Activation of Complement and Kinin Systems After Thrombolytic Therapy in Patients With Acute Myocardial Infarction
A Comparison Between Streptokinase and Recombinant Tissue-Type Plasminogen Activator

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Background We have previously shown that treatment with streptokinase induces abrupt complement activation and transient neutropenia in patients with acute myocardial infarction (AMI). The purpose of this study was to compare the effects of two different thrombolytic agents—streptokinase (SK) and recombinant tissue-type plasminogen activator (rTPA)—on activation of the complement and kinin systems in plasma of patients with AMI.

Methods and Results Forty-one patients with AMI who were eligible for thrombolytic therapy were studied. Twenty-three patients were treated with streptokinase (1.5 million IU IV over 60 minutes) and 18 were treated with rTPA (8 with bolus of 10 mg IV, followed by 50 mg infused over 60 minutes and then 40 mg infused over 120 minutes; 10 patients were administered rTPA and heparin according to the accelerated infusion protocol indicated by the GUSTO study). C4a and C3a were measured by radioimmunoassay, soluble terminal complement components (SC5b-9) and anti-SK IgG antibodies were measured by ELISA. Cleaved high molecular weight kininogen (HK) was quantitated in plasma by SDS-PAGE and immunoblotting analysis. C4a levels were significantly and similarly increased in both groups, whereas the levels of C3a and SC5b-9 after rTPA infusion were only slightly elevated and were significantly lower than after SK. No differences were observed between patients treated with slow or accelerated rTPA regimens. The titer of antibodies to SK was highly correlated with the levels of C3a and SC5b-9, whereas a lesser correlation was observed with C4a. Treatment with rTPA did not induce the transient neutropenia observed after SK infusion. The cleavage products of HK were significantly greater after SK than after rTPA infusion.

Conclusions Our results show that both thrombolytic agents activate the classic complement pathway and that plasmin could be the common trigger for this phenomenon. A significant activation of the complement common pathway (from C3 to terminal components) was observed only with SK infusion and is attributable to the rapid formation of immunocomplexes between SK and anti-SK antibodies present in plasma as a consequence of previous streptococcal infections. The minimal activation of C5 component of the common pathway explains the absence of leukopenia in patients treated with rTPA. Cleavage of HK, larger after SK than after rTPA infusion, represents a condition enhancing the generation of bradykinin by kallikrein. The recent experimental data that indicate a damaging effect of complement activation on the infarcted zone and the contrasting favorable effect consequent to bradykinin formation raise some questions about the clinical importance of the different biological consequences of SK versus rTPA. (Circulation. 1994;90:2666-2670.)

Key Words • complement • kinin • streptokinase • plasminogen activators

The inflammatory reaction attendant to acute myocardial infarction (AMI) is a topic of considerable interest and discussion. In addition to their well known action on coagulation, thrombolytic agents, routine treatment for AMI, activate other enzymatic systems that have proinflammatory effects. This aspect of thrombolytic therapy has been little investigated, and a study of the complement and contact systems could be an interesting approach.

It has been demonstrated that there is in vivo deposition of complement components in the myocardium of patients with AMI.1 Studies in animals have suggested that products derived from complement activation mediate irreversible myocardial cellular damage that could be diminished or prevented by inhibition of complement activation by soluble complement receptor type 1 (sCR1).2-4 Previous studies indicated that complement is activated in the plasma of patients with AMI,5,6 although two other studies (one, however, was limited to the early phase of AMI) did not report complement activation.7,8 We have recently been unable to detect signs of complement activation in plasma during the acute phase of uncomplicated myocardial infarction. However, we did show that thrombolytic treatment with streptokinase (SK) abruptly activates the complement system.9 Complement activation has also been demonstrated in five patients in the very early phase of

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thrombolytic treatment with recombinant tissue-type plasminogen activator (rTPA). As a consequence of complement activation, patients treated with SK have a transient leukenopia, but this was not evaluated in the rTPA study.

Moreover, recent experiments in animals suggest that bradykinin, a vasoactive peptide derived from high molecular weight kininogen (HK) during contact system activation, could reduce the area of infarct. The aim of the present study was to evaluate the extent of complement activation generated by SK and rTPA infusion by measuring plasma levels of anaphylatoxins (C4a and C3a) and soluble terminal complement components (SC5b-9) in patients with AMI. We also examined the effects of the two thrombolytic agents on contact system activation, measured as cleavage of HK.

Methods

Forty-one patients with AMI (31 men and 10 women; age, 39 to 82 years) who presented at the hospital within 6 hours of beginning the symptoms of AMI were considered to be eligible for thrombolytic treatment and were entered into the study. All patients gave informed consent, and the study protocol was approved by the Ethical Committee of the University of Milan.

Twenty-three patients were treated with SK (Streptase, Behringwerke; 1.5 million IU over 60 minutes). Eighteen were treated with rTPA (Actilyse, Boehringer Ingelheim), with two different regimens. Eight were given a bolus of 10 mg IV, followed by 50 mg infused over 60 minutes and then 40 mg infused over 120 minutes (slow regimen). Ten patients were treated according to the new accelerated rTPA-heparin infusion protocol suggested in the GUSTO study (15 mg rTPA IV bolus followed by 50 mg over 30 minutes and then 35 mg over 60 minutes, with a bolus of 5000 U of heparin IV followed by continuous infusion of 1000 U/hr) (fast regimen). Other conventional therapies (usually 6 to 50 mg/min nitroglycerin, 2 to 10 mg morphine IV, 50 to 100 mg atenolol PO) were similar in the different groups. Age and clinical status of the patients are reported in Table 1. Differences among various groups were not statistically significant. Plasma samples were obtained from blood collected into EDTA and EDTA-hexamethrine-bromide at admission, at 15 and 30 minutes after the start of the infusion of the thrombolytic agent, at the end of infusion, and 24 hours afterward. At the same time, white blood cell (WBC) counts were made. C3a and C4a were measured in plasma by radioimmunnoassay (Amersham), and SC5b-9 was measured by ELISA (Quidel). Normal values (median and range) of 20 healthy volunteers were 232 ng/mL (148 to 453 ng/mL) for C4a; 212 ng/mL (108 to 462 ng/mL), C3a; and 238 ng/mL (25 to 600 ng/mL), SC5b-9.

Specific anti-SK IgG antibodies were measured in serum by ELISA. Streptokinase (250 U/mL) (Streptase, Behringwerke) in phosphate-buffered saline (PBS), pH 7.2, was coated (50 μL) onto 96-well microtiter plates (Maxisorp, Nunc) by overnight incubation at 4°C. After thorough washing with 0.05% PBS-Tween 20 (pH 7.4), 50 μL of diluted plasma samples was added and incubated again for 60 minutes at room temperature. After washing, bound immunoglobin was identified by mouse monoclonal antibody to human IgG (Sigma Chemical) detected with peroxidase-conjugated anti-mouse IgG antibody. Bound peroxidase was detected by O-phenylenediamine dihydrochloride (OPD; Sigma Chemical). Absorbance at 492 nm was quantified with an automatic spectrophotometer (Titertek Twinreader, ICN-Flow). Results were expressed in arbitrary units (AU) referred to an internal standard (plasma collected from a patient with AMI 15 days after being treated with SK, arbitrarily considered to contain 10 000 U of antibodies).

Cleaved HK was evaluated with SDS-PAGE and immunoblotting analysis, using a method based on that described by Berrettini et al. Plasma was subjected to nonreducing SDS-PAGE (8% wt/vol). After electrophoretic transfer of proteins from gel onto a polyvinylidene difluoride membrane (Immobilon, Millipore), HK was identified with goat polyclonal anti-light-chain HK (Nordic) and visualized with a biotinylated donkey anti-goat antibody (GIBCO BRL). The apparent molecular mass of proteins was estimated by comparison with the high molecular weight protein markers from Bio-Rad Laboratories. With this method, native HK appears as a band with molecular weight of 130 000, and cleaved HK is represented by two bands with molecular weights of 107 000 and 98 000. Density of bands was quantified by computerized image analysis (Image Master, Pharmacia). The amount of cleaved HK (bands with molecular weights of 107 000 and 98 000) was expressed as percentage of total HK (sum of three

### Table 1. Age and Clinical Status of Patients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Streptokinase</th>
<th>rTPA (<em>Slow</em> Regimen)</th>
<th>rTPA (<em>Fast</em> Regimen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>23</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Age, y (mean±SEM)</td>
<td>62.2±2.5</td>
<td>52.7±3.4</td>
<td>64.3±1.5</td>
</tr>
<tr>
<td>Site, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>12</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Inferior</td>
<td>11</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Killip class &gt;2, n</td>
<td>5/23</td>
<td>0/8</td>
<td>2/10</td>
</tr>
<tr>
<td>Myocardial enzymes (mean±SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPK, U/L</td>
<td>2041±328</td>
<td>1612±344</td>
<td>2026±520</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>359±56</td>
<td>296±61</td>
<td>322±61</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>1916±260</td>
<td>1474±205</td>
<td>1646±288</td>
</tr>
<tr>
<td>Hours from pain to hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean±SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5±0.4</td>
<td>2.7±0.8</td>
<td>2.9±0.4</td>
</tr>
<tr>
<td>Mortality, n</td>
<td>3/23</td>
<td>0/8</td>
<td>0/10</td>
</tr>
</tbody>
</table>

rTPA indicates recombinant tissue-type plasminogen activator; CPK, creatine phosphokinase; AST, aspartate aminotransferase; and LDH, lactate dehydrogenase.
TABLE 2. Anaphylatoxin and Soluble Terminal Component Complement Levels in 23 Patients With AMI Treated With SK and in 18 Patients With AMI Treated With rTPA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Infusion</th>
<th>15 min</th>
<th>30 min</th>
<th>End of Infusion</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4a, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK</td>
<td>348</td>
<td>3600†</td>
<td>2264†</td>
<td>1796†</td>
<td>354</td>
</tr>
<tr>
<td></td>
<td>(70-615)</td>
<td>(780-10 008)</td>
<td>(244-6998)</td>
<td>(122-7164)</td>
<td>(47-977)</td>
</tr>
<tr>
<td>rTPA</td>
<td>323</td>
<td>1828†</td>
<td>1612†</td>
<td>845†</td>
<td>383</td>
</tr>
<tr>
<td></td>
<td>(35-1934)</td>
<td>(276-12 768)</td>
<td>(504-8056)</td>
<td>(259-3319)</td>
<td>(88-2184)</td>
</tr>
<tr>
<td>C3a, ng/mL</td>
<td>SK</td>
<td>213</td>
<td>2634†</td>
<td>2036†</td>
<td>1661†</td>
</tr>
<tr>
<td></td>
<td>(87-793)</td>
<td>(288-16 564)</td>
<td>(417-10 932)</td>
<td>(377-9688)</td>
<td>(117-963)</td>
</tr>
<tr>
<td></td>
<td>rTPA</td>
<td>186</td>
<td>596†</td>
<td>735†</td>
<td>574†</td>
</tr>
<tr>
<td></td>
<td>(80-830)</td>
<td>(190-2272)</td>
<td>(109-2856)</td>
<td>(133-1034)</td>
<td>(151-739)</td>
</tr>
<tr>
<td>SC5b-9, ng/mL</td>
<td>SK</td>
<td>250</td>
<td>1491†</td>
<td>1180†</td>
<td>1232†</td>
</tr>
<tr>
<td></td>
<td>(20-690)</td>
<td>(205-8365)</td>
<td>(151-8567)</td>
<td>(58-8907)</td>
<td>(14-882)</td>
</tr>
<tr>
<td></td>
<td>rTPA</td>
<td>105</td>
<td>145</td>
<td>255*</td>
<td>203*</td>
</tr>
<tr>
<td></td>
<td>(32-256)</td>
<td>(55-1298)</td>
<td>(24-502)</td>
<td>(12-924)</td>
<td>(10-526)</td>
</tr>
</tbody>
</table>

AMI indicates acute myocardial infarction; SK, streptokinase; and rTPA, recombinant tissue-type plasminogen activator. Wilcoxon matched-pairs sign rank test: *P<.01, tP<.001 vs before infusion.

bands); the normal value (median and range) of 20 healthy volunteers was 22% (14% to 36%).

Statistical analysis was performed by nonparametric tests because of the non-Gaussian distribution law of the recorded variables. Friedman nonparametric two-way ANOVA was performed to compare the overall pattern of the variables over time within each treatment (SK or rTPA) group. Paired comparisons over time (between preinfusion and postinfusion samples) were carried out thereafter with Wilcoxon's matched-pairs sign rank test. The effects of the two treatments on complement and contact system were compared at each point in time by Mann-Whitney U test. Correlations between complement parameters and WBC counts as well as SK antibody titers were assessed with Spearman rank coefficient. A value of \( P=.01 \) was considered statistically significant according to Bonferroni's procedure.

Results

Effects of SK and rTPA Infusion on Complement Activation and WBCs

Patients with uncomplicated myocardial infarction had plasma levels of C4a, C3a, and SC5b-9 on arrival at the hospital that did not differ from those of healthy volunteers. Basal values and those obtained during and after SK and rTPA infusion are reported in Table 2. Data concerning the different rTPA regimens were pooled together because they never differed. Levels of all complement parameters were significantly increased by thrombolytic treatment (Table 2). No significant differences in C4a levels were found between SK and rTPA infusion. On the other hand, C3a and SC5b-9 levels at the minutes 15 and 30 and at the end of the infusion were greater after SK than after rTPA (\( P<.001 \)); SC5b-9 levels were still higher (\( P<.001 \)) in the SK group than in the rTPA group 24 hours after thrombolysis. Peak levels of C4a and C3a were usually reached after 15 minutes from the beginning of SK infusion and 15 to 30 minutes from the beginning of rTPA infusion. SC5b-9 peak levels were reached by the end of the SK infusion, whereas a moderate increase of terminal components, within the normal values, was observed after rTPA. As depicted in Fig 1, peak levels of C4a were similar in the two groups, whereas those of C3a and SC5b-9 were markedly higher after SK than after rTPA.

Anti-SK antibodies were determined in plasma of patients undergoing SK infusion, both before and after
the end of the treatment. Pretreatment titers ranged from 5 to 620 AU (median, 122 AU) and were similar to the titers found in a group of 20 apparently healthy subjects (median, 111 AU; range, 2 to 1100 AU). Pretreatment titers were positively correlated with peak levels of C3a and SC5b-9 (r = 0.60, P = 0.001; r = 0.79, P < 0.001) and, to a lesser extent, with C4a (r = 0.53, P < 0.005). The anti-SK antibody titer fell after the infusion to 1.5 (range, 0.7 to 80) (P < 0.0001).

SK caused a transient but marked decrease in the number of WBCs 15 minutes after the beginning of the infusion (−32.2%; range, −87.9% to +9.3%; P < 0.001), which was not observed with rTPA infusion (+6.3%; range, −14.6% to +50.6%).

Effects of SK and rTPA Infusion on Cleavage of HK

Preinfusion levels of cleaved HK (median, 24%; range, 11% to 44%) were similar to those of healthy volunteers. Patients treated with SK showed a marked increase of cleaved HK (Fig 2). Peak levels of cleaved HK were 79% of the total protein (range, 67% to 93%) after SK and 44% (range, 17% to 82%) after rTPA infusion. The cleavage products of HK were significantly higher in the SK group than in the rTPA group both at peak levels and at 24 hours (P < 0.0001) (Fig 3).

Discussion

In several large trials published in 1986 through 1993, thrombolytic therapy was shown to reduce the mortality of AMI.12,14–17 However, little is known about the immunoinflammatory effects elicited by thrombolytic agents. Considering the importance of the inflammatory reaction in the evolution of AMI, the choice of the ideal thrombolytic agent should also involve these aspects. We evaluated the direct effects of SK and rTPA on complement activation and on the cleavage of HK as a marker of activation of the kinin system in patients with AMI. Our results confirm that, on arrival at the hospital, patients with AMI have no signs of complement activation, which instead become detectable after infusion of either thrombolytic agent. Nevertheless, the patterns of complement activation were different in the two groups: SK activates the entire system through the classic pathway, and rTPA gives a similar degree of activation of the classic pathway component C4 but only slightly activates C3 and the terminal components from C5 through C9 (Fig 1). The different pattern cannot be the consequence of the reported inhibitory effect of heparin on complement activation because limited complement activation was also observed after rTPA in patients who were not treated with heparin. After rTPA infusion, relatively higher titers of C4a than of C3a were also observed by Bennett et al.8 Previous in vitro data demonstrated that plasmin can activate the classic complement pathway, cleaving C1r.18 However, plasmin cannot generate a stable C3 convertase and thus cannot efficiently activate the terminal complement components. A similar reduction of C4 antigen levels was present after 15 minutes of both treatments (rTPA = −10%; SK = −7.8%), whereas C3 antigen levels were significantly reduced only after SK (−11.7%) (data not shown). Therefore, the generation of plasmin that occurs during rTPA treatment can account only for C4 activation. For activation of the entire complement system that characterizes SK infusion, an acceptable explanation might involve the rapid formation of SK–anti-SK immune complexes.19–21 Antibodies to SK, in fact, are widespread in the population as a result of previous streptococcal infections. The strong correlation we found between anti-SK antibody titer and the extent of complement activation along with the decrease in titer after infusion suggest that formation of SK–anti-SK immunocomplexes contributes to the generation of C3a and SC5b-9.
The transient leukopenia observed after SK infusion is complement mediated, as suggested by a significant inverse correlation between the extent of the decrease in WBCs and the increase in C3a and SC5b-9 released into plasma (r = -0.74, P < .001 and r = -0.68, P < .005, respectively). A similar phenomenon is classically observed during hemodiagnosis and cardiopulmonary bypass, and it is attributable to generation of the leukotactic anaphylatoxin C5a. Therefore, it is clear why rTPA infusion, which only minimally activates C5, does not lead to leukopenia.

The cleavage of HK is constant and important after SK infusion, whereas it is scarcely detectable after rTPA, indicating that the biological effects of the two thrombolytic agents also differ in this respect. It has been shown that in vitro activation of plasminogen by SK induces the cleavage of HK and increases bradykinin release by kallikrein. Because it is known that in vivo SK infusion activates larger amounts of plasminogen than infusion of rTPA,25 we suggest that the kinogenalytic activity of SK depends on its ability to generate more plasmin.

The series of events described in this article raise the obvious question of their clinical importance. Experimental data suggest that complement activation has a negative effect on myocardial infarction and that antagonizing the complement activation products with sCR1 reduces the infarct size. On the other hand, some investigators have claimed that when they increased plasma bradykinin levels during experimental myocardial infarction by administration of angiotensin-converting enzyme inhibitors, the necrotic area was reduced. The data available at present are not sufficient to draw conclusions about the clinical importance of the inflammatory effects of thrombolytic therapy. Nevertheless, because the effects clearly occur in vivo, we suggest that they be taken into account when doing risk-benefit evaluations of the treatments.

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References

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