Brief Rapid Communication

Coronary Thrombolysis With Recombinant Staphylokinase in Patients With Evolving Myocardial Infarction

Désiré Collen, MD, PhD, and Frans Van de Werf, MD

Background. Staphylokinase (STA), a protein with known profibrinolytic properties, is produced by transduced *Staphylococcus aureus* strains. In experimental animal models, recombinant staphylokinase (STAR) is less immunogenic and more active toward platelet-rich arterial blood clots than streptokinase.

Methods and Results. In the present study, 40 mg STAR given intravenously over 30 minutes was found to induce angiographically documented coronary artery recanalization within 40 minutes in four of five patients with acute myocardial infarction. Plasma fibrinogen and α2-antiplasmin levels were unaffected, and allergic reactions were not observed. Postinfusion disappearance of STAR antigen followed a biphasic mode with a t1/2α of 6.3±0.6 minutes (mean±SD) and a t1/2β of 37±15 minutes, corresponding to a plasma clearance of 270±100 mL/min. Neutralizing antibodies against STAR could not be demonstrated at baseline and up to 6 days after infusion, but STAR neutralizing activity, which did not cross-react with streptokinase, was consistently demonstrable in plasma at 14–35 days.

Conclusions. STAR can induce clot-selective coronary thrombolysis in patients with evolving myocardial infarction without concomitant induction of a systemic lytic state. STAR, a small protein that can be easily produced by recombinant DNA technology, may therefore offer promise for thrombolytic therapy in patients with thromboembolic disease. (Circulation 1993;87:1850–1853)

Key Words • thrombolytic therapy • acute myocardial infarction • staphylokinase • fibrin specificity • immunogenicity • pharmacokinetics

Thrombolytic therapy in patients with acute myocardial infarction has been shown to recanalize occluded coronary arteries, preserve left ventricular function, and reduce mortality. At present, coronary thrombolysis is primarily performed with streptokinase or with alteplase, a preparation of recombinant tissue-type plasminogen activator (rt-PA). The main advantages of streptokinase are its low cost and relative safety, whereas the main shortcomings are its limited efficacy for coronary recanalization and its immunogenicity, which predisposes to allergic reactions and to resistance to repeated administration. The main advantages of alteplase are its higher efficacy for coronary recanalization and its lack of immunogenicity, whereas its main disadvantage is its high cost. Thus, thrombolytic agents with higher thrombolytic potency, reduced side effects, and/or lower cost would be desirable.

Staphylokinase (STA), a protein of 136 amino acids that does not contain disulfide bridges, is produced by transduced *Staphylococcus aureus* strains and has been known for more than 40 years to have profibrinolytic properties. Its mechanism of action, its in vitro fibrinolytic properties, and its in vivo thrombolytic properties in experimental animal models have been evaluated to some extent. Like streptokinase, staphylokinase is not an enzyme, but it forms a 1:1 stoichiometric complex with plasminogen that then activates other plasminogen molecules. However, whereas the streptokinase–plasminogen complex undergoes an intramolecular transition without peptide bond cleavage, which results in exposure of a titrable active site in the plasminogen molecule, activation of the staphylokinase–plasminogen complex requires its conversion to plasmin. Furthermore, the staphylokinase–plasmin complex is very rapidly inhibited by α2-antiplasmin, whereas the streptokinase–plasmin complex is not.

The gene coding for staphylokinase has been cloned and expressed in *Escherichia coli* and in *Bacillus subtilis*, and the biochemical properties of recombinant staphylokinase (STAR) have been studied recently in some detail. Although the initial in vivo experiments with staphylokinase in dogs have yielded discouraging results, we have recently obtained evidence that STAR is fibrin specific, relatively more potent than streptokinase toward platelet-rich clots, and less immunogenic. Our studies suggested that several mechanisms may contribute to the fibrin specificity and the potency of staphylokinase in a plasma milieu. First, the plasmin–staphylokinase complex is rapidly neutralized by α2-antiplasmin (apparent second-order rate constant 2×104 M⁻¹·sec⁻¹) but this reaction is 130 times slower when the lysine binding sites of plasmin in the complex are either removed or saturated by binding to 6-amino-
hexanic acid or to fibrin.16 This would result in a protection of fibrin-bound complex against rapid inhibition and restriction of the plasminogen activation phenomenon to the clot surface. Second, staphylokinase dissociates in active form from the plasmin–staphylokinase complex after its inhibition by α2-antiplasmin and released staphylokinase molecules are recycled to other plasminogen molecules.15 Third, the plasminogen–staphylokinase complex is inactive and unable to convert to active plasmin–staphylokinase complex at appreciable rates in the presence of excess inhibitor.6 This observation may provide some explanation for the remarkable stability of plasminogen in plasma in the presence of relatively high concentrations of staphylokinase.10 Finally, fibrin disturbs this stability of staphylokinase in a plasma milieu, producing fibrin-specific clot lysis both in vitro systems as well as in in vivo situations.

In the present pilot study, we have evaluated the pharmacokinetic, thrombolytic, and immunogenic properties of STAR in five patients with acute myocardial infarction.

Methods

Recombinant Staphylokinase

STAR was produced from culture broth of E. coli cells transformed with recombinant pUC19 containing the 2.9 kb insert obtained by HindIII restriction enzyme digestion of genomic DNA obtained from a selected S. aureus strain.2 STAR was purified from 10-L batches by chromatography on SP-Sephadex with pH gradient elution, SP-Sephadex with NaCl gradient elution, and Sephacryl S-300 superfine gel filtration. It was obtained with a recovery of approximately 1.5 mg/L culture broth and a yield of 35%; it consisted of a single band on SDS-polyacrylamide gel electrophoresis with M, 18,000, and it had NH2-terminal sequence Ser-Ser-Ser-Phe-Asp-.17 After filtration on 0.22-μm millipore filters, the materials were sterile and virus free by routine screening for human pathogenic viruses; they were obtained at a concentration of ≈1 mg/mL, with a specific activity of 185,000 arbitrary units per milligram2 and an endotoxin content of 10±7 units/mg. Bolus injection at a dose of 3 mg/kg in mice did not produce weight loss or reduced weight gain within 8 days.17

Patients

Patients were studied after giving informed consent, and the protocol was approved by the Human Studies Committee of the University of Leuven. The following criteria were applied for inclusion: a history of chest pain typical of myocardial ischemia persisting for at least 30 minutes, a >2-mm ST-segment elevation in at least two frontal planes or two precordial ECG leads, and feasibility of initiating thrombolytic therapy within 6 hours of the onset of symptoms. Criteria for exclusion included major illness other than coronary artery disease, contraindications to thrombolytic agents, and concomitant anticoagulation.

After entry into the study in the emergency ward, the patient was immediately transferred to the cardiac catheterization laboratory, where selective coronary arteriography was immediately performed from the right femoral artery after injection of a bolus of 5,000 units of heparin. When coronary artery occlusion was documented, an intravenous bolus injection of 1 mg of STAR was given over 2 minutes followed by an infusion of the remaining 9 mg over 30 minutes. Arteriography of the occluded coronary artery was repeated at 10-minute intervals during the infusion, and the angiographic evaluation was terminated after the 40-minute angiogram. When coronary reperfusion was not obtained within 40 minutes, the patients were immediately treated with 100 mg rt-PA (Actilyse, Boehringer Ingelheim) given over 90 minutes. At 30 minutes, all patients were also given 320 mg aspirin, and a continuous intravenous infusion of heparin (1,000 units/hr) was started.

Blood Samples

Blood samples were collected on citrate (final concentration, 0.01 M) before and at 0, 10, 20, 30, 35, 40, 45, 60, 75, and 90 minutes after the start of the infusion of STAR. The fibrinogen and α2-antiplasmin were assayed as described elsewhere.14 Fibrin fragment D-dimer and STAR-related antigen levels in plasma were assayed with enzyme-linked immunosorbent assays using two murine monoclonal antibodies raised with the purified antigens. Additional blood samples were obtained at 4, 7, and 14–35 days for the determination of STAR-neutralizing antibodies using the staphylokinase reactivity assay with 350 μL citrated plasma, as described elsewhere.13

The pharmacokinetic parameters of the disposition of STAR from blood were derived from serial measurements of plasma antigen levels after the end of the infusion. The data describing the postinfusion disappearance of STAR-related antigen from plasma were plotted on semilogarithmic paper and fitted with a sum of two exponential terms, C(t)=Re−αt+Se−βt by graphical curve peeling.18 The variables A and B were first calculated (assuming steady state at the end of the infusion) using the formulas A=RXα/κs, and B=RXβ/κs, where Xs is the total administered dose and κs is the rate of infusion. Pharmacokinetic parameters were calculated from the coefficients A and B and exponents α and β using standard formulas derived by Gibaldi and Perrier.18 These pharmacokinetic parameters included initial half-life (in minutes): t1/2α=ln2/α; terminal half-life (in minutes): t1/2β=ln2/β; volume of the central (plasma) compartment (in liters): Vc=dose/(A+B); area under the curve (in micrograms per minute per milliliter): AUC=A/α+B/β; and plasma clearance (in milliliters per minute): Clc=−dose/AUC.

Results

Five patients with acute coronary occlusion were studied between June 25 and September 22, 1992. Relevant characteristics of the patients are shown in Table 1. Coronary artery recanalization within 40 minutes was achieved in four of five patients. The patient without recanalization at 40 minutes was immediately given 100 mg alteplase over 90 minutes without any clinical evidence of reperfusion during the infusion. Coronary angiography was repeated at 20–28 hours in four of the five patients. In one patient with early recanalization, reocclusion had occurred (clinically at 21 hours, when the activated partial thromboplastin time [aPTT] was normal notwithstanding continuous heparin infusion), whereas in the patient with persistent
TABLE 1. Response to Recombinant Staphylokinase in Five Patients With Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Infarct diagnosis</th>
<th>Locus of occlusion</th>
<th>Duration of symptoms* (hours)</th>
<th>Angiographic patency (TIMI grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>54</td>
<td>Inferolateral</td>
<td>Proximal RCA</td>
<td>3.8</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>59</td>
<td>Anterolateral</td>
<td>First diagonal</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>71</td>
<td>Inferolateral</td>
<td>Proximal RCA</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>71</td>
<td>LBBB, inferior</td>
<td>Middle RCA</td>
<td>4.0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>70</td>
<td>Anterior</td>
<td>Proximal LAD</td>
<td>2.5</td>
<td>0</td>
</tr>
</tbody>
</table>

TIMI, thrombolysis in myocardial infarction; LBBB, left bundle branch block; RCA, right coronary artery; LAD, left anterior descending coronary artery.

*Before admission to the emergency unit.
†Clinical reinfarction occurred at 21 hours when the activated partial thromboplastin time value was within the normal range.
‡Clinical recanalization occurred at 12 hours after infusion of thrombolytic agents.

angiographic occlusion after STAR administration, spontaneous recanalization had occurred (clinically at approximately 12 hours). All patients survived the episode of acute infarction without adverse effects or any apparent complication related to the infusion. The fifth patient with a major anterior infarction developed cardiogenic shock in the hours after recanalization of the infarct-related artery. The patient was not subjected to the 24-hour repeat angiography, and he died in cardiogenic shock after 30 hours.

Administration of STAR did not induce an overt systemic lytic state in any of the patients, as shown by the unchanged levels of fibrinogen and α2-antiplasmin (residual levels at 40 minutes, 90–95% of baseline) (Table 2). The aPTT remained above twice the baseline value during the 40-minute angiographic observation period. Fibrin fragment D-dimer increased significantly and progressively with infusion of STAR, from a median value of 150 ng/mL before to 1,500 ng/mL at 30 minutes and 2,200 ng/mL at 60 minutes, suggesting that detectable fibrin digestion occurred in vivo.

The concentration of STAR-related antigen in blood toward the end of the infusion of 10 mg STAR over 30 minutes increased to between 0.9 and 1.7 μg/mL (Table 3). After the end of the infusion, STAR-related antigen disappeared from plasma in a biphasic mode with an initial half-life of 6.3±0.6 minutes and a terminal half-life of 37±15 minutes, corresponding to a plasma clearance of 270±100 mL/min.

STAR-neutralizing activity in plasma was not consistently detectable at baseline and at 4–7 days after the STAR infusion (Table 2). However, at 14–35 days, neutralizing titers of 12–42 μg/mL plasma were found. The STAR-neutralizing activity consisted most likely of antibodies, because it could be quantitatively removed by adsorption on protein A-Sepharose. The antibodies induced after STAR administration did not cross-react with streptokinase.

### Discussion

This study evaluates the feasibility of intravenous infusion of 10 mg STAR for coronary thrombolysis in patients with acute myocardial infarction. The administration scheme used was based on our previous experience in baboons, which suggested that STAR had a similar potency as streptokinase for venous clot lysis but a higher potency for arterial clot lysis. Intra-coronary infusion of STAR at this dose induced coronary artery recanalization within 40 minutes in four of five patients. Recanalization was not associated with systemic fibrinolytic activation and fibrinogen breakdown.

Neutralizing antibodies could not be detected at baseline and within 1 week after administration of STAR but were consistently observed after 14–35 days. These induced antibodies did not, however, cross-react with streptokinase. Thus, with respect to immunogenicity, our initial observations in humans are not as encouraging as our experience in baboons, where three

### Table 2. Hemostasis Variables During and After Recombinant Staphylokinase Infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fibrinogen (g/L)</th>
<th>α2-Antiplasmin (%)</th>
<th>Activated partial thromboplastin time (seconds)</th>
<th>D-Dimer (μg/mL)</th>
<th>Neutralizing activity (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.7</td>
<td>3.3</td>
<td>67</td>
<td>60</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
<td>2.6</td>
<td>2.8</td>
<td>110</td>
<td>110</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>3.7</td>
<td>75</td>
<td>71</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
<td>2.3</td>
<td>97</td>
<td>81</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>3.8</td>
<td>3.6</td>
<td>95</td>
<td>96</td>
<td>...</td>
</tr>
<tr>
<td>Mean</td>
<td>3.3</td>
<td>3.1</td>
<td>89</td>
<td>84</td>
<td>40</td>
</tr>
<tr>
<td>SD</td>
<td>0.5</td>
<td>0.6</td>
<td>17</td>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

STAR, recombinant staphylokinase.

*Values >180 seconds were given a value of 180 seconds for calculation of the mean and SD.
of four animals did not even produce neutralizing antibodies after a five-times repeated administration of STAR at weekly intervals. However, we have recently obtained evidence (Declerck et al, unpublished) that the prevalence of anti-STAR antibodies in the general population is lower than that of antistreptokinase antibodies. Thus, the initial anticipation that STAR might produce fewer allergic reactions than streptokinase is not definitively invalidated by the present demonstration of its immunogenicity. Although the restriction to single use as a result of immunogenicity would probably apply to both streptokinase and STAR, the absence of cross-reactivity of induced antibodies suggests that the use of both substances would not be mutually exclusive.

Although the small number of patients studied precludes valid estimation of the frequency of coronary recanalization with STAR and of the adequacy of the dose used, this feasibility study suggests that intravenous infusion of 10 mg STAR may produce fibrin-specific coronary thrombolysis. Definition of the relative therapeutic benefit or lack thereof will require more detailed initial dose-finding studies followed by randomized clinical trials against other thrombolytic agents.

The STAR material used in the present study was obtained by expression of the staphylokinase gene cloned from the genomic DNA of a transduced S. aureus strain in E. coli using standard recombinant DNA techniques developed in the 1970s. The small size of the protein and its lack of disulfide bridges make it ideally suited for large-scale production in E. coli.

### Conclusion

The results of the present initial pilot study in humans confirm those of our previous studies in animals with venous or coronary artery thrombosis that STAR is an effective thrombolytic agent and extend these observations to patients with evolving myocardial infarction. The present preliminary experience may provide a basis for the design of future dose-finding studies and controlled clinical trials with this agent.

### Acknowledgments

The authors thank their collaborators (identified in the reference list) for their contributions to the production of STAR and its conditioning for use in humans. The authors also thank the catheterization laboratory personnel for their expert assistance and are grateful to the patients who volunteered to participate in this first human study with an experimental substance.

### References

Coronary thrombolysis with recombinant staphylokinase in patients with evolving myocardial infarction.
D Collen and F Van de Werf

Circulation. 1993;87:1850-1853
doi: 10.1161/01.CIR.87.6.1850

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/87/6/1850

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/