasminogen activator (t-PA) concentration reached 4434.8 (2117.8) and 4233.3 (2217.5) ng/ml within 10 minutes of each bolus and fell to 425.8 (288.3) ng/ml between boluses. The estimated peak concentrations at two minutes after boluses were 12 389 (8580) ng/ml and 10 811 (6802) ng/ml. The derived pharmacokinetic variables were volume of distribution 3.11 (1.89) l, clearance 21.3 (9.3) l/h, half life 5.9 (1.7) minutes.

**Conclusions**—This simple administration regimen achieved brief, high concentrations of plasma t-PA that were well tolerated. The regimen was associated with a high coronary patency rate at 90 minutes that was well maintained at 24 hours.

Thrombolytic therapy is now accepted as a standard treatment for acute myocardial infarction because studies have shown that it improves left ventricular function and reduces mortality.<sup>1-4</sup> The benefit of thrombolysis is improved by early treatment<sup>2,5</sup> and the re-establishment of coronary patency. Therefore, the ideal thrombolytic agent should be easily and rapidly administered by the intravenous route and should effectively and rapidly restore

coronary patency without adverse side effects. The ease of administration of the agent becomes increasingly important with the emphasis on immediate treatment on arrival in hospital and the possibility of starting treatment in the community.

Alteplase (recombinant tissue plasminogen activator) is successful in restoring coronary patency,<sup>6-11</sup> with higher patency rates than a conventional dose of intravenous streptokinase.<sup>6,7</sup> At present, the recommended dosage regimen for alteplase is a 10 mg bolus, with a tapering dose of 90 mg infused over three hours. This regimen was based on the apparent short half life of alteplase in the circulation,<sup>12</sup> but clearly this regimen is a cumbersome one to apply urgently.

We assessed the efficacy of two boluses of 35 mg alteplase administered intravenously 30 minutes apart in achieving angiographic coronary patency. We also studied the pharmacokinetics of this regimen.

## Patients and methods

Thirty three consecutive patients (23 men and 10 women, age range 40–74, mean age 56.3 years) were admitted to Stobhill Hospital coronary care unit and recruited into the study. Patients were eligible for inclusion if they presented with chest pain of at least 30 minutes' duration, could be treated within six hours of the onset of symptoms, and had electrocardiographic evidence of acute myocardial infarction (ST elevation >1 mm in two limb leads or >2 mm in two precordial leads). Patients were excluded if they were over 75 years of age, had a previous infarct in the same anatomical distribution, or had any of the recognised contraindications to thrombolytic therapy<sup>6</sup> or coronary angiography. The study protocol was approved by the local research and ethics committee.

Table 1 shows the baseline characteristics of the patients recruited. After the patients had given their written informed consent and the protocol had been discussed with accompanying relatives, the patients were given 35 mg alteplase intravenously over 30 seconds. This dose was repeated 30 minutes later. All patients received 150 mg aspirin orally immediately on entry to the study and daily thereafter. All patients were given an intravenous infusion of glyceryl trinitrate

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Table 1 Details of study population

Data	Result
Total	33
Sex:	
Male	23
Female	10
Mean age (yr)	56.3 (10.5)
Infarct related artery:	
LAD	12
RCA	15
LMCA	1
Cx	2
Angiography not performed	3
Mean (SD) time to therapy	208 (75) min
Mean (SD) time to first angiographic injection	49.4 (13.3) min
Mean weight	73.2 (12.8) kg
Dose of alteplase administered	70 mg
Mean dose of alteplase administered	0.984 (0.173) mg/kg

Cx, circumflex; LAD, left anterior descending; LMCA, left main coronary artery; RCA, right coronary artery.

starting at 0.25 mg/h unless there was a specific contraindication.

Coronary angiography was performed in the coronary care unit with an image intensifier (Siremobil 2N/2H) linked to a video tape recorder (JVC CR8200E). Details of this system have been described elsewhere.<sup>13</sup> The Seldinger percutaneous approach was used and a femoral sheath was left in situ. The coronary angiogram was started immediately after the second bolus of alteplase (30 minutes after the first bolus), and the infarct related coronary artery (as indicated by the admission electrocardiogram) was visualised as soon as possible after 30 minutes, then by selective injections at 60 and 90 minutes. The degree of perfusion was scored according to the Thrombolysis in Myocardial Infarction (TIMI) scale and reviewed by an independent experienced cardiologist: a score of 0-1 indicated non-patency and one of 2-3 indicated a patent artery.<sup>6</sup>

If the artery was occluded at 90 minutes, the study protocol allowed additional treatment to be given up to the total dose of 100 mg alteplase, as recommended by the product licence. The other coronary arteries were visualised in standard angiographic projections. The femoral sheath was left in situ and coronary angiography was repeated at 24 (8) hours after treatment to assess patency and reocclusion.

An intravenous heparin infusion was started in all patients 3-4 hours after onset of treatment, and the dose was titrated against the thrombin time to a therapeutic ratio of 2:3. No bolus of heparin was given at the start of alteplase treatment. In all patients heparin was stopped for a short period at the time of the second angiogram to allow removal of the

femoral sheath. Venous blood samples were collected via an indwelling venous catheter from the first 24 patients before treatment, and then 10, 20, 30, 40, 50, 60 minutes and 2, 4, 8, 12, 24 hours after the first bolus of alteplase, into sodium citrate. The samples were centrifuged immediately at 0°C, separated, and frozen at -40°C. Subsequent analysis for antigen was performed by an enzyme linked immunosorbent assay (ELISA).

## Results

### ANGIOGRAPHIC PATENCY

The infarct related artery was first visualised at 49.4 (13.3) minutes after the first bolus of alteplase. The artery was visualised within 55 minutes in 26 of 30 (86%) patients. In three patients an acute angiogram was not obtained: in one because of haemodynamic instability, in one because of death before 90 minutes, and in one it was not possible to inject the right coronary artery.

In three patients, although the entry criteria were satisfied, the serial electrocardiographic changes of acute myocardial infarction did not occur and cardiac enzymes were normal. In one of these patients the right coronary artery could not be visualised and this patient is not included in the angiographic data. In all other patients angiographic data were obtained within 90 minutes of start of treatment and the results presented are the patency rates of all patients in whom coronary visualisation was achieved at each time point. At the first injection of the infarct-related artery (49.4 (13.3) mins) 23 of 30 (77%, 95% confidence interval (CI) 61 to 92%) arteries were patent. At 90 minutes this had increased to 26 of 30 (87%, 95% CI 74 to 99%) arteries (table 2).

Two patients were given a further intracoronary dose of 30 mg alteplase after the 90 minute angiogram. In one there was intermittent reperfusion of the index artery during the period of angiographic observation. In the other, after occlusion of the left main coronary artery, there was penetration only of the circumflex at 90 minutes. In both patients, the arteries were patent at the 24 hour angiogram.

One patient did not undergo the 24 hour angiogram because of haematoma formation after splitting of the femoral sheath. At 24 hours, 24 of 29 arteries (83%, 95% CI 69 to 97%) were patent. Seven patients improved their angiographic appearance by at least one grade, with three achieving reperfusion. However, five deteriorated at least one grade with three arteries becoming reoccluded (10%, 95% CI 0 to 22%).

### ADVERSE EVENTS

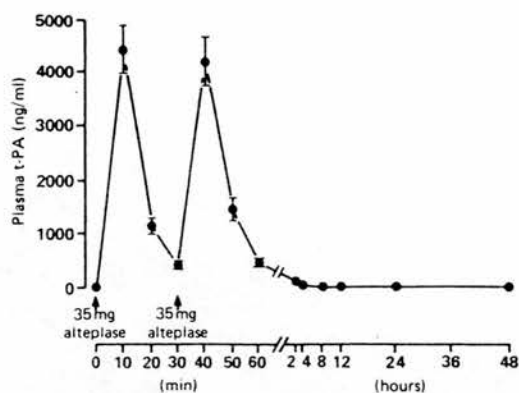
The only adverse events documented in our study group were bleeding complications. In five patients significant haematomata developed at the site of arterial access for coronary angiography. Two patients suffered minor coffee-ground haematemesis and one other bled from the gums. No patient required a blood transfusion or specific intervention.

One patient died during the study. This

Table 2 Angiographic details

	Number of patients			
	TIMI grade	First injection 49.4 (13.3) min	90 min	24-h
Non-patent	0	5	3	2
	1	2	1	3
Patent	2	15	18	10
	3	8	8	14
Reoccluded	—	—	0	0
Angiography not performed	—	3	3	4

ure Plasma t-PA concentrations (mean  $\pm$  SD) after two intravenous boluses of 35 mg alteplase.



patient had severe triple vessel disease and a previous myocardial infarction. Shortly after admission and treatment cardiogenic shock developed and he died despite inotropic support.

#### PLASMA t-PA CONCENTRATIONS

The mean (SD) pretreatment plasma t-PA concentration (20.1 (4.3) ng/ml) was significantly higher than our laboratory normal (3.6 (1.3) ng/ml). It rose to 4434.8 (2117.8) ng/ml within ten minutes of the first bolus of alteplase, fell to 425.8 (288.3) ng/ml before the second bolus, and rose again to a peak of 4233.3 (2217.5) ng/ml within 10 minutes of the second bolus (figure). Mean concentrations then fell rapidly and at four hours were about twice the pretreatment values (40.6 (28.6) ng/ml). After this t-PA concentrations were low, variable, and at approximately physiological values. This introduced considerable random variation in the pharmacokinetic fitting; therefore the data fitted were the baseline-subtracted concentrations confined to the four hour period after the administration of alteplase. This approach was adopted by others.<sup>12,14</sup>

The individual declines in t-PA concentration were fitted to one, two, and three compartment pharmacokinetic models, but a satisfactory fit was obtained only with the one compartment model. The pharmacokinetic results derived from this model were volume of distribution 3.11 (1.89) l (coefficient of variation (CV) 35.6 (26.7)%), clearance 21.3 (9.3) l/h (CV 14.1 (11.7)%), and half life 5.9 (1.7) min. The coefficients of variation are means (SD) of the coefficients of variation of the individual fitted data and express the quality of fit of the model (table 3). Using this model we estimated the plasma t-PA concentrations two minutes after the beginning of the intravenous boluses of alteplase, as a measure of the peak concentra-

tions achieved with this administration regimen. The peak concentration was 12 389 (8580) ng/ml two minutes after the first bolus and 10 811 (6802) ng/ml after the second bolus of alteplase.

#### Discussion

Our results show that a regimen of alteplase administered intravenously as two boluses of 35 mg at an interval of 30 minutes is effective in restoring coronary artery patency in acute myocardial infarction. To allow comparison with other patency studies, our primary end point of efficacy was patency at 90 minutes after treatment, but in addition we have angiograms earlier in the course of treatment to assess the time to reperfusion. At the time of first injection, at a mean of 49 minutes after treatment, 77% of our patients had patent index coronary arteries; this improved to 87% within 90 minutes, showing that patency is achieved rapidly and effectively.

Direct comparison with other studies of recombinant tissue-type plasminogen activator (r-tPA) is difficult because of several confounding factors. Other studies used different administration regimens with widely varying doses of two different preparations of rt-PA, with different specific activities.<sup>15,16</sup> In addition, whereas some workers studied patency others studied reperfusion, with a difference owing to sub-total coronary occlusion at presentation and spontaneous reperfusion.<sup>17</sup>

Verstraete and coworkers showed patency rates of 61% at 75–90 minutes after treatment using 0.75 mg/kg of double chain rt-PA over 90 minutes.<sup>11</sup> The same group using a 40 mg infusion of double chain rt-PA over 90 minutes achieved a coronary patency rate of 66% at 90 minutes.<sup>18</sup> Topol and the TAMI group achieved a patency rate of 68% with 70 mg of rt-PA over 90 minutes and one of 79% with a high dose (1.5 mg/kg) of alteplase over four hours in conjunction with a high dose of heparin.<sup>19–21</sup> In TIMI I workers used 80 mg of double chain rt-PA over three hours and found a reperfusion rate of 56%.<sup>6,7</sup> Williams *et al* used the same dose of 80 mg over three hours and found a similar reperfusion rate of 68%.<sup>8</sup>

Published reports of studies of bolus doses of alteplase are limited. Verstraete *et al* using single boluses of alteplase found that doses of 60 mg and 50 mg were associated with reperfusion rates at 90 minutes of 32% and 45% respectively, although a maximum dose of 70 mg achieved reperfusion in 72%.<sup>22</sup> Khan *et al* evaluated four boluses of double-chain t-PA of 25 mg given over 60 minutes and showed recanalisation in 11 of 14 patients; they suggested that this regimen achieves coronary patency more rapidly.<sup>23</sup> In our study, 70 mg divided into two boluses also achieved a high patency rate, and would be expected to be associated with fewer bleeding complications.<sup>24</sup>

In a recent small study Smalling *et al*, used rapid intravenous infusion of a weight adjusted dose of alteplase, and a median dose of 145 mg. They reported a 90 minute patency rate of 84%, which was significantly higher than the

Table 3 Pharmacokinetic variables

Variable	Mean	SD
Clearance (l/h)	21.4	9.3
Coefficient of variation of estimates of clearance (%)	14.1	11.7
Volume of distribution (l)	3.11	1.89
Coefficient of variation of estimates of volume (%)	35.6	26.7
Half life (min)	5.9	1.7

control group who received a conventional three hour infusion of 100 mg.<sup>25</sup> These results suggest that rapid administration of the thrombolytic agent achieves higher patency, and that it is the peak concentration of agent achieved that is important—an inference supported by our data.

The reported higher patency rates were achieved with higher weight adjusted doses of rt-PA. In our study the total dose of 70 mg alteplase represents a mean dose of 0.98 mg/kg. This contrasts with 1.5 mg/kg administered by Topol *et al.*<sup>20</sup> and the fixed doses of 150 mg used in the TIMI II study<sup>24</sup> or 100 mg used in the Australian National Heart Foundation Study.<sup>26</sup> The 100 mg dose is recommended by the manufacturers<sup>27</sup> and is now in regular use. Despite the lower dose we used, our patency rate of 87% at 90 minutes compares favourably with the published data.

Pharmacokinetic analysis of the plasma t-PA concentration-time profile after this bolus administration regimen of alteplase shows that very high, short lived concentrations of t-PA are achieved shortly after injection of the drug. The concentration of t-PA achieved at 10 minutes (4434.8 (2117.8) ng/ml) is 34% higher than the peak concentration attained by Seifried *et al.*, with 100 mg of single chain rt-PA delivered as a 10 mg bolus and 90 minute tapered infusion.<sup>14</sup> We found t-PA concentrations in excess of 2 300 ng/ml in all our patients and predicted concentrations two minutes after the boluses of alteplase in excess of 10 000 ng/ml. As would be expected, the period during which t-PA concentration was in excess of 1000 ng/ml is shorter with bolus administration than with prolonged infusion.

Despite the short duration of high plasma t-PA concentrations the reocclusion rate to 24 hours of three of 29 patients (10%) resembles previous experience.<sup>18</sup>

Our patients' pretreatment t-PA concentrations were raised, but 4–8 hours after alteplase concentrations fell to physiological values. After four hours in all patients, t-PA concentrations were low and variable, making fitting to pharmacokinetic models difficult. To eradicate this variable, we fitted the data for the first four hours only and used baseline-subtracted values. Despite taking frequent blood samples we were unable to define multiple phases of elimination, unlike others. Our data fitted our chosen model satisfactorily, as reflected in low standard deviations for each individually fitted variable. These individual standard deviations were expressed as coefficients of variation (CV) of their respective estimated variable and the CVs expressed as the mean. The mean CV of the estimate of mean clearance was 14% and that of the estimate of mean volume of distribution 36%, which indicates that the estimate of these mean variables was reliable. Previous studies have not reported details of the quality of fit of their models and therefore of the reliance that can be placed on their estimations.<sup>12 14</sup>

Our findings confirm the rapid clearance of t-PA from the circulation, with a half life of 5.9 (1.7) minutes and modest inter-patient

variability. These closely correlate with previous reports in patients, although they are somewhat slower than findings in normal volunteers with a lower total dose—possibly reflecting diminished cardiac output and hepatic blood flow in our patients with myocardial infarction. Our estimate of volume of distribution of 3.1 (1.9) l is very similar to previous estimates in both patients and volunteers.

Some workers have found a dose related rise in bleeding complications, in particular intracranial bleeding.<sup>24</sup> In our study, using a relatively small dose of alteplase, we saw bleeding complications in eight (24%) of 33, with five of these being related to arterial access for coronary angiography; there were three episodes of minor gastrointestinal bleeding (9%). No patients required transfusion or specific intervention. These results are similar to previously published data,<sup>6 9 10</sup> but our small study numbers do not allow firm conclusions to be drawn.

Our findings show that alteplase administered as two intravenous boluses of 35 mg 30 minutes apart reliably produced very high concentrations of t-PA in the plasma, which was rapidly cleared from the circulation and allowed a high coronary patency rate in acute myocardial infarction. The total dose administered was less than that used in many studies reporting high patency, yet reocclusion rates were similar to those reported before. The greater simplicity of administration and higher efficacy of this regimen may allow alteplase treatment to be safely started before admission to hospital with the advantages that such earlier administration would give.

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# comparison of the pharmacokinetic properties of streptokinase and anistreplase in acute myocardial infarction

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The pharmacokinetics of streptokinase (SK) and anistreplase in conventional dosage regimens of  $1.5 \times 10^6$  i.u. of SK infused over 60 min and 30 units of anistreplase over 5 min were studied in 24 consecutive patients presenting with acute myocardial infarction, using a functional bioassay to assess concentrations.

The two agents were found to have similar volumes of distribution (5.68 and 5.90 l), but SK was cleared significantly more rapidly than anistreplase, resulting in a shorter terminal phase half-life (0.61 vs 1.16 h) and a shorter mean residence time (0.76 vs 1.55 h).

**Keywords** streptokinase myocardial infarction pharmacokinetics anistreplase

## Introduction

The role of thrombolytic therapy in the treatment of acute myocardial infarction is well established, and the earlier treatment is initiated, the greater the clinical benefits (GISSI, 1986; ISAM, 1986; ISIS II, 1987; AIMS, 1988).

The ideal thrombolytic agent would therefore be easily and rapidly administered by the intravenous route and have pharmacokinetic properties allowing rapid onset of thrombolytic activity maintained for long enough to prevent early coronary reocclusion. Currently available agents and dosage regimens are not ideal, but pharmacokinetic studies may facilitate the development of optimal administration regimens.

Streptokinase binds to plasminogen (or plasmin) in the blood to form the complex SK-glu-plasminogen (-plasmin) which is an effective plasminogen activator (Anderson *et al.*, 1987). Anisoylated lys-plasminogen streptokinase activator complex (APSAC, anistreplase, Eminase, a trademark of Beecham Group p.l.c.) is a pro-enzyme giving rise to the plasminogen activator complex streptokinase-lys-plasminogen by deacylation. The activator complexes of the two agents are of comparable potency in the activation of plasminogen.

Deacylation of anistreplase occurs with a half-life of 105 min *in vitro* and is thought to be rate-limiting for the removal of anistreplase from the circulation, and to be slower than the elimination of SK-plasminogen or SK.

Previous pharmacokinetic studies are relatively few in number, were flawed by the paucity and timing of blood samples, and have used a variety of different assay methods. Despite these limitations, they have suggested that SK is rapidly eliminated with a concentration-time

profile variously fitted by mono- or bi-exponential functions (Kohler *et al.*, 1987, Mentzer *et al.*, 1986), while anistreplase is eliminated from the circulation much more slowly than SK (Been *et al.*, 1986; Kohler *et al.*, 1987). In this study, we have compared the pharmacokinetic properties of these agents directly using a functional bioassay.

## Methods

Twenty-four consecutive patients (18 male, six female, age range 48 to 72 years) with acute myocardial infarction as judged by strict ECG criteria, presenting within 6 h of onset of pain, and without contraindications to thrombolytic therapy were treated with either a conventional dose of  $1.5 \times 10^6$  i.u. of SK infused intravenously over 60 min or 30 u of anistreplase as a 5 min continuous intravenous injection. Blood samples were obtained from an indwelling venous catheter at frequent intervals up to 24 h after dosing (0, 6, 10, 20, 30, 45, 60, 75, 90 min, 2, 4, 6, 9, 12 and 24 h). They were collected into 0.1 volumes of 3.8% w/v sodium citrate, the plasma was separated immediately at 4° C and stored at -70° C.

Total fibrinolytic activity was measured as described by Been *et al.* (1986) and Nunn *et al.* (1987), and used as a functional bioassay of the plasma concentrations of the thrombolytic agents. The preparation of euglobulin fractions has been reported in detail elsewhere (Standring *et al.*, 1988). In brief, plasma samples were diluted with 0.011% v/v acetic acid and the resulting precipitates

**Table 1** Pharmacokinetic parameters

	Anistreplase (n = 12) Mean (s.d.)	SK (n = 12) Mean (s.d.)
$t_{\max}$ h	0.15 (0.07)	0.9 (0.21)**
$C_{\max}$ (M $\times 10^{-8}$ )	5.59 (2.22)	3.85 (1.18)†
<i>Volume of distribution (l)</i>		
Model-independent (V)	5.90 (1.91)	5.68 (2.29)NS
Computer-modelled ( $V_{ss}$ )	5.25 (1.49)	—
<i>Clearance (l h<sup>-1</sup>)</i>		
Model-independent (CL)	3.87 (1.52)	7.08 (2.91)*
Computer-modelled (CL)	3.72 (1.35)	—
<i>Terminal phase elimination half-life (h)</i>		
Model-independent $t_{1/2}$	1.16 (0.38)	0.61 (0.24)**
Computer-modelled $t_{1/2}$	1.15 (0.38)	—
<i>Time to half maximal fibrinolytic activity (h)</i>		
	1.12 (0.31)	0.48 (0.14)**
<i>Mean residence time (h)</i>		
	1.55 (0.48)	0.76 (0.31)**

† $P < 0.05$ , \* $P < 0.01$ , \*\* $P < 0.001$ , NS = Not significant.

were solubilized to give a 30-fold dilution of the original plasma. This dilution factor was found to abolish the interference from variable amounts of endogenous plasminogen and plasmin in the samples (Nunn *et al.*, 1987). Fibrinolytic activity was assayed by the lysis of fibrin plates prepared from human fibrinogen (containing 2  $\mu\text{g}$  plasminogen  $\text{mg}^{-1}$  fibrinogen) incubated at 37° C for at least 18 h. The plates were stained with bromophenol blue and lysis zones were measured with an AMS image analyser. Quadruplicate measurements were made at each time point and typical coefficients of variation ranged from 0.44 to 2.6% (mean 1.6%,  $n = 11$ ) for anistreplase and from 0.36 to 6.6% (mean 2.4%,  $n = 10$ ) for SK. The concentration of activator was calculated for each patient employing the appropriate standards diluted with autologous predose plasma, i.e. SK for SK treated patients and anistreplase for anistreplase treated patients. Standards were prepared using the patient's own pretreatment plasma to allow for any interpatient variability; the slopes of the standards ranged from 1.57 to 3.26 (mean 2.42) for SK, and 1.78 to 3.07 (mean 2.47) for anistreplase. SK and anistreplase standards gave linear responses with correlation coefficients of 0.9946 (percentage coefficient of variation, %CV 0.34) for SK ( $n = 12$ ) and 0.9938 (%CV 0.21) for anistreplase ( $n = 12$ ).

Internal standards were also included in each assay, in pretreatment, and 10 min to 2 h post-treatment samples, and processed concurrently. The average recovery for samples from the entire group of 12 patients was 91% for SK and 94% for anistreplase. The limit of reliable assay was  $0.08 \times 10^{-8}$  M (3.91 i.u.  $\text{ml}^{-1}$ ) for 10 SK treated patients and  $0.04 \times 10^{-8}$  M (1.95 i.u.  $\text{ml}^{-1}$ ) for the remaining two. For anistreplase the lower limit of assay was  $0.06 \times 10^{-8}$  M ( $7.81 \times 10^{-5}$  i.u.  $\text{ml}^{-1}$ ).

Plasma SK- and anistreplase-time data were fitted by one- or two-compartment models using non-linear regression as appropriate to each individual data set. Modelling was performed using the computer program, MODFIT, which employs a modified Danielson-Fletcher-

Powell algorithm (Allen, 1990). The same data sets were subjected to compartmental model-independent analysis calculating AUC using the linear trapezoidal method with extrapolation to infinity. Clearance (CL) and volume of distribution (V) were calculated by standard methods (Gibaldi & Perrier, 1982). The terminal phase half-life ( $t_{1/2}$ ) was also determined by regression analysis as was the time to loss of half the maximal fibrinolytic activity from the end of the dosing period. Instantaneous mean residence times (MRT), which compensate for the differences in the duration of dosing between SK and anistreplase, were determined by moment analysis (Riegelman & Collier, 1980).

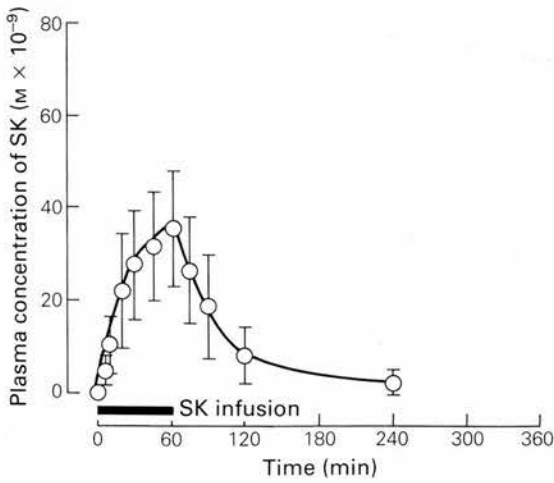
Pharmacokinetic parameters for anistreplase and SK (V,  $t_{1/2}$  and CL) were compared by one-way analysis of variance.

## Results

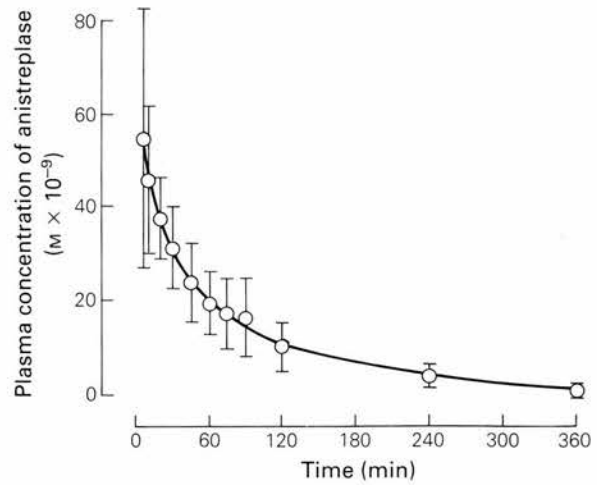
### Streptokinase

Derived pharmacokinetic parameters are summarised in Table 1, and mean concentration-time curves are shown in Figure 1. Concentration-time curves during and after infusion were analysed by model-independent methods only as the data could not be adequately fitted by a compartmental model. The terminal phase rate constant was determined using non-linear regression analysis with a weighting of concentration<sup>-2</sup>.

The maximum plasma concentration ( $C_{\max}$ ) occurred within 1.25 h ( $t_{\max}$ ) of the start of the infusion (mean 0.9 h). Subsequently concentrations declined rapidly to less than 15% of  $C_{\max}$  in all subjects by 4 h after the start of infusion, and in most subjects were below the limit of reliable measurement at this time. The post peak decline in concentration approximated to a mono-exponential fall (maximum % CV of the regression lines was 26%) as determined by MODFIT (Allen, 1989).



**Figure 1** Plasma concentrations of streptokinase (mean  $\pm$  s.d.) during and after intravenous infusion of  $1.5 \times 10^6$  i.u. of streptokinase over 60 min.



**Figure 2** Plasma concentrations of anistreplase (mean  $\pm$  s.d.) following intravenous injection of 30 u of anistreplase over 5 min.

The volume of distribution of SK was low, approximating to that of plasma proteins. In conjunction with a moderately rapid clearance this accounted for a short terminal phase half-life.

#### Anistreplase

The maximum total activity of anistreplase ( $C_{max}$ ) occurred within 20 min ( $t_{max}$ ) of the start of the 5 min intravenous injection (mean 0.15 h).

Activity fell to less than 15% of maximum by 4 h. In six of the 12 patients, an early rapid decline phase of the concentration-time curve could be delineated, conforming to a two-compartment model. In five subjects the data sets conformed to a one-compartment model. In one subject, neither model provided a satisfactory fit and these data were analysed by model-independent methods only. The derived parameters were similar regardless of which model was most appropriate, and were comparable with parameters derived from model-independent analysis.

Pharmacokinetic parameters are summarised in Table 1 and mean total activity-time curves are shown in Figure 2.

The volume of distribution of anistreplase and its deacylated product was relatively low, consistent with anistreplase and its metabolite being confined largely to the systemic circulation. Clearance was modest and in conjunction with a small volume of distribution resulted in a longer elimination phase half-life ( $1.16 \pm 0.38$  h) than that of SK.

#### Discussion

Thrombolytic therapy in acute myocardial infarction achieves infarct related artery patency, limitation of infarct size and improves mortality. Coronary reperfusion rates are highest when the therapy is administered early in the course of myocardial infarction (Kennedy *et al.*,

1985; Khalilullah *et al.*, 1984; Lew *et al.*, 1986; Weinstein, 1982), and is associated with greatest clinical benefits (AIMS, 1988; Anderson *et al.*, 1984; GISSI, 1986; ISAM, 1986; ISIS II; 1987, Simoons *et al.*, 1985). For earlier institution of therapy, a suitable thrombolytic agent must be administered in a simple regimen allowing easy administration, applicable even outside the hospital environment. The ideal regimen should therefore achieve a high early concentration, and resulting fibrinolytic activity (short  $t_{max}$  and high  $C_{max}$ ), a slow elimination phase, allowing maintenance of adequate concentrations from a single 'bolus' injection (long  $t_{1/2}$ , low CL) for an appropriate period of time, thus minimising early re-occlusion.

Early recommendations for the use of SK were for a prolonged low dose infusion, but more recent usage in myocardial infarction has adopted the now standard, but essentially empirical dose of  $1.5 \times 10^6$  i.u. over 60 min. The recommended dose of anistreplase was based on being equivalent to 1– $1.5 \times 10^6$  i.u. of SK. Following the administration of SK and anistreplase in their standard treatment regimens for myocardial infarction, anistreplase achieves a significantly earlier and higher peak concentration as would be predicted from its more rapid infusion.

In this study, we have found that SK and anistreplase both have a low volume of distribution, approximately twice that of plasma volume and similar to the volume of distribution of plasma proteins (Rowland & Tozer, 1980). This would be consistent with both agents behaving as proteins with no specific carrier mechanism.

The observed elimination phase half-life of SK of 0.61 h was similar to previous estimates based on fibrin plate lysis assay in acute myocardial infarction (Kohler *et al.*, 1987) and slightly longer than estimates based on other functional assays of 0.3 h (Martin, 1982) and 0.38 h (Mentzer *et al.*, 1986). These small differences may be accounted for by variation in assay specificity and pharmacokinetic analysis. Claims of a considerably longer terminal phase, with a half-life of 1.38 h (Fletcher *et al.*, 1958; Grierson & Bjornson, 1987), are based on radio-immunoassay or amidolytic assay methods for SK.

These methods do not differentiate between active and inactive SK fragments, or take account of *in vivo* deiodination. Similarly, the amidolytic assays based on the lysis of chromogenic substrate do not evaluate the fibrinolytic sites of the activator molecule, and also therefore cannot differentiate active and inactive SK fragments, or fragments bound by circulating inhibitors such as  $\alpha_2$ -antiplasmin. The results of previous studies based on the lysis of bovine fibrin are also problematic, in that this assay is particularly sensitive to endogenous human plasminogen.

In this study, both drugs have been assayed by the same fibrinolytic bioassay based on human fibrin plate lysis. It has been shown that administration of either thrombolytic agent does not completely deplete circulating plasminogen. Therefore, all of the SK present would be in its fibrinolytically active complexed form. Thirty-fold dilution of the euglobulin fractions eliminates the influence of endogenous plasminogen on the assay procedure (Fears, 1989). Therefore, this assay method

measures the functional moiety and is relevant to the clinical application of these drugs.

We have shown that SK and anistreplase have similar low volumes of distribution, but that SK is effectively cleared from the circulation twice as fast as anistreplase ( $7.08$  vs  $3.87$  l h<sup>-1</sup>,  $P < 0.01$ ). *In vitro* studies of the deacylation of anistreplase in human blood or plasma have shown a deacylation half-life of 1.76 h (Ferre 1987). Our results would therefore support the concept that the deacylation of anistreplase is the rate-limiting step in its elimination.

Both streptokinase as a 60 min infusion of  $1.5 \times 10$  i.u. (Verstraete *et al.*, 1985) and anistreplase as a 5 min injection of 30 units (Been *et al.*, 1985; Hillis *et al.*, 1987; Ikram *et al.*, 1986; Timmis *et al.*, 1987) are well-tolerated and effective in restoring coronary patency in acute myocardial infarction. In this study, we have confirmed that anistreplase in this dosage schedule achieves earlier higher and more prolonged levels of total thrombolytic activity.

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