Chapter I

General introduction and aims of the thesis

1. The origin of myocardial infarction

Already in ancient history it was noted that sudden pain in the chest could be a harbinger of death. The actual cause of death was not yet understood. First the circulation had to be described which was done by Harvey in the 17th century. Medical science developed slowly. Only at the beginning of the 20th century theories emerged on the pathophysiology of myocardial infarction (MI), which was defined as necrosis of heart muscle tissue due to persisting ischemia.

In 1910, Russian pathologists described five patients with acute MI of whom three showed coronary thrombosis at autopsy (Obraztsov, 1910). Subsequently, in 1912, Herrick wrote a publication on the syndrome of acute MI. He suggested that "hope for the damaged myocardium lies in the direction of securing a supply of blood .. so as to restore as far as possible its functional integrity" (Herrick, 1912). So it was hypothesized for the first time that obstruction of the blood stream was the cause of subsequent necrosis and dysfunction of a part of the heart. The issue of thrombosis did not receive much attention for several decades. Clinicians and pathologists continued to argue about the question whether coronary thrombosis was a cause or a consequence of MI (Roberts, 1972; Baroldi, 1976; Silver, 1980). The introduction of cardiac catheterization settled this dispute. Using this technique, a thrombus was shown in patients with symptoms and ECG signs of MI (DeWood, 1980). This confirmed the view that acute thrombotic occlusion was the cause of MI. In addition, Falk and Davies showed that focal arterial lesions, in particular a fissured atherosclerotic plaque, were the origin of the thrombotic process (Falk, 1983; Davies, 1985). Adherence and aggregation of thrombi at the site of the culprit lesion preceded the development of fibrin (Davies, 1979). Currently, the process of plaque rupture is more precisely understood (Richardson, 1989; Chesebro, 1991). This is depicted in Figure 1.

2. Management of myocardial infarction

Acute death in patients with MI is usually caused by extensive ischemia leading to pump failure, ventricular arrhythmias, in particular ventricular fibrillation, and cardiac rupture. Initially, attention focused on the reduction of secondary complications. During the last decade, however, emphasis in treatment of patients with MI has shifted to strategies aimed at reducing the extent of necrosis. This was based on improved understanding of the underlying mechanisms in evolving MI and supported by developments in the field of antithrombotic and thrombolytic treatment. In the following paragraphs major developments will be summarized.

2.1. Coagulation and fibrinolytic system

Clotting is a process that includes the conversion of soluble fibrinogen into fibrin by
Figure 1  Diagram of coronary artery plaque rupture and its possible consequences: A) complete occlusion of the lumen leading to regional transmural myocardial necrosis, B) subtotal or intermittent occlusion of the lumen which may result in unstable angina or non transmural infarction, C) complete endogenous lysis of the thrombus, D) organization of the thrombus which may lead to progression of coronary artery disease, or E) distal embolization (adapted from Chesebro, 1991).
the action of thrombin. Thrombin is formed by proteolytic cleavage of the proenzyme prothrombin which is produced by activated factor X (Xa) in the presence of calcium. Activation of factor X may occur by either one of two separate pathways, the extrinsic or the intrinsic pathway. In the extrinsic pathway, a tissue factor, released from damaged cells (tissue thromboplastin) activates factor X in the presence of factor VII (and calcium). In the intrinsic pathway, the contact of blood with a foreign surface such as collagen (or, in vitro, glass) activates factor XII. Cascadian activation of other coagulation proteins finally leads to activated factor X (Xa). Hemostatic platelet plugs in the injured vessel wall are stabilized by the fibrin network. Activated platelets release proaggregatory substances and catalyse the coagulation process. Thrombin, in its turn, is the most potent and physiologically important activator of platelets and is pivotal in the process of platelet recruitment into thrombus formation after vascular injury (Harker, 1992). The close interaction between platelet membrane receptors and the coagulation cascade is schematized in Figure 2.

In homeostasis, coagulation is in balance with fibrinolysis. Like coagulation, fibrinolysis is a complicated interplay between activators and inhibitors. In static blood, inhibition outweighs activity. If blood is allowed to clot in a test-tube and incubated at 37°C, the clot will remain solid for days or weeks (Fearnley, 1961). The fibrinolytic system is depicted in Figure 3.

2.2. Anticoagulants

Theoretically, there were several reasons to assume that anticoagulants might be beneficial in patients with MI: a) anticoagulants might halt or slow the progression of the development of a thrombus, b) anticoagulants might be expected to inhibit the subsequent formation of mural thrombi, and c) anticoagulants might reduce the incidence of deep venous thrombosis, and eventually subsequent pulmonary embolism, in immobilized patients. Antithrombotic treatment with heparin was propagated in the 1970s following publication of a number of randomized trials in the USA (Chalmers, 1977). However, the discussion continued and in 1984 one could read in Braunwalds’ textbook Heart Disease that "Despite several decades of evaluation, the results of the treatment of acute MI with anticoagulants are inconclusive" (Sobel, 1984). Subsequently, the role of heparin in conjunction or adjunction to thrombolytic therapy has gained in importance due to the supportive findings (MacMahon, 1988; Yusuf, 1988). Its intravenous use is currently advocated in conjunction with recombinant tissue-type plasminogen activator (rt-PA), whereas its use might be delayed for several hours in those patients with MI who have been treated with streptokinase (SK) (Prins, 1991). This will be discussed further in the chapters I and IV.

2.3. Plasminogen activators

In 1933 it was reported that filtrates of broth cultures of certain strains of hemolytic streptococci contained a substance capable of inducing fibrinolysis in human plasma clots (Tillett 1933, 1934). At first this substance was called streptococcal fibrinolysin. Christensen suggested that it be renamed into streptokinase (SK) because the streptococcal
Figure 2  Diagram showing activated platelets exposing specific receptor sites (left) and the intrinsic and extrinsic system of the coagulation cascade (right). A two-way interaction may occur: activated platelets enhance coagulation whilst thrombin is a most potent platelet activating agent. Ca$^{++}$ = calcium ion, vWF = von Willebrand factor, Ia = glycoprotein Ia, Ib = glycoprotein Ib, IIb/IIIa = glycoprotein IIb/IIIa (adapted from Chesebro, 1991).
Figure 3  Schematic representation of the fibrinolytic system
product was an activator rather than a fibrinolysin. Actually, it activated a "lytic factor" present in plasma globulins, which Kaplan identified as plasminogen (Kaplan, 1944). Another plasminogen activator found in human urine was called urokinase (Sobel, 1952). Additional research on components of the fibrinolytic enzyme system was carried out by Astrup and Permin. They showed that animal tissue contained an agent which could activate plasminogen (Astrup, 1947). This trypsin-like serine protease was t-PA. It was a poor plasminogen activator in the absence of fibrin but after binding to fibrin in a clot it activated plasminogen several hundred-fold more efficient than in the circulation. This characteristic was called fibrin-specificity. Investigators of the Genentech company succeeded in the manufacturing of t-PA by means of recombinant DNA technology (Pennica, 1983). rt-PA is known as alteplase or duteplase. Because of its specificity for fibrin and its nonallergenic structure, hope arose that bleeding and anaphylactic complications would decrease compared to those occurring after SK.

The knowledge, that SK-plasmin was a highly effective plasminogen activator guided research to manufacture a thrombolytic agent that contained such an enzyme complex. It was intended to control its action by a specific temporary chemical protection of its catalytic centre by inserting a p-anisoyl group. The complex would reactivate at physiological pH following spontaneous deacylation. Such a compound was developed by Beecham pharmaceutical research and it was named anisoylated plasminogen streptokinase activator complex (APSAC or anistreplase) (Smith, 1981).

As depicted in Figure 3, exogenous plasminogen activator converts plasminogen, which is a single-chain glycoprotein consisting of 790 amino acids, into plasmin. Actually, the Arg560-Val561 peptide bond in plasminogen is hydrolysed whereafter plasmin is formed. Lysine binding sites mediate its adherence to fibrin which is subsequently digested to soluble degradation products. Plasmin is the key enzyme of the plasma fibrinolytic system, which is predominantly inhibited by the serine protease inhibitor α2-antiplasmin (Collen, 1976, 1980).

2.4. Thrombolytic therapy

Administration of plasminogen activators is usually called thrombolytic therapy. This term will also be used in the following paragraphs and chapters.

2.4.1. Introduction of thrombolytic therapy, the first trials

The fibrinolytic properties of SK in patients with MI were demonstrated for the first time in the late 1950s (Fletcher, 1958, 1959). The first controlled clinical trial with the purified compound SK was conducted in Europe (European Working Party, 1971). In this trial patients with MI of less than 24 hours’ duration were randomly allocated to receive intravenous SK (initial dose 250,000 U, maintenance dose 100,000 U hourly) or heparin for 24 hours. After 24 hours and 6 weeks the mortality rate in patients treated with SK were significantly lower (10.6% and 19.0%, respectively) compared to patients given heparin (17.8% and 27.4%, respectively). However, these results were not generally accepted, reflecting doubts about the causal connection between coronary thrombosis and MI (Sherry, 1989). During the early 1980s these doubts diminished because another trial
again showed a significant reduction in mortality in patients with MI treated with i.v. SK (European Cooperative Study Group, 1979) whereas other investigators showed rapid recanalization of obstructed coronary vessels in patients with MI treated with intracoronary SK (Rentrop, 1981). As intracoronary thrombolysis is not widely applicable because of its dependence on catheterization facilities, intravenous administration was considered to be the only realistic approach (Verstraete 1966, 1985). Clinical investigation showed that 1.5 million U SK, administered intravenously during 1 hour, appeared appropriate for patients with MI (Schröder, 1983). Subsequently, this dosage regimen was used in nearly all clinical studies. Among these was the landmark TIMI-trial (TIMI, 1985). In this trial the efficacy of a 1-hour i.v. infusion of 1.5 million U of SK was compared with a 3-hour infusion of 80 mg t-PA in patients with MI. The primary end point was angiographic grade 2 or 3 patency at 90 minutes from start of the infusion, in patients who had grade 0 initially. Thus, patency was measured 30 minutes after infusion of SK and after 50 mg of the total dose of 80 mg t-PA. Sixty percent of 99 patients assigned to t-PA had 90-minutes grade 2 or 3 patency, as compared with only 40% of 115 patients assigned to SK (p <0.01). Because of this substantial, statistically significant difference in recanalization rate, phase I of the trial was stopped by the TIMI policy advisory board. Since this trial t-PA therapy was considered to be more efficacious than treatment with SK in the USA. Recently, this difference in time to reperfusion between t-PA and SK has been confirmed (Ganz, 1992).

In the early thrombolytic trials in patients with MI, mortality was chosen as the study end point. Because of the inverse relation between low residual left ventricular ejection fraction (LVEF) and post-MI mortality rate (Taylor, 1980; Norris, 1984), several subsequent trials focused on changes in this indicator of morbidity. In one of these studies it was found that patients with MI treated with SK had a significantly higher LVEF (57%) compared to those who were given placebo (54%) (ISAM, 1986). Another smaller study showed corresponding results: SK recipients had a 18 ml smaller end-systolic volume, indicating decreased dilatation of the heart after MI treated with SK (White, 1987). These investigators also found no differences between SK and rt-PA treatment with respect to preservation of LVEF (White, 1989). These studies suggest that thrombolytic treatment in patients with MI does not only reduce the mortality rate but also decreases morbidity as indicated by a higher residual LVEF.

2.4.2. Towards the thrombolytic era, placebo controlled megatrials

The "Schröder regimen" of SK administration was used in patients with MI in the first Italian megatrial (GISSI-1, 1986). In this trial, a 3-week mortality rate of 10.7% in the SK recipients was found compared to 13% in the control patients (reduction in risk 18%; p=0.002). Treatment was safe, as the incidence of life threatening bleeding was only 0.2%. The first randomized placebo-controlled mortality trial with rt-PA in patients with suspected MI was the Anglo-Scandinavian Study of Early Thrombolysis (ASSET). Patients received a bolus of 10 mg, followed by an infusion of 50 mg in the 1st hour and then 20 mg in each of the next 2 hours (or placebo). All patients received i.v. heparin. At 1 month the overall case fatality rates were 7.2% in 2516 patients given 100 mg rt-PA and 9.8% in 2495 patients given placebo, a relative reduction of 26%. Major bleeding was observed in 1.4% of the patients treated with rt-PA versus 0.4% in those treated with placebo. The
incidence of stroke was similar: 1.1% in the rt-PA group and 1.0% in the placebo group (ASSET, 1988).

The results of the APSAC intervention mortality study (AIMS, 1988) were also presented in 1988. In this randomized, placebo-controlled, double-blind trial 1004 patients with proven MI received 30 U of APSAC or placebo. Mortality after 30 days was 6.4% in patients treated with APSAC and 12.2% in those treated with placebo, a 47% reduction of relative risk. Adverse events were few. Unexpectedly, 5 cerebrovascular bleedings occurred in the placebo group versus 2 in those patients who received APSAC. The fourth thrombolytic megatrial, also published in 1988, was the ISIS-2 study. In this randomized study 17,187 patients with suspected MI received SK, aspirin 160 mg daily, both, or neither (placebo). SK alone and aspirin alone each produced a highly significant reduction in 5-week vascular mortality. The combination of SK and aspirin was significantly better than either agent alone. Absolute mortality rates after treatment with SK plus aspirin and placebo were 8% and 13.2%, respectively (ISIS-2, 1988). Reductions in the odds of early death in various trials are summarized in Figure 4.

2.4.3. The thrombolytic era, comparative trials and adjuncts to therapy

Thus, these thrombolytic drugs share the ability to reduce mortality in patients with MI, but they differ in several characteristics, especially speed of action, infusion time, fibrin specificity, and also price. Suggested superiority in efficacy or safety of one drug to the other led to comparative trials. With this purpose, 20,891 and 41,299 patients with MI were studied in GISSI-2 (and its international extension) and ISIS-3, respectively (GISSI-2, 1990; The International Study Group, 1990; ISIS-3, 1992). In these megatrials, all patients were given aspirin because this was shown to elicit an independent reduction of mortality in the ISIS-2 study. No statistical difference in mortality rate between the thrombolytic agents was found in these trials. In ISIS-3, stroke, including intracranial bleeding, was more common in patients treated with APSAC or rt-PA compared to those receiving SK therapy. Both GISSI-2 and ISIS-3 included a group of patients who were treated with twice daily s.c. heparin, initiated 12 and 4 hours, respectively, after infusion of the thrombolytic agent to meet criticism against rt-PA monotherapy. This attempt was not successful and the heparin controversy flared up (White, 1990a). The latest thrombolytic megatrial in patients with MI bears the acronym GUSTO (Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries) (GUSTO, 1993). In GUSTO, 41,021 patients were randomized over 4 treatment arms: SK (1.5 million U over 60 min) with either s.c. or i.v. heparin, front-loaded rt-PA (15 mg bolus, 50 mg over 30 minutes and an additional 35 mg over the next 60 minutes) plus i.v. heparin, and a combination of rt-PA (≤ 90 mg) with SK (1.0 million U) plus i.v. heparin. All patients received aspirin. After combining the s.c. and i.v. heparin branch of patients who received SK, 30-day mortality rates were 7.3, 6.3, and 7.0% for the SK, rt-PA and rt-PA/SK treatment arm, respectively. Subgroup analysis revealed that rt-PA therapy was superior to both of the SK regimens in patients with one (or more) of the following characteristics: age under 75 years, anterior myocardial wall localization or duration of chest pain shorter than 4 hours. A substudy recently showed that front-loaded
**Figure 4** Reductions in the odds of early death among patients treated within 6 hours: overview of currently available data from 5 large randomized controlled trials of thrombolytic versus control therapy in patients with MI (adapted from Granger, 1992).

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial name</th>
<th>Deaths/patients</th>
<th>Odds ratio (± 95% CI)</th>
<th>Odds reduction (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>active</td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Streptokinase</td>
<td>GISSI</td>
<td>495/4865</td>
<td>623/4878</td>
<td>23% ± 6</td>
</tr>
<tr>
<td></td>
<td>ISAM</td>
<td>50/842</td>
<td>61/868</td>
<td>16% ± 8</td>
</tr>
<tr>
<td></td>
<td>ISIS-2</td>
<td>471/5350</td>
<td>648/5360</td>
<td>30% ± 5</td>
</tr>
<tr>
<td>Anistreplase</td>
<td>AIMS</td>
<td>32/502</td>
<td>61/502</td>
<td>50% ± 16</td>
</tr>
<tr>
<td>Alteplase</td>
<td>ASSET</td>
<td>182/2516</td>
<td>245/2495</td>
<td>28% ± 9</td>
</tr>
<tr>
<td>Overall: any fibrinolytic</td>
<td></td>
<td>1230/14075</td>
<td>1638/14103</td>
<td>27% ± 3</td>
</tr>
</tbody>
</table>
rt-PA therapy was associated with earlier patency and higher LVEF which might explain the lower mortality (GUSTO Angiographic Investigators, 1993). The findings support the "open-artery" hypothesis which means that more rapid and complete restoration of coronary flow through the infarct related artery will result in improved LVEF and lower mortality among patients with MI (Braunwald, 1993).

3. Factors that determine the success of thrombolytic therapy

Apart from dosage, speed of administration and/or type of agent, several other factors may determine the success of thrombolytic therapy, especially a) morphology and localization of the culprit lesion, b) time delay to thrombolytic therapy, and c) hematological factors.

3.1. Morphology and localization of the lesion

One reason for failure of thrombolytic therapy may be that the composition of the obstruction is not susceptible to a thrombolytic agent. In other words, not a thrombus but something else is present, such as discharged atheromatous material from a cracked plaque or an intimal flap obstructing the coronary lumen. These rare occurrences have been called "plaque disasters" (Falk, 1991). Clots rich in platelets with few fibrin, and therefore probably less susceptible to fibrinolytic therapy, were also recognized as a cause of treatment failure (Fuster, 1988). Experiments in a rabbit model showed that such thrombi, compared to erythrocyte-rich clots, were intrinsically more resistant to thrombolysis (Jang, 1989). Depth and length of the underlying plaque fissure were suggested to be contributing factors (Richardson, 1989). Furthermore, occlusions in the anterior descending artery were recanalized more frequently than those in the left circumflex or right coronary artery and proximal occlusions showed higher opening rates than distal lesions after intracoronary ostial thrombolysis (Tendera, 1985).

3.2. Delay from onset of complaints to initiation of therapy

Duration of chest pain before seeking medical attention is of great predictive value for the outcome of thrombolytic therapy. The sooner patients are treated in the hospital, the greater the reduction in mortality compared to non-thrombolytic treatment. This was clearly shown by the pooled results of the megatrials GISSI-1 and ISIS-2. The absolute (odds) reduction in mortality in hour 1, hours 2-3, hours 4-6, and hours 7-12, amounted 6.5% (48.5%), 2.7% (25.4%), 2.5% (21.5%) and 1.3% (11.8%), respectively (Gersh, 1993). Time as a determinant for success of thrombolytic therapy will be discussed in chapter II.

3.3. Hematological factors

It was already known in the 1950s from in vitro experiments that failure or delay of clot lysis was due to the binding of SK to inhibitory plasma components or antibodies, thereby creating a complex which rendered no plasminogen activation. The origin of these antibodies in human plasma was sought in streptococcal infections which induced the
immunosystem to create anti-streptococcal antibodies. It took a long time before other blood constituents were found which played a role in the regulation of fibrinolysis, and possibly in the failure of thrombolytic therapy. In 1983, the presence of a naturally occurring, fast-acting inhibitor of t-PA, plasminogen activator inhibitor (PAI), was reported (Kruithof 1983,1984). The third factor that was identified as a possible factor that might impair fibrinolysis was lipoprotein(a) [Lp(a)]. After a striking homology was shown between the protein moiety apo(a) of Lp(a) and plasminogen (Berg,1963; McLean,1987; Eaton,1987), several authors suggested that Lp(a) can inhibit the binding of plasminogen to immobilized fibrinogen or to specific binding sites of the endothelial cell (Scott,1989; Harpel,1989). So both molecules may compete for the same binding site as schematized in Figure 5. These in vitro findings (Edelberg,1989; Rouy 1991), however, have not yet been confirmed clinically.

Thus, the plasma constituents anti-streptokinase antibodies (aSKa), plasminogen activator inhibitor (PAI) and lipoprotein(a) [Lp(a)], may play a role in the efficacy of thrombolytic therapy. These factors will be discussed more extensively in chapter III.
Figure 5  The endothelial cell fibrinolytic system. N-terminal Glu-plasminogen, Glu PLG; N-terminal Lys-plasminogen, Lys PLG; Lys Plasmin, Lys PM; plasminogen-binding site, PLG BS; tPA-binding site, tPA BS; α2-plasmin inhibitor, α2-PI; plasminogen activator inhibitor-1, PAI-1. 1: Conversion of Glu PLG to Lys PLG by surface-localized plasmin-like diisopropylfluorophosphate-sensitive serine protease. 2: Activation of plasminogen by tPA (adapted from Scott, 1989).
4. Aims of the thesis

In the following chapters the conclusions of our studies will be placed in a more general context. Three topics are under discussion which involve most of the aforementioned factors that determine the success of thrombolytic therapy. The first relates to delay from onset of symptoms to initiation of thrombolytic therapy (Chapter II). Special attention will be given to prehospital thrombolysis. Next, the significance of hematological parameters in the outcome of therapy will be addressed with attention focused on endogenous inhibitors of SK and plasminogen activation (Chapter III). Finally, the subject of early and late vessel reocclusion will be illuminated (Chapter IV). Following the summary and conclusions (Chapter V), the main studies, which have been published or are submitted for publication, are added as appendices. These include data on: a) logistical problems in prehospital thrombolysis (no. 1), b) a characteristic condition of the coagulation system referred to as "the systemic lytic state" (no. 2), c) measurement and clinical role of anti-streptokinase antibodies (nos. 3, 4 and 5), d) the contribution of plasminogen activator inhibitor (PAI) and lipoprotein(a) [Lp(a)] to failure of thrombolytic therapy (nos. 6 and 7), and e) the 3 months reocclusion rate after thrombolytic therapy with anistreplase (no. 8).