Abbreviations and acronyms

APSAC  Anisoylated Plasminogen Streptokinase Activator Complex (anistreplase) [Eminase™]

aPTT  activated partial thromboplastin time

aSKa  anti-streptokinase antibodies

AST  antistreptolysin titre

CCU  coronary care unit

ECG  electrocardiogram/electrocardiographic

ECSG  European Cooperative Study Group

ELISA  enzyme-linked immunosorbent assay

EMIP  European Myocardial Infarction Project

FPA  fibrinopeptide A

GISSI  Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico

GREAT  Grampian Region Early Anistreplase Trial

GUSTO  Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries

ISIS  International Study of Infarct Survival

i.v.  intravenous(ly)

Lp(a)  lipoprotein(a)

LVEF  left ventricular ejection fraction

MI  myocardial infarction

MITI  Myocardial Infarction Triage and Intervention

PAI  plasminogen activator inhibitor

PTCA  percutaneous transluminal coronary angioplasty

REPAIR  REPerfusion in Acute Infarction Rotterdam

RIA  radio-immuno assay

rt-PA  recombinant tissue-type plasminogen activator (alteplase) [Actilyse™]

s.c.  subcutaneous(ly)

SK  streptokinase [Kabikinase™/Streptase™]

TIMI  Thrombolysis In Myocardial Infarction

TAMI  Thrombolysis and Angioplasty in Myocardial Infarction

TAT  thrombin-antithrombin III complex

U  Unit(s)

vs  versus
Logistic Problems in Prehospital Thrombolysis

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Eur Heart J 1989:10;122 (abstract)

Abstract

In this study we compared efficacy and safety of prehospital with in-hospital thrombolytic treatment with anistreplase in patients with acute myocardial infarction (AMI). Three-hundred and fifty patients with chest pain were screened for eligibility by the municipal ambulance staff and/or the general practitioner. Patients were included in absence of contraindications and if the telephone-transmitted ECG showed AMI. In a 6 months 16 patients (5%) were eligible, but only seven were randomized. Age over 70 years, duration of chest pain for longer than 4 h and logistic problems were the major factors responsible for the low inclusion rate. The mean time spent at home with and without the ECG procedure amounted 38±14 and 14±8 minutes, respectively (p< 0.001). These results demonstrate that in a medium sized town prehospital delivery of intravenous thrombolytic therapy by paramedics and/or the general practitioner is not feasible, leads to unnecessary time delay and may therefore yield no clinical benefits.

Introduction

Thrombolytic therapy in patients with acute myocardial infarction (AMI) reduces cardiovascular morbidity and mortality and efficacy is increased by early administration (1). Often time is lost before therapy is initiated (2), but if the selection of patients was performed by a physician in a mobile care unit, administration of prehospital thrombolytic therapy might shorten the ischemic period (3). On the basis of these data, we designed a study to determine the efficacy and safety of therapy at home with anistreplase in patients with AMI. Because the ambulance staff in The Netherlands does not usually include a physician, we sought support of the general practitioner (GP) and the paramedical ambulance staff for selection purposes and initiation of thrombolytic treatment. In this paper we describe our experience with prehospital thrombolysis.

Methods

The study was performed in Groningen, The Netherlands, a town of 200,000 inhabitants, from July 1988 to January 1989. Prior to the study, the ambulance staff was extensively educated and trained and the GPs were asked for their participation.

If there was suspicion of AMI on the basis of a telephone call by a patient to the GP,
an ambulance, equipped with an ECG device (Marquette Electronics) and the study medication, was sent to the spot. Subsequently, the patient was subjected to a list of 20 questions which was preferentially checked by the GP or otherwise by a member of the ambulance staff. Inclusion criteria for the study were age less than 70 years, chest pain of over 20 minutes duration, but less than 4 h, unresponsiveness to sublingual nitroglycerin, and absence of contraindications for thrombolytic therapy. If the patient was eligible, a 12-lead ECG was made and transmitted by telephone for review to the hospital. ECG ST-segment elevation of over 0.1 mV in more than one of the standard leads or over 0.2 mV in more than two of the precordial leads was required before permission was given to proceed. Treatment was started with 100 mg prednisolone and 100 mg lignocaine. Subsequently, either 30 U of anistreplase (Eminase, Trade Mark of the Beecham Group plc) or placebo was given intravenously (i.v.) over 4-5 min in a double blind randomized fashion. After admission to one of the three local participating hospitals, the second vial of the study medication, containing either placebo or anistreplase, was infused. Thus, each randomized patient received anistreplase. Infusion of heparin was started 4-6 h after admission and continued for 48 h when coronary angiography was performed. The study was approved by the ethical committee, provided that no more than 20 min were lost due to inclusion procedures.

Results for continuous variables are presented as mean ± standard deviation. Student’s 2 sample t-test was used to assess differences between the pre- and in-hospital treated patients.

Results

During 6 month period 350 patients were screened. The mean ambulance travel time to the patient’s house after a telephone call was 10±7 min.

In only 24 cases (7%) was an ECG transmitted and an AMI was diagnosed in 16 (5%) of these recordings. The most important reasons for ineligibility were age over 70 years (over a third of the patients), chest pain of more than 4 h and maximal delay at home exceeded. Following diagnosis of AMI, nine of the 16 eligible patients were not randomized because of: telephone communication problems (four times); inability to achieve i.v. access (three times); chaotic situation (once); in the last case the reason is unknown. Consequently, no more than seven patients (2%), with a mean age of 57±7 years, who had complaints for 125±55 minutes, received the study medication. Patency of the affected vessel was found in six of these seven patients (86%).

For the 24 patients in whom an ECG was registered at home, the time from arrival of the ambulance to completion of the procedure, including review of the ECG, was 38±14 minutes. For the other patients, who were not eligible for the study, the time spent at home was 14±8 minutes, which was significantly shorter (p< 0.001). Transportation of a patient to the emergency room lasted 9±5 minutes, irrespective of inclusion.

Three patients experienced ventricular fibrillation prior to infusion of the study medication. Minor bleeding was seen in two and hypotension in one patient. One patient showed a mild allergic reaction. None of the patients died.

Discussion
In the prehospital studies published so far, a considerable reduction of the time prior to thrombolytic treatment of patients with AMI has been reported: 40-45 minutes (4-7), 60 minutes (8,9) and 73 minutes (10). Results concerning clinical benefits of this early intervention are ambiguous. In an earlier study, a higher left ventricular ejection fraction was shown in those patients who were treated less than 1.5 h after the onset of pain (3). However, this result was not confirmed in larger, more recent studies (4,7,8). Only one study showed a decrease of mortality in the prehospital treated patients (5). Nearly all prehospital thrombolysis protocols prescribed an ECG which was assessed on the spot by a physician (3,5,7,8), or, as in the REPAIR study, by a computer (6).

In our study, thrombolytic therapy at home was administered by the GP and paramedics following remote ECG assessment. In the field only 16 out of 350 patients (5%) had AMI and seven patients (2%) were treated. Because of these small numbers, the efficacy of prehospital versus in-hospital treatment cannot be compared. The low percentage of patients eligible for prehospital thrombolysis is in accordance with two similarly designed studies in which 107 out of 2472 patients (4%) (10) and 3 out of 85 patients (3.5%) (11) were eligible.

Due to the ECG procedure, eligible patients spent on average 24 minutes more at home. This time was well in excess of the mean transportation time of 9 minutes to the hospital.

Our study demonstrates that prehospital delivery of i.v. thrombolytic therapy by paramedics and/or GP is not feasible, leads to unnecessary time delay and may therefore render no clinical advantages. This may, in part, be due to the size of our town. In contrast to a metropolis, we do not experience regular traffic jams and the travel time to a hospital is short. Moreover, due to the low number of eligible patients, each paramedic dealt with too few patients with AMI to maintain competence of skills, a problem that was also noted by others (11). In our view, the conditions in Groningen are representative for many European medium-sized towns. In these circumstances, patients with chest pain must be delivered to hospital immediately, which is also the current opinion of the British Heart Foundation Working Group (12).

References


A Systemic Non-lytic State and Local Thrombolytic Failure of Anistreplase (Anisoylated Plasminogen Streptokinase Activator Complex, APSAC) in Acute Myocardial Infarction

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J Am Coll Cardiol 1990;15:3A (abstract)

Abstract

The relation between coronary thrombolysis and coagulation variables after administration of anistreplase (anisoylated plasminogen streptokinase activator complex, APSAC) was studied in patients with an acute myocardial infarction. Fifty-eight consecutive patients with acute myocardial infarction were given 30 U of anistreplase intravenously within 4 hours of the onset of symptoms. A fall in the plasma concentration fibrinogen level to < 1.0 g/l, within 90 minutes after administration of anistreplase was considered to reflect a systemic lytic state. Coronary angiography was performed 48 hours after thrombolytic treatment. The overall patency rate was 74% (43/58). Patency rates were significantly different in patients with a systemic lytic [83% (43/52)] and a systemic non-lytic state [0% (0/6)]. The absence of a systemic lytic state after anistreplase administration seemed to be highly predictive of the failure of coronary thrombolysis. Coagulation studies showed evidence of inhibition of anistreplase induced fibrinolytic activity which may explain the failure of thrombolytic treatment in patients with evidence of a systemic non-lytic state.

Introduction

Thrombolytic drugs reduced mortality in patients with acute myocardial infarction treated within 6, 12 or even 24 hours after onset of symptoms (1-3). When treatment was started within the first 4-6 hours after the onset of chest pain, reperfusion was shown in most of the infarct related coronary arteries. However, in up to 30-40% of the patients no reperfusion could be achieved (4). Failure of thrombolytic treatment has been reported irrespective of the drug used (4). The configuration of coronary obstruction may be an important determinant of the success of treatment (5), but inhibition of drug activity has never been ruled out.

In general, streptokinase and anistreplase (anisoylated plasminogen streptokinase activator complex, APSAC) caused comparable changes in hematological variables such as fibrinogen, plasminogen, and α2-antiplasmin during the first 24 hours after they were given (6). These changes were ascribed to systemic effects. Some patients, however,
showed no substantial decrease in plasma-fibrinogen after anistreplase or streptokinase administration (7). This suggests resistance to these drugs. It has been suggested that a systemic lytic state, defined as a low plasma concentration of fibrinogen after thrombolytic treatment, is a prerequisite for local thrombolytic efficacy (8).

To investigate the possibility of drug resistance as an explanation for failure of thrombolytic treatment, we performed a retrospective study to assess the relation between the systemic fibrinolytic effects and the local efficacy of anistreplase in patients with acute myocardial infarction.

**Patients and methods**

**Patients:** We studied 58 consecutive patients (47 men, 11 women), mean age 57 years (range 34-71), who presented within 4 hours of the onset of chest pain. Selection criteria for thrombolytic treatment included the presence of characteristic symptoms of myocardial infarction and ST-segment elevation of at least 0.1 mV in one or more of the standard leads or at least 0.2 mV in two or more of the precordial leads in a 12-lead electrocardiogram and the presence of symptoms unresponsive to sublingual glyceryl trinitrate. We excluded patients with contraindications for thrombolytic treatment and those who had been treated with streptokinase or anistreplase within the previous 6 months.

**Study Protocol:** Patients were treated with 30 U of anistreplase (Eminase™, SmithKline Beecham) administered intravenously in 4-5 minutes. Infusion with heparin (30,000 U in 24 hours) was started 4-6 hours after thrombolytic treatment and was continued until an adequate level of anticoagulation had been achieved with oral acenocoumarol, which was started after 48-72 hours. To assess patency of the infarct related artery, coronary angiography was performed 48 hours (range 36-60) after the administration of anistreplase in all patients. In the first 30 consecutive patients patency was also assessed after 90 minutes (range 1 to 3 hours). Patency was documented according to the score used in the thrombolysis in myocardial infarction (TIMI) trial (9). Scores of grade 0 or 1 indicated occlusion of the infarct related vessel and grades 2 and 3 patency.

**Coagulation analyses:** Coagulation and fibrinolytic variables were studied immediately before and 90 minutes and 48 hours after anistreplase administration. Venous blood samples were collected on ice in a 1/10 volume 3.05% trisodium citrate for measurements of fibrinogen, plasminogen, α2-antiplasmin, reptilase time and euglobulin clot lysis time. Assays were performed immediately or plasma was stored at -80°C for analysis later. Fibrinogen was measured according to the method of Clauss (10). Plasminogen and α2-antiplasmin assays were performed with a synthetic chromogenic substrate (Kabi) according to the method of Friberger et al (11). Reptilase time was determined by the method of Soria et al. (12) and euglobulin clot lysis times by the method of Buckell (13). The assay for fibrinogen/fibrin degradation products was carried out on serum collected at the times mentioned above with a latex agglutination kit (Wellcome) according to the method of Pitcher (14).

A systemic lytic state was defined as a decrease of the plasma concentration of fibrinogen to below 1.0 g/l, measured 1.5 hours after the administration of anistreplase.

**Statistical analysis:** Plasma concentrations of fibrinogen, plasminogen, and α2-
antiplasmin were expressed as mean (SD). Statistical comparisons between patients showing a systemic lytic state and a systemic non-lytic state were performed by means of the Student’s t-test for independent samples. Comparisons within the groups were made with the paired Student’s t-test.

Measurements of reptilase time, euglobulin clot lysis time, and fibrinogen/fibrin degradation products were expressed as median (range). Patient groups were compared by the Mann-Whitney U/Wilcoxon rank sum test. Differences within the groups were tested by the Wilcoxon matched paired signed ranks test. We used Fisher’s exact test to compare the result of treatment in terms of patency and the presence of a systemic lytic state. A two-tailed p-value of <0.05 was regarded as statistically significant.

Results

Coagulation data: Fifty-eight patients were retrospectively classified into two groups. Fifty-two showed a systemic fibrinolytic state and in six patients plasma fibrinogen did not decrease below 1.0 g/l. Initial values of fibrinogen, plasminogen, α2-antiplasmin, reptilase time, euglobulin clot lysis time, and fibrinogen/fibrin degradation products were similar in the two groups (Table I).

After 90 minutes, fibrinogen, plasminogen, and α2-antiplasmin concentrations were significantly reduced in both the lytic and the non-lytic groups. Mean plasma concentrations of fibrinogen in the lytic and the non-lytic groups were 0.0 g/l and 2.3 g/l (normal range 1.7-3.5); of plasminogen 11% and 57% (normal range 70-130); and α2-antiplasmin 4% and 35% (normal range 90-130) respectively. These differences were statistically significant. Individual values for fibrinogen in the six non-lytic patients before and 90 minutes after treatment with anistreplase were: 3.3 vs 2.7; 2.7 vs 2.3; 2.4 vs 1.1; 3.0 vs 2.2; 2.4 vs 1.8, and 3.7 vs 3.6 g/l respectively. The reptilase time was considerably prolonged in the lytic group from 19 to 109 seconds, but did not change in the non-lytic group (19 vs 24 seconds). Euglobulin clot lysis time was shortened from >120 before to <10 minutes after the administration of anistreplase in both groups (normal value >120 minutes). Serum concentrations of fibrinogen/fibrin degradation products remained within normal ranges (<8 μg/ml) in the non-lytic group, whereas they were considerably increased in the lytic group (median value >256 μg/ml).

These changes declined after 48 hours. At that time mean plasma concentrations of fibrinogen, plasminogen, and α2-antiplasmin were still significantly lower in the lytic group, and, with the exception of fibrinogen, below the normal ranges. Values for reptilase time, euglobulin clot lysis time, and fibrinogen/fibrin degradation products were normal or almost normal.

Patency: Ninety minutes after anistreplase administration angiography showed patency in 20 (67%) of 30 patients. No early reocclusion occurred in these patients. Overall patency at 48 hours was achieved in 43 (74%) of 58 patients. The patency rate was 83% (43/52) in the patients showing a systemic lytic state and 0% (0/6) in those showing a non-systemic lytic state (Table II). The relation between systemic non-lytic state and non-patency of the infarct related vessel was statistically significant (p <0.001).
### Table I  Coagulation variables of all patients stratified according to fibrinolytic state

<table>
<thead>
<tr>
<th>variable</th>
<th>lytic</th>
<th>non-lytic</th>
<th>p-value&lt;sup&gt;@&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>fibrinogen (g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>3.1 (0.96)</td>
<td>2.9 (0.48)</td>
<td>NS</td>
</tr>
<tr>
<td>1.5 h after</td>
<td>0.0 (0.15)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.3 (0.78)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>48 hrs after</td>
<td>2.5 (0.68)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.2 (0.84)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>plasminogen (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>97 (18)</td>
<td>104 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>1.5 h after</td>
<td>11 (13)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>57 (9)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>48 hrs after</td>
<td>55 (13)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>78 (12)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>α&lt;sub&gt;2&lt;/sub&gt;-antiplasmin (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>before</td>
<td>93 (14)</td>
<td>90 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>1.5 h after</td>
<td>4 (5)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>35 (2)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>48 hrs after</td>
<td>80 (16)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>99 (8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>reptilase time (sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>19 (10-27)</td>
<td>19 (18-20)</td>
<td>NS</td>
</tr>
<tr>
<td>1.5 h after</td>
<td>109 (44-201)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>24 (18-31)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>48 hrs after</td>
<td>19 (15-23)</td>
<td>20 (19-21)</td>
<td>NS</td>
</tr>
<tr>
<td>euglobulin clot lysis time (sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>&gt;120 (&gt;120)</td>
<td>&gt;120 (&gt;120)</td>
<td>NS</td>
</tr>
<tr>
<td>1.5 h after</td>
<td>&lt;10 (&lt;10)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;10 (&lt;10-15)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>NS</td>
</tr>
<tr>
<td>48 hrs after</td>
<td>&gt;120 (95-&gt;120)</td>
<td>&gt;120 (&gt;120)</td>
<td>NS</td>
</tr>
<tr>
<td>fibrinogen/fibrin degradation products (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>&lt;8 (&lt;8)</td>
<td>&lt;8 (&lt;8)</td>
<td>NS</td>
</tr>
<tr>
<td>1.5 h after</td>
<td>&gt;256 (&lt;8-&gt;256)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;8 (&lt;8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>48 hrs after</td>
<td>36 (&lt;8-&gt;256)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>&lt;8 (&lt;8)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<sup>*</sup> p <0.01 vs baseline, <sup>#</sup> p <0.05 vs baseline, <sup>@</sup> p value for between group comparison.

Fibrinogen, plasminogen and α2-antiplasmin expressed as mean (SD). Values of reptilase time, euglobulin clot lysis time and fibrinogen/fibrin degradation products as median (range).
**Table II**  Relation of coagulation variables to patency of infarct related vessel 48 hours after treatment with anistreplase

<table>
<thead>
<tr>
<th></th>
<th>lytic state</th>
<th>non-lytic state</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>patency</td>
<td>43</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>non-patency</td>
<td>9</td>
<td>6</td>
<td>0.00012</td>
</tr>
<tr>
<td>total</td>
<td>52</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The predictive value of a systemic lytic state for the efficacy of thrombolytic treatment with streptokinase, urokinase, or anistreplase was the subject of several previous studies. White et al. did not find that systemic hematological markers of fibrinolysis were helpful in explaining the success or failure of intracoronary thrombolysis (15). In contrast, Rothbard et al. showed a close relation between a systemic lytic state and reperfusion of the infarct related vessel (8). Burket et al. stated that a systemic lytic state, rather than being considered an adverse effect of treatment, might serve as a reasonable clinical goal when thrombolysis is attempted (16). Lew et al. showed that high residual fibrinogen concentrations identified patients in whom thrombolytic treatment was relatively ineffective (17). It is difficult to compare the results of these studies. In the first three studies streptokinase or urokinase was given, whereas in Lew et al’s study streptokinase was administered intravenously. The dosages of streptokinase varied widely as did the interval between onset of chest pain and thrombolytic treatment. Finally, a systemic lytic state was defined differently in these studies -as a reduction in fibrinogen of at least 50% (15), at least 10% (8) or to below 0.5 g/l (17), and Burket et al. did not define a cut off point (16). Marder et al. studied 106 patients treated with streptokinase or anistreplase (7). A systemic lytic state was defined as a fall of >20% in plasma fibrinogen concentration. In 4 of the 58 patients treated with 30 U of anistreplase a systemic non-lytic state was found and none of these patients achieved reperfusion. None the less, no statistically significant relation between a systemic non-lytic state and failure of reperfusion was found. In the remaining 48 patients treated with a low dose of intracoronary streptokinase there was also no statistical correlation. Despite the presence of a systemic non-lytic state reperfusion occurred in 10 patients. This discrepancy was partly explained by local thrombolytic effects of intracoronary streptokinase.

In our patients we regarded a systemic lytic state as being likely if the concentration of plasma fibrinogen was <1.0 g/l 90 minutes after the administration of anistreplase. This value was chosen because it is commonly accepted as the hemostatic concentration of fibrinogen (18). In the lytic group there was an almost complete depletion of fibrinogen, plasminogen, and α2-antiplasmin, associated with a short euglobulin clot lysis time, considerably prolonged reptilase time, and high concentration of fibrinogen/fibrin degradation products. In both the lytic and the non-lytic patients there was a comparable shortening of the euglobulin clot lysis time. Euglobulin clot lysis time reflects the fibrinolytic activity of plasma after inhibitors have been removed. Apparently, the fibrinolytic system was activated by anistreplase in all patients. The moderate decrease in plasminogen and α2-antiplasmin in the non-lytic group also accorded with activation of the fibrinolytic system. Because neither reptilase time nor the concentration of fibrinogen/fibrin degradation products changed, whereas the fibrinogen concentration decreased but remained within normal ranges, it seems likely that inhibition was responsible for the limited expression of fibrinolytic activity.

None of the patients in the non-lytic group showed reperfusion of the infarct related vessel. Thus fibrinolytic inhibition seems to be restricted not only in terms of systemic effects but also for local thrombolytic failure. Initial plasma concentrations of α2-antiplasmin plasma were similar in both groups. Therefore, it is unlikely that this potent
inhibitor is responsible for the supposed fibrinolytic inhibition. Anti-streptokinase antibodies from earlier treatment with streptokinase or anistreplase can be excluded because none of the patients had previously received one of these drugs. There may have been naturally occurring anti-streptokinase antibodies (19), but they were not sought in our patients.

A systemic non-lytic state 90 minutes after the administration of anistreplase in a proportion of patients with myocardial infarction predicted failure of thrombolysis. The absence of systemic and local fibrinolytic activity was probably due to fibrinolytic inhibitors. These compounds are currently under investigation. The reported findings are relevant not only to explain the mechanism of thrombolytic failure but may also have implications for clinical practice. A simple and rapid laboratory test to detect thrombolytic failure of anistreplase would lead to the option of additional treatment.

Acknowledgment

We thank Beecham Research Laboratories, The Netherlands, for providing us with the thrombolytic drug (anistreplase, Eminase™).

References


**Rapid Enzyme Immunoassay of Anti-Streptokinase Antibodies in Human Plasma**

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Thromb Haemost 1991;65:1268 (abstract)

**Abstract**

A simple enzyme immunoassay for determination of anti-streptokinase antibodies (aSKa) in plasma is described. Commercially available reagents have been used for the assay, which is calibrated with a reference preparation of aSKa containing 100 AU/ml. The assay is specific and reproducible with a variation coefficient of 4.8%. In healthy individuals a broad range of values between 4 and 291 AU/ml was observed with a large difference between the mean and median value (55 and 27 AU/ml, respectively). Data from a study on 21 patients with myocardial infarction treated with the streptokinase derivative anistreplase suggest that a high titre of aSKa before treatment is associated with failure of thrombolytic therapy. The assay procedure can be shortened to 0.5 h to screen patients for a high aSKa level. This assay allows a more routine assessment of aSKa in the clinic.

**Introduction**

Streptokinase (SK), a protein produced by group C β-hemolytic streptococci, is now routinely used in thrombolytic treatment of myocardial infarction (1-4). The mechanism by which SK restores patency in occluded vessels is based on formation of a complex between SK and circulating plasminogen that acts as an activator of both free and thrombus-bound plasminogen, resulting in dissolution of the thrombus by formed plasmin (5,6). An acylated form of the complex, p-anisoyl plasminogen streptokinase activator complex (APSAC, anistreplase, Eminase™) has been introduced as a second generation thrombolytic agent.

Though early administration of SK or APSAC significantly reduces the mortality due to myocardial infarction, reperfusion studies have shown that 20-40% of patients fail to respond, for reasons that have remained largely unclarified (3,4,7,8). One interesting but still controversial explanation is that high levels of circulating antibodies to SK may reduce the efficacy of the thrombolytic therapy (8-11).

Circulating anti-SK antibodies (aSKa) are found in most people. They are probably induced by Streptococcal infections (11,12) which might also explain the considerable
variation in aSKa levels observed previously (2,8,13). In general these levels have been measured by a functional assay, the SK-resistance (SKR) test (1,9-11) which is based on neutralization of SK activity in a clot-lysis system. Though this assay can be performed rapidly, it demands appreciable technical skill to obtain reproducible results and also its specificity is limited. Alternatively, aSKa has been measured by radioimmunoassay (8,13) which requires a long assay period, radioactive reagents and expensive instruments, thereby hampering its routine application in clinical laboratories. A non-radioactive immuno assay has also been described, however the assay was only poorly characterized (12).

In this report we describe in detail a rapid and simple enzyme immunoassay of aSKa (14,15), which has been used to assess aSKa levels in healthy individuals and patients with myocardial infarction treated with anistreplase.

Materials and Methods

Materials

Microtiter plates of high protein-binding capacity were obtained from Nunc, Copenhagen, Denmark. Bovine serum albumin (grade V) was obtained from Sigma Chemical Co., St Louis, MO, USA. Streptokinase was purchased from Behringwerke, Marburg, Germany (Streptase™: 100,000 IU; 133-174 mg or 750,000 IU; 150-160 mg) and from KabiVitrum, Stockholm, Sweden (Kabikinase™: 100,000 IU, 26-27 mg) who also supplied human fibrinogen. Eminase™ (anistreplase, APSAC) is a registered trademark of Beecham Pharmaceuticals, Great Burgh, Epsom, England. Human thrombin was purchased from the Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. Peroxidase-conjugated rabbit antibodies to human IgG (P214) and tablets of ortho-phenylenediamine dihydrochloride (OPD) were obtained from Dakopatts, Glostrup, Denmark. Tween 20 was purchased from Pierce Chemical Co., Rockford, IL. Other chemicals were of high purity and obtained from Merck, Darmstadt, Germany or BioRad Laboratories, Richmond, VA.

Plasmas were prepared from venous blood samples anticoagulated with 1:10 volume of a 0.109 M solution of trisodium citrate, pH 6.0. After centrifugation at 2200 x g, the supernatants were rendered platelet free by subsequent centrifugation at 16,000 x g (Eppendorf centrifuge 5415-C). Aliquots were stored at -80°C. Blood samples were obtained from healthy individuals and patients referred to the emergency ward of our hospital because of acute myocardial infarction. Normal plasma pools were made of plasma samples of 50-59 healthy hospital staff members and one of these pools was assigned as the reference preparation of aSKa.

Enzyme immunoassay of aSKa

The assay was performed similarly to standard ELISA procedures. First the wells of a microtiterplate were coated with 100 µl of a 10 IU/ml solution of SK (routinely Streptase™: 100,000 IU, 133-174 mg) dissolved in PBS (0.15 M NaCl, 0.01 M sodium phosphate pH 7.2) during 1 hr at 37°C. Afterwards the solution was aspirated and the
wells were washed and incubated with PBS+ (PBS supplemented with 0.35 M NaCl, 0.002 M EDTA, 1 g/l of bovine serum albumin and 1 ml/l of Tween 20) during 5 min at room temperature to block remaining binding sites, and washed again, before 100 µl of plasma sample dilutions in PBS+ were incubated in duplicate for 2 hr.

Thereafter non-bound material was removed and the wells were washed 3 times with PBS+. The amount of bound aSKa was determined by incubation with 100 µl of a solution of peroxidase-conjugated anti-human IgG diluted 1:3000 in PBS+ at room temperature for 1 hr.

After washing again, colour was developed by incubation with 100 µl of OPD/H₂O₂ reagent (8 mg OPD in 12 ml 0.065 M sodium phosphate, 0.035 M citric acid (pH 5.0), supplemented with 5 µl of a 30% solution of H₂O₂) for 5 min. The reaction was stopped by adding 150 µl of 3M H₂SO₄ and the absorbance read at 492 nm using a microplate reader.

The results were calculated using a standard dilution curve of reference plasma, which was plotted against the absorbance on a double-logarithmic scale. The reference plasma had an assigned value of 100 AU/ml. Plasma samples were tested in at least 2 dilutions in PBS+, routinely 1:100 and 1:200.

In the rapid "cito" procedure a pre-coated microtiterplate (5 IU of SK per well; keeping quality ≥1wk at 4°C) is used. This plate is incubated with 3 sample dilutions (1:100, 1:200, 1:400) together with 4 reference plasma dilutions (1:50, 1:100, 1:200, 1:500) for only 10 min in the first stage and with conjugate 1:500 for only 5 min in the second stage of the assay.

**Streptokinase resistance (SKR) test**

This assay was performed as described by Moran et al. (13). In short, 20 µl of a SK dilution in 0.9% NaCl was added to 160 µl test plasma and the mixture was vortexed immediately. Then 20 µl of a thrombin solution (50 IU/ml) were added and the mixture was vortexed again. Clot formation occurred within 30 sec and lysis was established after 10 min. The SKR titre is defined as the lowest plasma concentration of SK that results in complete lysis of the clot within 10 min and is expressed in IU/ml of SK (16).

**Patency assessment**

Patency of the infarct related vessel was assessed by coronary angiography performed 48 hr after thrombolytic therapy. According to the thrombolysis in myocardial infarction (TIMI) trial (17) scores of grade 0 or 1 indicated occlusion and scores of grade 2 and 3 indicated patency.

**Statistical analysis**

Correlation analysis was done by Spearman rank correlation test and a p-value < 0.01 was considered to indicate significance.

**Results**
Characterisation of the enzyme immunoassay of aSKa

The enzyme immunoassay of aSKa was performed using a typical ELISA incubation scheme, starting with immobilized streptokinase to capture the analyte, in casu aSKa, and with enzyme-labeled rabbit anti-human IgG antibodies for the final analysis. The calibration curve with reference plasma containing 100 AU/ml is sigmoidal on a double-logarithmic scale as shown in Figure 1. Parallel dilution curves were obtained with 2 other pooled plasmas containing 46 and 115 AU/ml of aSKa, respectively, and with most individual plasmas. Some individual plasmas showed non-parallelism at dilutions below 1:100 (i.e. at 1:50 to 1:10). This is probably due to interferences which are commonly encountered in immunoassays at low dilutions (18). Therefore, plasma samples were routinely tested at dilutions of 1:100 and 1:200 and the corresponding results (defined as differing less than 15%) were averaged. At these dilutions the detection limit of the assay is 1 AU of aSKa per ml of plasma.

Most experimental conditions of the assay were not very critical, i.e. SK could be used at a 5-fold higher concentration or after coating overnight at 4°C in stead of 1 hour at 37°C; also the amount of conjugate could be varied 2- to 3-fold with only minor adaptation of the color development time. The final conditions as described under Materials and Methods have been chosen based on the highest, most reproducible signal-to-noise ratio at the lowest costs. Under these conditions the inter-assay coefficient of variation for a sample containing 44 AU/ml of aSKa was determined to be 4.8% (n=6).

For rapid application, i.e. for patients who present with chest pain and electrocardiographic characteristics indicating acute myocardial infarction, the assay can be shortened to 0.5 hour to give quantitative values of aSKa in the range of 40 to 400 AU/ml (see Materials and Methods). The variation coefficient of this rapid "cito" assay for a sample containing 137 AU/ml of aSKa was found to be 1.7% (n = 6).

The specificity of the assay was established in five different ways. Firstly, preincubations of several plasmas with an excess of SK showed a reduction of the measured aSKa level of at least 50-fold. Secondly, the use of a SK preparation of a 7.5-fold higher specific activity or of SK obtained from another manufacturer did not affect the results. Thirdly, when SK as coating ligand was replaced by other proteins like bovine serum albumin and human fibrinogen, the analytes were unreactive in the assay (though the background was slightly higher). Fourthly, mixtures of plasmas with high and low aSKa titres in ratios of 3:1, 1:1 and 1:3 all gave nearly expected values: for nine mixtures the mean deviation was 1 AU/ml (range -7 to 7 AU/ml). Finally, in 3 patients having a high aSKa level, this level decreased by on the average 92% within 1.5 hr after treatment with the SK-derivative anistreplase (19) [see below].

Distribution of aSKa levels in a human population

The concentration of aSKa was determined in the plasmas of 63 healthy volunteers aged 18-59 years. A broad range of values was observed between the extremes of 4 and 291 AU/ml. The distribution, given as a histogram in Figure 2, was evidently not normal with
Figure 1  Calibration curve for the enzyme immunoassay of aSKa. The reference plasma containing 100 AU/ml of aSKa was diluted in PBS+ buffer and assayed under standard conditions as described under Materials and Methods. The standard dilution of 1:50 was taken to represent 100 AU/ml.
Figure 2  Distribution of plasma levels of aSKa in 63 healthy individuals.
a mean and a median value of 55 and 27 AU/ml, respectively. This was mainly due to some rather high plasma levels of aSKa: in 10% of the individuals aSKa levels were 5-fold higher than the median value.

**Correlation between aSKa level and SKR titre**

The aSKa level was compared with the SKR titre in 57 different plasmas as shown in Figure 3. There was a statistically significant correlation: $r=0.6975$, $p<0.001$. However, large individual discrepancies were noticed, i.e., a SKR titre of 24 IU/ml was found in plasmas containing 20-180 AU/ml of aSKa. Also three different normal plasma pools differed in their ratio of SKR titre to aSKa level, ranging from 0.30 to 0.78 (mean 0.48).

**aSKa levels in patients with myocardial infarction treated with anistreplase**

In a retrospective study we analyzed the plasma levels of aSKa in 21 patients with myocardial infarction before onset of the thrombolytic therapy. The results together with the angiographical findings with respect to patency or non-patency of the infarct-related vessel are shown in Figure 4. Three patients (14%) were found to have an aSKa level above 135 AU/ml, so 5-fold higher than the median normal value, and these patients did not respond to the thrombolytic treatment with anistreplase. The mean aSKa level in these patients dropped from 321 AU/ml to 25 AU/ml within 1.5 hr after administration of the anistreplase (results not shown, see ref. 19), which is most likely explained by a strong reactivity to the SK-derivative. Patency was demonstrated in the remaining patients, all having a lower aSKa level.

**Discussion**

The enzyme immunoassay of aSKa reported here is very simple and rapid to perform in any laboratory familiar with standard ELISA techniques. All reagents are commercially available and most of the assay conditions are not very critical. The "cito" procedure is rapid enough (0.5 hr) to be valuable for clinical practice. The assay seems specific and is accurate as indicated by variation coefficients of 4.8 and 1.7% for the standard and "cito" assay, respectively. In our opinion this assay is superior to the radioimmunoassay described by Moran et al. (13), not only because of its rapidity and elimination of radioactive materials, but also because incubation of plasma with free SK, resulting in a rapidly degradable SK-plasminogen complex to which aSKa have to bind, is avoided by the use of immobilized SK; also the use of protein A, which does not react with all subclasses of immunoglobulin G, is eliminated.

The results of the aSKa assay have been expressed in an arbitrary unit (AU/ml), relative to the aSKa concentration in a home-made pooled normal reference plasma. As three different plasma pools (each made of at least 50 individual plasmas) were found to vary considerably in their aSKa content (between 46 and 115 AU/ml), it is obvious that an international standard would be necessary to compare results between different laboratories. In the SKR test, a pooled plasma, containing 100 AU/ml of aSKa, has on average an inhibitory potential of 48 IU/ml of SK.
Figure 3  Correlation of SKR titre to aSKa level in 57 different plasmas.
Figure 4  Distribution of aSKa in patients with myocardial infarction before treatment with anistreplase and patency of the infarct related vessel 48 hr after the thrombolytic treatment.
The aSKa level in plasma was found to be significantly correlated to the SKR titre, though a wide scatter in individual ratios was observed. This is similar to what has been observed by others using different assay techniques to measure aSKa (7,13). Also a 2.6-fold variation of the SKR/aSKa ratio (0.30-0.78) was observed between three different normal plasma pools. A lower SK neutralizing capacity than expected from the aSKa level might be explained by the presence of low-affinity antibodies (which do not fully exert their effect within 10 min. in the SKR test) and by the presence of weakly- or non-neutralizing antibodies. Besides, interfering plasma factors may have affected (some) SKR titres and hence the ratios.

A useful application of the enzyme immunoassay of aSKa might be the screening of patients with myocardial infarction who will be treated with SK or anistreplase. In a retrospective study we analyzed 21 patients and found plasma levels of aSKa above 135 AU/ml (5-fold higher than the median normal value) in 3 (14%) of them; this frequency of high aSKa levels in the patients was in good agreement with that (10%) among healthy individuals. All three patients with high plasma aSKa levels failed to respond to thrombolytic treatment with anistreplase. In these patients we also observed a decrease of the mean aSKa level of 321 AU/ml to 25 AU/ml within 1.5 hour after treatment, which is likely to be due to reactivity of aSKa to the SK-derivative. The combined data strongly suggest that aSKa had neutralised anistreplase, at least partly. With a presumed plasma volume of 3000 ml, these patients would have a mean plasma SK neutralization capacity of 462,000 IU, which constitutes 31% of the administered dose of anistreplase (6).

Our findings support the hypothesis that high concentrations of aSKa can reduce the efficacy of anistreplase in vivo. However, Hoffmann et al. (8) did not find a correlation between a high aSKa level and failure of anistreplase therapy in a group of 32 patients. Whether this discrepancy is related to the radio-immunoassay they used to measure aSKa (vide supra) needs further investigation. The availability of the reported rapid "cito" aSKa assay would be relevant for clinical practice if high aSKa levels in patients are predictive for the outcome of anistreplase or SK therapy. In such patients either a higher dose or another thrombolytic agent like tissue-type plasminogen activator or urokinase might be preferred, primarily or additionally.

In conclusion, the simplicity, rapidity and reliability of the enzyme immunoassay of aSKa as well as its possible relevance for clinical practice make this assay valuable for clinical laboratories.

Acknowledgements

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References


Anti-Streptokinase Antibodies Inhibit Fibrinolytic Effects of Anistreplase in Acute Myocardial Infarction

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Thromb Haemost 1991;65:1095 (abstract)

Abstract

Twenty-one patients with acute myocardial infarction (MI) were treated with anistreplase (Eminase™) within 4 hours after onset of symptoms. Coagulation parameters and the IgG antibody level to streptokinase (aSKa) were determined in pretreatment blood samples and after 1.5 and 48 hours. Coronary angiography was performed after 48 hours. A systemic non-lytic state was characterized by plasma fibrinogen in excess of 1.0 g/liter 1.5 hours after therapy. A high pretreatment aSKa level was found in 3 of the patients (mean 321 Arbitrary Units/ml). In these patients no systemic lytic state was induced and coronary angiography showed non-patency of the infarct related vessel. When aSKa levels were within the normal range (mean 55 Arbitrary Units/ml), a systemic lytic state was always obtained and a high patency rate was seen (16/18 patients). Binding of aSKa to the streptokinase component of anistreplase was suggested by a temporary decline of its level following anistreplase administration. Thus, the pretreatment IgG aSKa level is of major importance to achieve a systemic lytic state and subsequent local thrombolysis when anistreplase and probably streptokinase, is used in patients with MI.

Introduction

Streptococcal fibrinolysin, later renamed streptokinase (SK), has been used widely in patients with acute myocardial infarction since publication of the GISSI (Gruppo Italiano per lo Studio della Streptochinasi nell’Infarto Miocardio) trial (1). However, this therapy is not always successful. Thirty to 40% failure of reperfusion has been reported (2). This may, among other causes, be due to systemic inactivation of the drug by immunoglobulin G antibodies to streptokinase (aSKa). These antibodies may be present as a result of previous infections with streptococci, or previous treatment with SK (3-5). Plasma of patients who had been treated with SK showed resistance to the action of a repeat dose in the course of 1 to 2 months after initial therapy (6). In the late 1950s, it was thought that local coronary thrombolysis was the result of a systemic lytic state. Two decades later, this point of view was advocated again by Rothbard et al. (7). They showed that a systemic
lytic state, as characterized by a fibrinogen decrease of >10% after SK administration, was related to the success of therapy. Recently, we described a strong relation between a systemic non-lytic state (fibrinogen >1 g/liter) and local thrombolytic failure of the SK-containing thrombolytic drug anistreplase in acute myocardial infarction (8). It was hypothesized that a systemic lytic state was not achieved, because of the presence of a high aSKa level. To verify this hypothesis, in the present study we determined the aSKa level in plasma of patients who had been treated with anistreplase.

Methods

In 21 patients (17 men, 4 women; mean age 56 years, range 34-70) with electrocardiographically documented acute myocardial infarction, an intravenous bolus injection of 30 U anistreplase (Eminase™, SmithKline Beecham) was administered ≤4 hours after the onset of chest pain. Adequate anticoagulation with intravenous heparin (30,000 U/24 hours) was begun 1-4 hours after thrombolytic treatment and continued for 48 hours. Subsequently, coronary angiography was performed, and patency of the infarct related artery was documented according to the score used in the Thrombolysis in Myocardial Infarction trial (9). Grade 0 or 1 was considered to reflect occlusion, whereas grade 2 or 3 was considered to indicate patency. Venous blood samples were obtained before treatment, and after 1.5 and 48 hours, and placed on ice in 1/10 volume 3.05% trisodium citrate for measurement of fibrinogen, plasminogen, α2-antiplasmin and aSKa. Fibrinogen was determined according to the method of Clauss (10); plasminogen and α2-antiplasmin were determined according to the method of Friberger (11). A decrease in the fibrinogen level to <1.0 g/liter 1.5 hours after administration of anistreplase was considered to reflect a systemic lytic state. Human aSKa was determined using a newly developed enzyme-linked immunosorbent assay (12). The assay was performed similar to standard enzyme-linked immunosorbent assay procedures using SK-coated microtiterplates. Plasma sample dilutions were incubated in duplicate for 2 hours, and then the nonbound material was removed. The amount of bound aSKa was quantified spectrophotometrically after 1-hour incubation with a peroxidase conjugated to anti-human immunoglobulin G. Results were calculated using a standard dilution curve of reference plasma with an assigned value of 100 Arbitrary Units/milliliter (AU/ml). In addition to plasma samples of patients, blood samples were obtained from healthy hospital staff members as a reference to determine aSKa levels in the population.

Results

After administration of anistreplase, levels of fibrinogen, plasminogen and α2-antiplasmin decreased by a variable extent. The patient group was dichotomized according to the fibrinogen level 1.5 hours after treatment, resulting in 18 patients being characterized with a lytic state and 3 with a non-lytic state. Sixteen of 18 lytic patients (89%) had a patent infarct related artery and aSKa levels before treatment in the range of 1 to 79 AU/ml (mean 42). The remaining 2 lytic patients had a non-patent coronary artery and aSKa levels before treatment of 51 and 54 AU/ml, respectively. In the 3 patients with a non-lytic state, the infarct-related vessel was found to be non-patent; the plasma of these
patients before treatment showed high aSKa levels (171, 343 and 450 AU/ml, respectively [mean 321 AU/ml]). Values before, and 1.5 and 48 hours after treatment are summarized in Table I and depicted in Figure I. One and a half hours after treatment with anistreplase, a decrease in the aSKa level was found in both groups of patients. The mean and median aSKa plasma levels in 63 healthy hospital staff members were 55 and 27 AU/ml, respectively, with a broad range of between 4 and 291 AU/ml. Thus, the aSKa levels in the systemic lytic patients corresponded to the mean value in healthy subjects.

Discussion

Since the late 1950s, the plasma of patients with thromboembolic disease was assayed for its content of SK inhibitory constituents (6); depending on the measured level, patients received a varying dose of SK. The concept of SK inactivation by aSKa was revived in 1984 when Lew et al. described clinical failure of SK in a patient with acute myocardial infarction due to a high titer of aSKa (13). That patient had only a minimal decrease in serum plasminogen and no decrease in serum fibrinogen after therapy; thus, in contrast to most other patients, neither a systemic lytic state nor local coronary thrombolysis occurred. Although the definition of a systemic lytic state varies among investigators, it generally corresponds to a certain degree of fibrinogen decrease after administration of SK. It was shown previously that a systemic lytic state is associated with successful therapy (7,8,14). In a Dutch study, it was observed that the degree of systemic lysis correlated inversely with the preexisting aSKa level, but a causal relation between a high preexisting aSKa level and unsuccessful thrombolytic therapy with SK or anistreplase, was not found (15).

In our study, we found a high aSKa level before treatment in 3 of 21 patients (14%) with acute myocardial infarction. These patients had relatively high levels of fibrinogen, plasminogen and α2-antiplasmin after administration of anistreplase, and coronary angiography showed non-patency of the infarct related vessel. When aSKa levels were in the normal range, a systemic lytic state was always obtained; a high level of aSKa apparently prevented the induction of such a state and subsequently, local thrombolysis. The binding of aSKa by the SK component of anistreplase was clearly shown by the temporary reduction in their levels after its administration. This corresponds to the virtual disappearance of aSKa due to treatment with SK, as was shown recently (16,17). Unsuccessful therapy may be avoided by the administration of a higher dosage of SK or anistreplase, or administering supplementary tissue-type plasminogen activator; assessment of the aSKa level before treatment in patients with acute myocardial infarction enables the identification of patients in whom this strategy should be considered. Several investigators used the radioimmunoassay of Moran et al. (18) to quantify the aSKa level. We developed an enzyme-linked immunosorbent assay for aSKa, which was originally performed in 3 hours (12). Simplification and shortening of the assay has made it possible to perform this quantification in 1 hour. A quicker procedure is currently being investigated so that routine clinical application would be more worth while. It is concluded that high aSKa levels may result in the failure of SK-containing thrombolytic agents, because a systemic lytic state is not induced; however, as was shown in 2 patients

Table I Patients dichotomised according to the fibrinogen level 1.5 hours after treatment with anistreplase. Mean values are expressed. Systemic lytic status represents fibrinogen
<1.0 g/L, and non-lytic state is characterized by higher values. Corresponding values of coagulation parameters, aSKa level, and number of patients with a patent and a non-patent vessels are shown.

<table>
<thead>
<tr>
<th>coagulation status</th>
<th>lytic (n=18)</th>
<th>non-lytic (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>time (hours)</td>
<td>0 1.5 48</td>
<td>0 1.5 48</td>
</tr>
<tr>
<td>fibrinogen (g/l)</td>
<td>3.3 0.0 2.8</td>
<td>3.2 2.7 4.4</td>
</tr>
<tr>
<td>plasminogen (%)</td>
<td>100 15 58</td>
<td>102 59 76</td>
</tr>
<tr>
<td>α2-antiplasmin (%)</td>
<td>94 4 84</td>
<td>96 37 102</td>
</tr>
<tr>
<td>aSKa (AU/ml)</td>
<td>42 16 25</td>
<td>321 25 88</td>
</tr>
<tr>
<td>patency (n)</td>
<td>- - 16</td>
<td>- - 0</td>
</tr>
<tr>
<td>non-patency (n)</td>
<td>- - 2</td>
<td>- - 3</td>
</tr>
</tbody>
</table>

aSKa = anti-streptokinase antibody, 0 = before treatment, AU/ml = Arbitrary Units/milliliter
Figure I Relation of coagulation status (lytic or non-lytic) 1.5 hours after thrombolytic therapy and its angiographic result (patency or non-patency) with corresponding values of the anti-streptokinase antibody (aSKa) level before treatment (t=0) and after 1.5 and 48 hours.
with normal aSKa levels in whom a systemic lytic state was induced, even this does not always guarantee success.

References


Anti-streptokinase antibodies are of clinical importance and can be measured quantitatively in 0.5 hr using a simple enzyme-linked immunosorbent assay

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Br Heart J 1994, in press (letter)

Sir, - Buchalter (1) and Patel et al. (2) stated that the relation between anti-streptokinase antibodies and lytic efficacy of streptokinase or its derivate anistreplase in patients with acute myocardial infarction is unknown. Furthermore, the authors appeared not to be aware of a rapid assay for anti-streptokinase antibodies. However, these issues have been described recently. Firstly, a strong relation between a systemic non-lytic state and angiographic non-patency of the infarct related vessel in patients with myocardial infarction has been reported in this Journal (3) and elsewhere (4). Secondly, high levels of anti-streptokinase antibodies before thrombolytic therapy with anistreplase have been shown to lead to failure in achieving a systemic lytic state and subsequently, infarct related vessel patency (5). Finally, following the development of a simple enzyme-linked immunosorbent assay, the level of these antibodies can be measured in half an hour (6). As this method is quick and can be performed easily, its use might have beneficial clinical consequences since additional or alternative thrombolytic therapy, potentially leading to early coronary artery reperfusion, could be applied.

It has been advocated that anti-streptokinase antibodies should be searched for routinely in patients undergoing thrombolytic therapy in myocardial infarction (7). However, the commonly used radio-immunoassay is time consuming and unpractical and therefore not suitable to guide clinical strategies in critically ill patients. In order to identify patients in whom delayed or failed reperfusion is likely to occur due to the presence of anti-streptokinase antibodies, some clinicians routinely measure serum fibrinogen immediately after administration of streptokinase (8). Whereas this method is still useful, a quick and easy measurement of anti-streptokinase antibodies can now be performed for screening purposes and/or to guide clinical treatment.

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Tissue-type Plasminogen Activator and Plasminogen Activator Inhibitor in Patients With Acute Myocardial Infarction Treated With Streptokinase

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submitted

Abstract

We studied 46 patients with acute myocardial infarction (MI) who were treated with intravenous streptokinase (SK) and heparin ≤4 hours after onset of chest pain. Coronary angiography was performed about 24 (≤48) hours after therapy. Blood samples for assessment of fibrinolytic and coagulation parameters were collected on ice before therapy and on days 1 and 2 at 12.00 AM in order to eliminate circadian fluctuations in plasma levels. Patency of the infarct related vessel was found in 36/46 (78%) patients. Baseline t-PA antigen and PAI activity levels were lower in patients with a non-patent compared to those with a patent coronary artery: 6.0 vs 9.3 ng/ml, p=0.04, for t-PA antigen and 1.9 vs 3.5 AU/ml, p=0.06, for PAI activity. On the first day after MI all patients showed an increased level of t-PA antigen and PAI-activity. Thus, in patients with MI treated with SK, systemic endogenous t-PA levels are somewhat lower in patients with a non-patent vessel.

Introduction

The endogenous fibrinolytic system includes on the one hand the activators urokinase-type and tissue-type plasminogen activator (t-PA) and on the other the inhibitors α2-antiplasmin and plasminogen activator inhibitor (PAI) (1). t-PA is localized in endothelial cells and is released into the blood in response to a variety of stimuli, such as thrombin formation, fibrin deposition, venous occlusion or ischemia. The level of t-PA activity in blood is regulated by fast-acting PAI liberated from platelet α-granules and/or from endothelial cells. PAI is present in complex with t-PA or in a free active form. Total PAI content can be measured by the PAI antigen assay while PAI activity reflects functional free PAI (2,3). Plasma concentrations of both t-PA and PAI show a diurnal variation (4,5). Elevated PAI levels have been associated with non-successful therapy with recombinant t-PA (rt-PA) in acute myocardial infarction (MI) (6).

It is unknown if plasma levels of endogenous t-PA and PAI affect the outcome of
treatment with streptokinase (SK) in patients with MI. These levels were determined in a prospective study in which coronary angiography was performed to determine success of therapy.

Methods

Forty-six patients (age 28-78, mean 56 years) with electrocardiographically proven MI and symptoms less than 4 hours, were treated with 1.5 million Units (U) SK intravenously. SK was administered for 1 hour whereafter heparin was started in a dosage of 25,000 U/24 hours for at least 1 day. Thereafter coronary angiography was performed to determine patency of the infarct related vessel. Patients with TIMI-grades 0 or 1 were considered to have occlusion of the infarct related vessel whilst those with grades 2 or 3 to be patent (7).

Blood samples were collected on ice before therapy and at 12.00 AM on days 1 and 2 in order to eliminate the circadian fluctuations of fibrinolytic parameters. Measurement of t-PA activity and t-PA antigen was performed on euglobulin fractions using the Coaset t-PA™ assay (KabiVitrum, Stockholm, Sweden) (8), and the Asserachrom t-PA™ enzyme-linked immunosorbent assay (ELISA) (Boehringer, Mannheim, Germany), respectively. PAI-1 antigen and PAI activity was performed by use of an ELISA purchased from Biopool AB, Umea, Sweden, and by the chromogenic substrate method of the Berichrom-PAI™ assay (Behringwerke, Marburg, Germany) (9), respectively. Fibrinogen was determined according to the method of Clauss (10). Plasminogen and α2-antiplasmin assays were performed using a synthetic chromogenic substrate (11).

Statistical comparisons were calculated with the Student t-test.

Results

Repeated coronary angiography after 24 hours revealed patency of the infarct related artery in 36 of 46 patients. Following thrombolytic therapy, plasma levels of fibrinogen, plasminogen and α2-antiplasmin decreased in all patients irrespective of angiographic patency at 24 hours (Table I). There was no significant difference in the extent of decrease in these coagulation parameters between patients showing patency or non-patency of the infarct related vessel. Before therapy, t-PA antigen levels were significantly lower in patients with a non-patent vessel (6.0 vs 9.3 ng/ml, p=0.04) and PAI activity levels showed this as a trend (1.9 vs 3.5 AU/ml, p=0.06). However, PAI levels did not exceed the normal range. Within both groups a significant increase in t-PA antigen levels from baseline to day 1 occurred (p<0.01). A similar increase of PAI antigen in both groups did not reach statistical significance.

Discussion

During the first 2 to 3 days following treatment with SK because of MI, a temporary decrease of fibrinogen, plasminogen and α2-antiplasmin in the plasma of patients has been shown previously (12). Our results are in agreement with those findings. A significant increase in the level of t-PA antigen and PAI activity, leading to a decrease of t-PA
**Table I** Fibrinolytic parameters (mean±sd) in patients with acute myocardial infarction before (0) and at day 1 (D1) and day 2 (D2) following treatment with streptokinase.

<table>
<thead>
<tr>
<th>state</th>
<th>patency (n=36)</th>
<th>non-patency (n=10)</th>
<th>reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>0</td>
<td>D1</td>
<td>D2</td>
</tr>
<tr>
<td>fibrinogeen (g/l)</td>
<td>3.5±1.0</td>
<td>1.2±0.7</td>
<td>3.1±1.2</td>
</tr>
<tr>
<td>plasminogeen (%)</td>
<td>104±16</td>
<td>51±15</td>
<td>70±17</td>
</tr>
<tr>
<td>α2-antiplasmin (%)</td>
<td>97±11</td>
<td>67±16</td>
<td>90±16</td>
</tr>
<tr>
<td>t-PA antigen (ng/ml)</td>
<td>9±7*</td>
<td>21±13*</td>
<td>13±9</td>
</tr>
<tr>
<td>t-PA activity (IU/ml)</td>
<td>1.5±2.1</td>
<td>0.9±0.4</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>PAI antigen (ng/ml)</td>
<td>17±17</td>
<td>24±18</td>
<td>13±8</td>
</tr>
<tr>
<td>PAI activity (AU/ml)</td>
<td>3.5±3.8</td>
<td>4.8±7.2</td>
<td>2.4±2.7</td>
</tr>
</tbody>
</table>

[t-PA and PAI reference values were adapted from Angleton (ref. 5) and Sane/Loskutoff (ref. 17,18), respectively]

* : values between patency and non-patency, p=0.04
+ : baseline versus day 1 values within the groups, p<0.01
activity, has been observed following surgery. This has been called the postoperative fibrinolytic shutdown (13). The same investigators showed a rapid increase in PAI activity in patients with MI which illustrated the acute phase characteristics of PAI. In the Physicians’ Health Study cohort, it was shown that study participants who developed MI had a significantly higher mean t-PA antigen level (14). Hence, it was suggested that the endogenous t-PA antigen level increased as a consequence of coronary artery disease (15). We found pretreatment t-PA antigen levels in correspondence to the figures found in patients with coronary artery disease (14,15). Interestingly, t-PA antigen levels in patients with a non-patent vessel were lower compared to those patients with a patent vessel at coronary angiography.

Elevated PAI activity levels have been reported in patients showing a non-patent infarct related coronary artery 3 days after treatment with rt-PA or urokinase (6,16). The TAMI-investigators, who studied thrice the number of patients compared to Barbash et al., found only a weak correlation between a low PAI activity level and patency of the infarct related vessel when determined 90 minutes after rt-PA therapy (17). The limited level of agreement between these findings was suggested to relate to the different points in time at which angiography was performed. Average physiologic PAI antigen and PAI activity levels amount approximately 27 ng/ml and <5 IU/ml, respectively (17,18). In contrast, pharmacologic rt-PA levels range from 1 to 3 µg/ml (order of multiplication >1000). Therefore it is likely that PAI activity levels are overwhelmed during rt-PA treatment. However, because of the short half-life of the systemic rt-PA concentration following therapy, it remains possible that PAI plays a role after termination of the infusion of this drug. A local high PAI concentration, due to release of this protein by thrombin-mediated activated platelets at the culprit lesion, could be responsible for blunting the initial thrombolytic success. Supporting evidence for this theory can be derived from recent experiments in a rabbit model of thrombosis which showed that monoclonal antibodies to PAI enhanced rt-PA mediated thrombolysis (19).

Our study showed that in patients with MI treated with SK, systemic levels of t-PA antigen are lower in case of non-patency of the infarct related vessel. This may explain the higher coronary patency rate after a combination of rt-PA and SK in patients with MI compared to treatment with a single thrombolytic agent (20,21). Unfortunately, however, the hypothesis of superiority of a combination of thrombolytic agents versus thrombolytic monotherapy was not confirmed in the recent GUSTO-trial (22). Systemic PAI levels do not appear of significant importance for the outcome of thrombolytic therapy with SK. Whether or not systemic levels of t-PA affect the patency rate of infarct related vessels following SK therapy in patients with MI needs further study.

References


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Lipoprotein(a) Levels in Myocardial Infarction Treated with Anistreplase: No Prediction of Efficacy but Inverse Correlation with Plasminogen Activation in Non-Patency

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Thromb Res 1992;65:S98 (abstract)
Eur Heart J 1992;13:27 (abstract)
Int J Cardiol 1994, in press

Abstract

The aim of this study was to investigate whether failure of thrombolytic treatment might be due to inhibition of fibrinolysis by high lipoprotein(a) levels. Fifty-eight patients with acute myocardial infarction were treated intravenously within 4 hours after onset of symptoms with anistreplase (30 U) and heparin (30,000 IU/24 h). Blood samples for measurement of coagulation parameters were taken before and 1.5 hours after treatment. Coronary angiography was performed after 48 hours. Levels of lipoprotein(a) were measured 6 months after discharge from hospital. The patency rate was 74% (43/58). Median lipoprotein(a) levels were not different between the patients with a patent and those with a non-patent vessel (10 and 8 mg/dl, respectively), however, in patients with a non-patent infarct related vessel, a significant inverse correlation was found between the lipoprotein(a) level and the decrease of plasminogen in the first 1.5 hours after treatment. It is concluded that high lipoprotein(a) levels, although not directly associated with a poor outcome of anistreplase therapy, might contribute to insufficient fibrinolysis in patients with a non-patent infarct related vessel.

Introduction

Lipoprotein(a) is a genetically determined plasma lipoprotein variant firstly described by Berg in 1963 (1). The lipid composition is similar to that of low-density lipoprotein. The protein moiety consists of apolipoprotein B-100 linked covalently to apolipoprotein(a), which is a glycoprotein with a striking homology to plasminogen (2). The distribution of lipoprotein(a) plasma concentrations in the Caucasian population is independent of age and diet and highly skewed for both men and women, with mean levels of 17.0 and 15.5 mg/dl, respectively. Two-thirds of white subjects have lipoprotein(a) levels below 16 mg/dl (3,4). The lipoprotein(a) level is a stable biological characteristic which is not influenced by life style or drug use (5). Lipoprotein(a) is an independent risk factor for
It has been shown in vitro that lipoprotein(a) competes with plasminogen for the same binding sites on endothelial cells and fibrin (9-11). In addition, several authors have claimed interference of the streptokinase and tissue-type plasminogen activator mediated activation of plasminogen by lipoprotein(a) (12-15). Until now, no studies have reported on the influence of lipoprotein(a) levels on the clinical efficacy of the streptokinase containing drug anistreplase.

The aim of the study was to assess, firstly, the relation between steady state lipoprotein(a) levels and patency of the infarct related vessel after thrombolytic treatment, and secondly, the influence of lipoprotein(a) levels on coagulation parameters, in particular plasminogen activation.

Materials and Methods

Fifty-eight consecutive caucasian patients (47 men, 11 women) with proven acute myocardial infarction (mean age 57 years, range 34-71), who were treated with an intravenous bolus-injection of 30 U anistreplase (SmithKline Beecham), within 4 hours after the onset of chest pain, were studied. Adequate anticoagulation with intravenous heparin was started 4-6 hours after thrombolytic treatment and continued for 48 hours. Subsequently coronary angiography was performed to assess patency of the infarct related artery. Patency was documented according to the score used in the Thrombolysis in Myocardial Infarction (TIMI) trial (16). Patients with grade 0 or 1 were considered to have occlusion of the infarct related vessel whilst those with grades 2 or 3 to be patent.

Venous blood samples were collected before treatment, and after 1.5 hours, and placed on ice in 1/10 volume 3.05% trisodium citrate for measurement of fibrinogen, plasminogen and α2-antiplasmin. Assays were performed immediately or plasma was stored at -80°C for later analysis. Fibrinogen was determined according to the method of Clauss (17), plasminogen and α2-antiplasmin were determined according to the method of Friberger (18).

Approximately 6 months after myocardial infarction, blood samples for lipoprotein(a) assay were collected in tubes containing citrate. Specimens were stored at 2-8°C until assessment. Levels of apo(a) were measured by a two-site apolipoprotein(a) immuno radiometric assay kit purchased from Pharmacia diagnostics AB, S-75182 Uppsala, Sweden. The lipoprotein(a) detection limit is 0.02 Unit apolipoprotein(a)/dl whereas 1 Unit of apolipoprotein(a) is equal to 0.7 mg lipoprotein(a). A concentration of up to 5 g/l of plasminogen gives no measurable crossreactivity in the assay. Intra-assay variability amounts 6.3%.

Statistical analysis: Values of the normally distributed coagulation parametes are expressed as mean (SD). Lipoprotein(a) levels, which distribution is skewed, are expressed as median (range). Levels of coagulation parameters within the two groups were compared with a paired t-test, for comparisons between the groups an unpaired t-test was used. Lipoprotein(a) values between the patients with a patent and a non-patent coronary artery were compared with the Mann-Whitney U-test. For comparison of the normally distributed hemostatic parameters with the skewed distribution of lipoprotein(a), the Spearman rank
correlation (R) coefficient was calculated. Because the logarithm of lipoprotein(a) with value zero does not exist, zero was replaced for 0.14 because that was the detection limit of the assay. P-values of less than 0.05 were considered significant.

Results

Fifty-eight patients were retrospectively classified into 2 groups. Forty-three patients showed a patent whilst 15 patients showed a non-patent infarct related artery (Table I). The patients were comparable for initial values of fibrinogen (normal range 1.7-3.5 g/l), plasminogen (normal range 70-130 %) and α2-antiplasmin (normal range 90-130 %). In both the patent and the non-patent groups there was a statistically significant decrease of these parameters between the baseline value and the value at 1.5 hours after anistreplase therapy. At 1.5 hours, there were statistical significant differences between the 2 groups for each of these 3 parameters. Mean values of fibrinogen in the patent and the non-patient groups were 0.0 versus 0.9 g/l, respectively; plasminogen 11 versus 31 %, respectively; and α2-antiplasmin 3 versus 19 %, respectively. Fifty-five of the 58 patients were available for lipoprotein(a) assessment. The remaining 3 patients, who did show a patent vessel at angiography, were not sampled due to logistical reasons. The distribution of lipoprotein(a) levels among the subject population was highly skewed. Median and mean lipoprotein(a) levels were 14 and 27 mg/dl (range 0-184), respectively. Levels of lipoprotein(a) below the limit of detection, which is approximately 0.014 mg/dl, were found in 14 subjects, 10 with a patent and 4 with a non-patent coronary artery, respectively. Unmeasurable lipoprotein(a) levels were assigned 0.14 before log transformation was performed. Extremely high levels of lipoprotein(a) were found in 2 patients with a patent coronary artery (116 and 184 mg/dl) and in 1 patient with a non-patent coronary artery (112 mg/dl). Lipoprotein(a) levels were not statistically different between patients with successful and unsuccessful thrombolysis. For patients with a patent vessel the median level of lipoprotein(a) was 10 mg/dl (range 0-184) and for patients with a non-patent vessel, 8 mg/dl (range 0-112). The decrease (delta) of fibrinogen, plasminogen and α2-antiplasmin levels from before to 1.5 hours after thrombolytic therapy was not significantly rank correlated with the lipoprotein(a) levels in patients with a patent vessel. In these patients, the coefficients of rank correlation (R) of lipoprotein(a) with delta-fibrinogen, delta-plasminogen and delta-α2-antiplasmin were -0.09, 0.03 and 0.05, respectively. However, in patients with a non-patent vessel, the coefficients of rank correlation (R) were -0.38 (delta-fibrinogen), -0.57 (delta-plasminogen, p<0.05) and -0.52 (delta-α2-antiplasmin, p<0.10). The log transformed lipoprotein(a) level and the associated delta-plasminogen percentage in patients with a patent and non-patent vessel are depicted in Figure I.

Discussion

The relation of cardiovascular disease with elevated plasma lipoprotein(a) levels is considered to be due to antifibrinolytic effects of the plasminogen-like apolipoprotein(a) in lipoprotein(a) (9). Plasma lipoprotein(a) levels are steady during life, but in patients with myocardial infarction, its level is raised, reflecting the acute phase characteristics of the
**Table I** Coagulation parameters before and at 1.5 hours after thrombolytic therapy, and lipoprotein(a) levels at 6 months after myocardial infarction, in patients stratified to patency of the infarct related artery 48 hours after treatment.

<table>
<thead>
<tr>
<th></th>
<th>patent (n=43)</th>
<th>non-patent (n=15)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>fibrinogen (g/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>3.1 (1.1)</td>
<td>3.0 (0.4)</td>
<td>NS</td>
</tr>
<tr>
<td>after</td>
<td>0.0 (0.2)*</td>
<td>0.9 (1.2)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>plasminogen (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>97 (19)</td>
<td>100 (13)</td>
<td>NS</td>
</tr>
<tr>
<td>after</td>
<td>11 (14)*</td>
<td>31 (25)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>α2-antiplasmin (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before</td>
<td>93 (15)</td>
<td>92 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>after</td>
<td>3 (5)*</td>
<td>19 (21)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>lipoprotein(a) (mg/dl)</strong></td>
<td>10 (0-184)</td>
<td>8 (0-112)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* p<0.01 vs baseline (before therapy); # p-values for patent and non-patient comparisons. NS denotes not significant. Fibrinogen, plasminogen and α2-antiplasmin are expressed as mean (SD); lipoprotein(a) is expressed as median (range).
Figure 1  Relation between log transformed lipoprotein(a) [Lp(a)] level (mg/dl) and the percentage decrease (delta) of plasminogen from before to 1.5 hours after thrombolytic therapy with anistreplase. Patients with a non-patent infarct related vessel (■); patients with a patent vessel (▼).
compound (19). In case of thrombolytic treatment, plasma lipoprotein(a) levels temporarily decrease about 50% (20). Following myocardial infarction, lipoprotein(a) levels return to baseline after at least 3 months (21). In order to avoid these confounding effects on lipoprotein(a) levels, we measured the steady state level at 6 months after acute myocardial infarction.

Anistreplase is a long-acting streptokinase derivate which use has been associated with a very low early recocclusion rate (22). Its efficacy appeared not to be influenced by conjunctive intravenous heparin therapy (23). Therefore, coronary angiography 48 hours after administration of anistreplase was considered appropriate.

Interference of lipoprotein(a) with the thrombolytic activity of streptokinase and tissue-type plasminogen activator has been demonstrated in vitro by several authors (12-15). In clinical practice, however, no relation between the lipoprotein(a) level and outcome of thrombolytic therapy has been shown (24-26). Our results are in agreement with these findings as the median lipoprotein(a) level in patients with a patent and a non-patent infarct related vessel were about the same. That lipoprotein(a) levels are in some way affecting endogenous fibrinolysis in the clinical situation was recently shown in survivors of myocardial infarction who did not receive thrombolytic therapy (27). In plasma samples obtained 23 months after the event, significantly elevated lipoprotein(a) levels were found in patients with a non-patent infarct related coronary artery compared to those who showed patency. It was suggested that, probably due to an elevated level of lipoprotein(a), intrinsic fibrinolysis was impaired resulting in non-patency of the infarct related vessel. Our finding of an inverse correlation between lipoprotein(a) levels and the decrease of plasminogen levels in the first 1.5 hours following thrombolytic therapy in patients with a non-patent coronary vessel, supports this hypothesis of impaired fibrinolysis. Thus, lipoprotein(a) may interact with fibrinolysis following thrombolytic treatment, but only in a complex manner which does not necessarily lead to failure of therapy. Other factors which impair or inhibit coronary thrombolysis are probably morphology related and/or due to plasma constituents such as anti-streptokinase antibodies and plasminogen activator inhibitor (28). The relative contribution of lipoprotein(a) compared to these other parameters needs to be determined.

Acknowledgment

We are grateful to Dr. GJM Boerma, Dept. of Clinical Chemistry, Academic Hospital Dijkzigt Rotterdam-The Netherlands, for determining the lipoprotein(a) levels.

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Reocclusion Three Months after Successful Thrombolytic Treatment of Acute Myocardial Infarction with Anisoylated Plasminogen Streptokinase Activator Complex (APSAC)

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Eur Heart J 1988;9:10 (abstract).

Abstract

Thirty consecutive patients with acute myocardial infarction (AMI) were treated with anisoylated plasminogen streptokinase activator complex (APSAC) within 4 hours after onset of symptoms. After 1½ and 48 hours, patency of the infarct related vessel and the quantitative degree of residual diameter stenosis were studied by selective coronary angiography. Ventriculograms were made to determine the global left ventricular ejection fraction. Patients showing patency at 48 hours were reevaluated angiographically after 3 months. At 1.5 and 48 hours after APSAC administration patent vessels were demonstrated in 65 and 69% of patients respectively. Mean residual stenosis decreased significantly from 56±11% at 1.5 hours to 46±13% at 48 hours (p<0.01). Patients not responding to thrombolytic therapy showed significant deterioration of the left ventricular function during the first 48 hours after AMI. Side effects were minor and mainly associated with invasive procedures. Despite adequate oral anticoagulation, angiographically documented reocclusion at 3 months amounted 28%. Reocclusion, however, was neither associated with clinically documented reinfarction, nor with a decrease in the left ventricular ejection fraction. Our study shows that APSAC is an effective thrombolytic agent in AMI but that late reocclusion may occur. Oral anticoagulants appear to be less effective in the prevention of reocclusion in the treatment regimen after thrombolysis.

Introduction

Coronary thrombosis is the commonly accepted cause of acute myocardial infarction (AMI) (1). Intravenous thrombolytic treatment with streptokinase is well established as effective therapy for improving survival (2,3) and preserving left ventricular function (4,5). During the last decade, new intravenous thrombolytic drugs such as recombinant tissue-type plasminogen activator and anisoylated plasminogen streptokinase activator complex (APSAC) have emerged. Each of these drugs is effective (6,7). Only APSAC can be given as a slow bolus injection. Because of its long plasma half-life, APSAC exhibits prolonged
action, a property that has been related to a low incidence of early reocclusion (8). However, little is known about reocclusion in the subsequent period. We therefore studied 3-months reocclusion rate in patients with AMI treated with APSAC.

Methods

Patients: Patients who were 70 years or younger were eligible if they had the onset of symptoms suggestive of AMI within the previous 4 hours. In addition, electrocardiographic ST-segment elevation of at least 0.1 mV in ≥1 of the standard leads or at least 0.2 mV in ≥2 of the precordial leads in a 12-lead registration was required. Symptoms had to be unresponsive to sublingual glyceryl trinitrate. Patients were excluded if contraindications for thrombolytic treatment were present.

Study Protocol: Treatment was started with nitroglycerin and lignocaine infusion followed by a bolus injection of 100 mg prednisolone. Subsequently, 30 U of APSAC (Eminase™-Beecham) was given intravenously in 4-5 minutes. Intravenous therapy with heparin was supplied 4-6 hours after APSAC in a dosage of 30,000 IU in 24 hours and was continued until an adequate level of anticoagulation had been achieved with acenocoumarol which was started after 48-72 hours. Oral anticoagulation was continued for at least 3 months. Antiplatelet therapy was not part of the treatment regimen. Heart catheterisation by means of the Judkins technique was performed 1.5 (range 1 to 3) and 48 hours (range 36 to 60) after the administration of APSAC in all patients. Patency and percentage of residual diameter stenosis of the infarct related vessel were assessed by coronary angiography. Patency was documented according to the score used in the Thrombolysis In Myocardial Infarction Study (6). Patients with grade 0 or 1 perfusion were classified as occluded, those with grades 2 and 3 as patent. Quantification of coronary residual stenosis was realized using the computer-assisted cardiovascular angiography analysis system (9,10). Global left ventricular ejection fraction was assessed by quantitative analysis of the left ventricular angiograms in the right anterior oblique projection. In patients with a patent vessel at 48 hours, heart catheterisation was repeated at 3 months. Twelve lead electrocardiograms were made and blood samples were collected for creatine kinase level determination before treatment and serially after the administration of APSAC.

Statistical analysis: Results for continuous variables are presented as mean ± standard deviation. The Student’s unpaired t-test was used to assess differences between patients with patent and non-patent vessels. Comparisons within each group were made with the paired Student t-test. A p-value <0.05 was considered significant.

Results

Thirty consecutive patients (23 men, 7 women) ranging in age from 33 to 70 years (mean 55) with AMI (15 anterior, 15 inferior) participated in the study. The mean overall delay between onset of symptoms and thrombolytic treatment was 2.6±0.9 hours. One patient died in cardiogenic shock within 1.5 hours after treatment with APSAC. Three patients were not subjected to angiography at 1.5 hours for logistic reasons. Seventeen infarct related coronary vessels in 26 patients at 1.5 hours were patent (65%). Forty-eight
hours after the administration of APSAC, patency was found in 20 of 29 patients (69%), including the aforementioned 3 patients (Table I). No reocclusion occurred between 1.5 and 48 hours. There was no significant relation between outcome of thrombolytic therapy and duration of symptoms. One patient, with a patent right coronary artery, underwent emergency bypass surgery soon after the coronary angiography at 48 hours because of symptoms of anterior myocardial ischemia. Another patient with a patent left anterior descending artery at 48 hours died suddenly at home within 3 months. Two patients with a non-patent infarct related vessel died between the second day and the end of the 3 months follow up period. They both had cardiac failure.

Angiography after 3 months was repeated in 18 patients who had a patent vessel after 48 hours. The patient who underwent bypass surgery was excluded. Patency was demonstrated in 13 (72%). Reocclusion was found in 5 patients (1 right coronary artery, 4 left anterior descending coronary artery). In none of these 5 patients did a documented reinfarction occur.

The mean percentage diameter stenosis in patent infarct related arteries decreased significantly between 1.5 and 48 hours from 56±11 to 46±13% (p<0.01). No further significant improvement was found after 3 months when 40±14% residual coronary diameter stenosis was demonstrated in the 13 patent vessels.

Left ventricular ejection fraction differed significantly between patients with a patent and a non-patent vessel at 1.5 hours (67±14 and 49±22%; p<0.05) and 48 hours (59±16 and 41±20%; p<0.02) after treatment with APSAC. Patients with an occluded vessel also showed a significant decrease between 1.5 and 48 hours after treatment (Table II). These patients with an occluded vessel were not reassessed at 3 months. No significant decrease of the left ventricular ejection fraction was found in 5 patients demonstrating reocclusion at 3 months; values at 48 hours and 3 months were 53±14 and 57±14%, respectively.

Neither creatine kinase levels, nor creatine kinase-MB levels were significantly different in patients with a patent or an occluded vessel. However, the time to peak level of each was significantly shorter in patients with a patent vessel.

Mild bleeding, mainly at puncture sites, occurred in 9 patients. Two patients received a blood transfusion for moderate bleeding. A purpuric rash developed in 1 patient at the 5th day after treatment. A skin biopsy showed this to be due to a non specific vasculitis.

Discussion

In our study 3 patients did not undergo angiography at 1.5 hours. However, these patients showed rapid relief of chest pain, quick improvement of electrocardiographic repolarization changes and an early peak of the creatine kinase-MB. These findings have been shown to be strongly predictive for patency of an infarct related vessel (11). Therefore, it seems likely that in case of angiography of all patients, the patency results at 1.5 and 48 hours after APSAC would have been similar. Forty-eight hours after treatment with APSAC 69% of the patients in our study had a patent infarct related vessel. This figure corresponds to the 60 to 70% of thrombolytic success reported in a review by Marder and Sherry (8). These authors estimated the incidence of reocclusion after treatment with APSAC to be 10%. However, this low percentage was derived from a
### Table I  Patency rates in course of 3 months

<table>
<thead>
<tr>
<th>coronary artery</th>
<th>time of angiography</th>
<th>1.5 hours</th>
<th>48 hours</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD</td>
<td>No.</td>
<td>14</td>
<td>8/11</td>
<td>11/14</td>
</tr>
<tr>
<td>RCA</td>
<td>11</td>
<td>6/11</td>
<td>6/11</td>
<td>4/5</td>
</tr>
<tr>
<td>CX</td>
<td>4</td>
<td>3/4</td>
<td>3/4</td>
<td>3/3</td>
</tr>
<tr>
<td>patency (%)</td>
<td>17/26* (65)</td>
<td>20/29 (69)</td>
<td>13/18# (72)</td>
<td></td>
</tr>
</tbody>
</table>

LAD: Left anterior descending, RCA: Right coronary artery, CX: Left circumflex

*: no angiography was obtained from 3 living patients because of logistic reasons, 1 patient died <1.5 hours

#: only patients with a patent infarct related vessel at 48 hours were examined 3 months after treatment, 1 patient underwent coronary artery bypass surgery and 1 patient died
Table I  Global left ventricular ejection fraction (%)

<table>
<thead>
<tr>
<th>time</th>
<th>result of coronary angiography</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>patent</td>
<td>occluded</td>
</tr>
<tr>
<td>1.5 hours</td>
<td>67±14</td>
<td>49±22</td>
</tr>
<tr>
<td>48 hours</td>
<td>59±16</td>
<td>41±20</td>
</tr>
<tr>
<td>3 months</td>
<td>62±15</td>
<td></td>
</tr>
</tbody>
</table>

# = between group comparisons, * = p <0.01 vs baseline
limited number of patients in whom angiography was repeated 24 hours after treatment (12,13). We found no reocclusion between 1.5 and 48 hours. A similar result was obtained in a study of 99 patients with AMI also treated with APSAC. In these patients angiography was performed at 1.5 and 24 hours after treatment (14). The low rate of early reocclusion after treatment with APSAC is probably due to a long lasting extensive fibrinogenolysis. In such a hypocoagulable state a more complete lysis of the residual thrombus can be elicited, as was also demonstrated in our study. Thorough lysis was one of the factors postulated to be preventive for reocclusion (15).

Only 1 study reported reocclusion data 4 weeks after therapy with APSAC (16). These investigators found reocclusion in 5 of 37 (14%) of the infarct related vessels. However, data of some of the patients were lacking, which may partly explain the low percentage. In our study angiographic reocclusion at 3 months was found in 5 of 18 patients (28%). This relatively high rate, despite adequate oral anticoagulation, is disappointing. Recently it was shown in animal experiments that platelets have a vital role in reocclusion after thrombolysis (17). Thus, instead of oral anticoagulants, platelet inhibitors might have been a more appropriate approach to prevent reocclusion. Since the Second International Study of Infarct Survival, the issue of aspirin in the treatment of AMI is also acknowledged (3).

It should be emphasized that none of the patients with reocclusion at 3 months had documented reinfarction or decrease of the left ventricular ejection fraction. Obviously, reocclusion after initial successful therapy with APSAC appears not to be as deleterious as complete failure of treatment. This could be explained by the restriction of the cardiac area at risk in the acute phase through reperfusion and subsequent adaptation of the ventricle by collateral blood supply.

The significant decrease in coronary residual diameter stenosis in the first 48 hours after successful treatment corresponds with the findings in studies with streptokinase (18) or recombinant tissue-type plasminogen activator (19). Their thrombolytic effect persists for a longer period than might be expected from their plasma half-life.

Thrombolytic therapy of AMI with APSAC has been shown to preserve the left ventricular ejection fraction compared to conventional heparin therapy (20). In our study this only occurred in patients responding to APSAC, whereas a significant decrease of the left ventricular function during the first 48 hours was seen in patients resistant to thrombolytic treatment. Thus, it appears highly desirable to achieve reperfusion of an occluded coronary vessel in the acute phase of AMI in order to improve myocardial function.

Bleeding complications were infrequent and considered not serious in most patients. One case of vasculitis, which resolved quickly and spontaneously, was seen. Vasculitis results from APSAC’s antigenic properties and has been described earlier in a limited number of patients (21).

In this study APSAC proved to be effective and safe. Early reocclusion was absent but reocclusion after 3 months was found in 28% of the patients. Failure to achieve patency, however, was more deleterious than failure to maintain patency. To avoid reocclusion, therapy with aspirin might be more appropriate instead of oral anticoagulants. This should be confirmed in a controlled trial in patients with AMI after thrombolytic therapy.

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References


