Abrupt Complement Activation and Transient Neutropenia in Patients With Acute Myocardial Infarction Treated With Streptokinase

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Background  Whether and to what extent complement is activated in acute myocardial infarction (AMI) and how it contributes to inflammation of the ischemic area are not yet clear. Fibrinolytic agents used for thrombolysis are known to activate complement in vitro and may contribute to its activation in vivo. The aim of this study was to measure the extent of complement activation in AMI patients, some treated and some not treated with streptokinase. In addition, because abrupt complement activation in vivo is usually associated with leukocyte margination, plugging of cells in the microcirculation, and hypotension, we correlated complement activation with leukocyte numbers and mean arterial pressure.

Methods and Results  Forty AMI patients were studied: 20 were treated with streptokinase (1.5 million IU IV over 60 minutes), and 20 were not given any fibrinolytic agent. The extent and severity of AMI were not significantly different in both groups. Blood samples were drawn on arrival at the hospital, during streptokinase infusion, and then daily for 1 week. Time-matched samples were also drawn from patients not treated with streptokinase. We measured plasma levels of anaphylatoxin C4a, C3a, and C5a by radioimmunoassay and membrane attack complexes SC5b-9 by enzyme immunoassay. Leukocytes and arterial pressure also were measured when samples were obtained. C4a, C3a, and SC5b-9 levels increased about 10-fold (P<.0001) during infusion of streptokinase. There were no significant increases in complement catabolic products in AMI patients not treated with streptokinase. There was a significant transient leukopenia (mean±SEM, −29.5±7.0%; P=.001) and decreases in systolic and diastolic pressures (systolic, −29.3±3.2%; P<.0001; diastolic, −27.5±3.4%; P<.0001) after 15 minutes of streptokinase infusion in coincidence with the peak of anaphylatoxins in plasma.

Conclusions  Streptokinase treatment of AMI causes abrupt activation of the complement system, whereas no significant complement activation can be detected in plasma of AMI patients not treated with fibrinolytic agents. Complement activation causes a transient leukopenia, as reported for other clinical conditions as dialysis and cardiopulmonary bypass, and possibly contributes to the hypotension observed during streptokinase treatment. (Circulation. 1994;89:76-80.)

Key Words  • fibrinolysis • leukocytes • hypotension • streptokinase

Deposition of complement components has been demonstrated in sections of infarcted myocardium of animals and humans. Recent studies may indicate that complement is activated in the plasma of patients with acute myocardial infarction (AMI), although two other studies (one, however, limited to early phases of AMI) did not find complement activation. In addition, fibrinolytic agents, now commonly used for therapy of AMI, activate complement in vitro. In vivo complement activation has been demonstrated only for recombinant tissue plasminogen activator (rt-PA). The in vivo effects of streptokinase are not known. Moreover, the consequences of complement activation by fibrinolytic agents have not been studied. Indeed, the abrupt complement activation observed in experimental and clinical situations, such as hemodialysis or cardiopulmonary bypass, entails leukocyte sequestration in the pulmonary vascular bed, peripheral neutropenia, and hypotension.

We measured the levels of anaphylatoxins and SC5b-9 complexes in streptokinase-treated and untreated AMI patients to evaluate separately the effects of the thrombotic event and the fibrinolytic treatment on the complement system. Parallel white blood cell (WBC) counts and blood pressure measurements were performed to discover whether complement activation was associated with WBC sequestration and hypotension. Anaphylatoxin-mediated vasoconstriction or plugging of WBCs in the microcirculation plus a fall in the arterial pressure might impair blood flow in such critical circumstances as AMI.

Methods  Forty AMI patients (26 men and 14 women), aged 42 to 89 years, were studied. Diagnosis was confirmed by typical ECG patterns and cardiac enzyme elevation. Twenty patients were treated with streptokinase (1.5 million IU IV over 60 minutes) within 30 minutes after arrival at the hospital plus other conventional therapies (usually 6 to 50 mg/min nitroglycerin and 2 to 10 mg morphine IV, 50 to 100 mg atenolol PO). Similar conventional drugs were given to 20 AMI patients who served as control subjects. Streptokinase was not administered to this group because of delayed arrival at the hospital (more than 6 hours after the onset of chest pain) or the presence of specific contraindications (age >80 years, cerebrovascular accidents, or risk of bleeding). Age and clinical status for the
patients of the two groups are reported in the Table. Twelve angina patients without evidence of myocardial injury were also studied.

Plasma samples were collected at hospital admission and daily thereafter for 1 week. Additional samples were collected at 15 minutes, 30 minutes, and 60 minutes during streptokinase infusion and 1 hour later. Time-matched samples were also drawn from the control subjects (angina pectoris and AMI patients not treated with streptokinase). At the same time, WBCs were counted and blood pressures measured.

C3a, C4a, and C5a were measured in plasma by radioimmunoassay (Amersham; Amersham, England) and SC5b-9 by enzyme immunoassay (Quidel; San Diego, Calif). Native complement components C4 and C3 were determined in serum by radial immunodiffusion (Nor Partigen, Behringwerke AG; Marburg/Lahn, Germany). Mean normal values of 20 healthy volunteers were the following: C4a, 251±22 ng/mL; C3a, 219±29 ng/mL; C5a <10 ng/mL; SC5b-9, 345±65 ng/mL; C4, 33±2 mg/dL; and C3, 86±3 mg/dL. All data are expressed as mean±SEM.

Results

At hospital admission, mean plasma anaphylatoxin and SC5b-9 levels in all AMI patients were C4a, 369±32; C3a, 241±45; C5a, 12±3; and SC5b-9, 353±42 ng/mL. They were similar in both fibrinolytic-treated and untreated AMI patients (C4a, 350±33 versus 388±56; C3a, 234±33 versus 247±85; C5a, 11±3 versus 12±5; and SC5b-9, 340±45 versus 372±67 ng/mL) and were not significantly different from those observed in anginal patients (C4a, 396±75; C3a, 248±73; C5a <10; and SC5b-9, 355±68 ng/mL) and normal healthy volunteers, although levels slightly higher than the normal range were occasionally observed.

There were striking increases in C4a, C3a, and SC5b-9 during streptokinase infusion (Fig 1). The highest levels of C3a (3219±844 ng/mL) and C4a (3699±656 ng/mL) were measured 15 minutes after starting streptokinase, whereas the highest SC5b-9 levels (2204±626 ng/mL) were measured at the 60th minute of infusion. These complement parameters were still significantly high 1 hour after the end of the streptokinase infusion (Fig 1). There were no increases in plasma levels of C4a, C3a, and SC5b-9 24 hours later and up to 7 days later (data not shown). No significant
changes in plasma C5a levels could be detected during streptokinase infusion. Only minor and nonsignificant changes in C4 and C3 levels (mg/dL) were observed (before streptokinase treatment: C4, 36±3; C3, 83±4; 15 minutes after beginning of streptokinase infusion: C4, 35±2; C3, 77±6; the day after: C4, 33±2; C3, 72±5).

Patients not treated with streptokinase had no significant increases in anaphylatoxins or SC5b-9 plasma levels, either in plasma collected at times matching those during streptokinase infusion (Fig 1) or in the following week. Angina patients also did not show any signs of complement activation (observation limited to the first 24 hours after arrival at the hospital).

A transient leukopenia (−29.5±7.0%; paired t test, P<.001) was observed 15 minutes after the beginning of streptokinase infusion because of a reduction of circulating polymorphonuclear cells (PMNs), followed by an increase in the number of circulating cells (Fig 2). The extent of the decrease in WBCs and the C3a release into plasma were significantly inversely correlated (r=.73, P=.001). No transient leukopenia was observed in streptokinase-untreated patients.

Streptokinase-treated patients had significant decreases in both systolic and diastolic pressures after about 15 and 30 minutes of infusion (systolic, 136.8±3.6 to 96.4±4.9 mm Hg; diastolic, 83.9±3.5 to 60.8±3.7 mm Hg; paired t test, P<.0001). The calculated values for mean arterial pressure (MAP) were 105.0±4.1 to 72.7±3.4 mm Hg (paired t test, P<.0001). A slight decrease was also observed in control subjects at the same time (MAP, 105.0±4.1 to 96.6±4.00 mm Hg; paired t test, P<.05). The short-term blood pressure fall was, however, significantly greater in fibrinolytic agent-treated patients (t test, P<.0001) and was independent of nitrate treatment. The extent of blood pressure fall in the streptokinase-treated group did not correlate with C3a increase in plasma.

Discussion

The main information provided by our study is that streptokinase given for AMI therapy abruptly activates the complement system and produces a transient leukopenia. We observed, in fact, striking increases during streptokinase treatment in three different complement activation byproducts: C4a, C3a, and SC5b-9 complexes. Complement activation appeared to be drug related because complement catabolic peptide levels in a group of AMI patients, comparable for extent of the infarcted area, severity, and clinical outcome but not treated with streptokinase, were not significantly increased. The group of angina patients also had no signs of complement activation.

Previous observations have clearly demonstrated an in situ deposition of complement components in the myocardia of patients who died because of AMI or animals with ligated coronary arteries. However, the lack of an increase of complement anaphylatoxins or SC5b-9 in non–streptokinase-treated AMI patients does not rule out that complement activation may have occurred in the ischemic area. Such activation might not cause detectable levels of complement catabolic peptides in the peripheral circulation. Previous observations may indicate that ischemia by itself is able to cause enough complement activation to be detected in the circulation. However, one of these publications indicates an activation that did not involve the different complement components proportionally. Differences in the severity of the clinical condition might explain the discrepancies between our data and those of the other study (we also found baseline levels of anaphylatoxins and SC5b-9 higher than the normal range in two non–streptokinase-treated patients who died from cardiogenic shock). On the other hand, our data appear to agree with those of a previous report of normal plasma anaphylatoxin levels in AMI. Moreover, Bennett et al reported normal plasma anaphylatoxin levels in the early phases of AMI before thrombolysis and a significant increase after rt-PA administration, before any angiographically detectable reperfusion. Although our study did not include an angiographic demonstration that complement activation by streptokinase preceded reperfusion, the hypothesis that complement activation is due to reperfusion injury appears to be unlikely when one examines the difference in the time courses of the complement activation and coronary reperfusion. Signs of complement activation were found in a few samples collected as early as 5 minutes after streptokinase infusion was started (data not shown).

The group of patients treated with streptokinase had higher, although not significantly, peak levels of CPK. However, the fact that the levels of C4a, C3a, and SC5b-9 on arrival at the hospital were not significantly elevated in both streptokinase-untreated and treated patients makes it hardly conceivable that the high levels of complement components measured during streptokinase infusion were due to larger infarcts in this group.

The complement activation caused by streptokinase infusion involves the classic pathway, since C4a levels are clearly increased. The activation involves C3 as well as the terminal components C5 to C9. The increases in C3a and C4a started immediately after the beginning of infusion and peaked 15 minutes later, whereas, as expected, the SC5b-9 peak was slightly later. Streptokinase infusion did not deplete native complement com-
ponent, since C4 and C3 levels were not significantly modified.

Complement probably is activated by streptokinase through generation of plasmin. Previous in vitro data demonstrate that plasmin can directly activate C5r and consequently trigger the classic complement cascade. Direct C3 activation by plasmin, with formation of chemotactic peptide, indistinguishable from C3a, also has been described.7 The role of plasmin is strengthened by previous observations in six patients that rt-PA infusion also was followed by a moderate increase of complement classic pathway activation.5 These observations, however, do not exclude the recently proposed importance of streptokinase-antistreptokinase immunocomplexes in complement activation.18 We also measured anaphylatoxins and SC5b-9 in a limited number of patients treated with rt-PA. Our data, albeit preliminary, confirm that rt-PA activates complement, although probably to a lesser extent than streptokinase. In these patients, interestingly, no significant leukopenia or changes in blood pressure levels were observed (data not shown).

In streptokinase-treated patients we observed transient decreases in the number of WBCs (PMNs) in coincidence with the maximum increase in anaphylatoxins. The significant inverse correlation between the extent of the decrease in WBCs and the C3a release into plasma suggests that leukopenia is anaphylatoxin mediated. Moreover, the transient decrease in the number of leukocytes is followed by a rebound of the number of circulating cells, as is invariably observed when PMNs are exposed to chemotactic agents.19

The relation between complement activation and leukopenia is a well-documented phenomenon that has been observed in animals after injection with C5a or zymosan-activated plasma containing large amounts of this anaphylatoxin. C5a binds to specific receptors on PMNs, increasing cell adhesion and consequent margination along vessel walls. The degree of neutropenia is dose dependent, and continuous C5a production is needed to keep the PMNs marginated.12,20 A complement-mediated neutropenia also has been observed in clinical conditions in which complement is acutely activated, such as hemodialysis and other extracorporeal circulation systems.8,9 Because C5a binds to the receptors, it is not surprising that plasma C5a levels in the extracorporeal circulation and in streptokinase-treated AMI patients are not modified, even though an increase in generation of this anaphylatoxin has been documented by formation of SC5b-9 complexes.

Both complement activation and neutrophil stimulation may contribute to myocardial ischemic disfunction. C5a recently has been demonstrated to produce coronary vasoconstriction through thromboxane A2 and leukotriene production.13 Moreover, complement-stimulated PMNs are known to adhere to the vessels of different organs, including lung and heart microcirculations, contributing to a reduction of regional blood flow.8,10,21 Inhibition of complement activation by the use of soluble recombinant complement receptor type 1 reduced tissue injury size in a rat model of myocardial infarction.15

We found a decrease in MAP during streptokinase treatment. This phenomenon has been reported previously, and it is independent of the size and site of the infarcted area.22 This decrease coincides in time with the release of anaphylatoxin. This suggests that hypotension may be at least partially complement mediated, even if a quantitative correlation between the extent of complement activation and blood pressure fall is lacking. Other mechanisms may contribute to the streptokinase-mediated hypotension. Plasmin, for example, facilitates bradykinin generation by acting on high-molecular-weight kininogen.23 We are currently studying the role of bradykinin in the pathogenesis of streptokinase-mediated hypotension.

Conclusions

Our study demonstrates that (1) uncomplicated AMI does not cause activation of the complement system detectable in the blood stream; (2) streptokinase treatment causes abrupt complement activation through the classic pathway; and (3) complement activation entails transient leukopenia and possibly contributes to hypotension during streptokinase infusion.

References


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