Streptokinase-Induced Platelet Aggregation
Prevalence and Mechanism

Douglas E. Vaughan, MD; Elisabeth Van Houtte; Paul J. Declerck, PhD; and Désiré Collen, MD, PhD

Background. Streptokinase (SK) is a bacteria-derived protein and one of the plasminogen activators that is currently available for therapeutic use. Exposure to SK induces synthesis of specific antibodies that may initiate platelet aggregation and paradoxical clot propagation during treatment.

Methods and Results. Using platelet-rich plasma (PRP), we found that SK (5,000 units/ml) but not urokinase (2,500 units/ml) or recombinant tissue-type plasminogen activator (2,500 units/ml) caused platelet aggregation in PRP from 14 of 100 normal volunteers. In 13 consecutive patients treated with SK for acute myocardial infarction, SK-mediated platelet aggregation was induced in five patients within 1 week after treatment. SK-mediated platelet aggregation was associated with significantly increased titers of both anti-SK antibodies and SK-neutralizing activity in plasma; it was partially inhibited by aspirin (1 mM) and by aprotinin (500 kallikrein inhibitor units/ml) and completely inhibited by tranexamic acid (1 mM) and by prostaglandin E1 (9 μM). Addition of SK (1,000 or 5,000 units/ml) induce a statistically significant dose-dependent thromboxane B2 release in mixtures of PRP with plasma from subjects with SK-induced aggregation but not in samples of PRP mixed with plasma from nonresponders; addition of recombinant tissue-type plasminogen activator (1 or 50 μg/ml) did not induce thromboxane B2 release. Mixing experiments with PRP and immunoglobulin G from reactive and nonreactive donors revealed that SK-induced aggregation requires the presence of anti-SK antibodies. When 125I-SK (50 nM) was used, platelets preincubated with plasminogen (0.5 μM) bound 9,500±600 (mean±SEM, n=6) molecules SK/platelet, which increased to 25,000±3,100 molecules/platelet after thrombin stimulation. Tranexamic acid (1 mM) blocked specific binding of SK to resting platelets.

Conclusions. These data demonstrate that SK-induced platelet aggregation is initiated by the binding of anti-SK antibodies to the SK–plasminogen complex located on the platelet surface. SK-induced platelet activation may limit the therapeutic effectiveness of the drug, and in view of the high prevalence of aggregation in a normal population, prospective evaluation of the effects of platelet aggregation during treatment with SK is warranted. (Circulation 1991;84:84–91)

Streptokinase (SK) is one of the plasminogen activators currently available for therapeutic use, but it is derived from nonhuman sources. Exposure to SK induces specific antibody formation and provokes allergic responses in up to 5% of patients receiving SK. SK has been reported to induce thromboxane A2 synthesis and platelet activation when administered to patients with acute myocardial infarction, and it may initiate platelet aggregation by a mechanism involving specific anti-SK antibodies. Theoretically, platelet activation brought about by SK may limit the therapeutic effectiveness of the drug and may contribute to the pathogenesis of early reocclusion. In experimental studies, inhibition of platelet function has been shown to enhance the thrombolytic response to SK and to prevent reocclusion.

The present study was designed to 1) define the prevalence of SK-mediated platelet aggregation in a control population and in a population after SK infusion, 2) correlate platelet aggregation with anti-SK antibody titers, and 3) elucidate the mechanism of SK-induced platelet aggregation.

Methods

Materials

Three different preparations of SK were used: albumin-free reagent quality SK from Sigma Chemical Co., St. Louis, for coating of enzyme-linked

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immunosorbent assay (ELISA) plates and for quantitative binding studies; Kabikinase from Kabi Diagnostica, Stockholm, Sweden; and Streptase from Hoffmann-La Roche, Basel, Switzerland. These three SK preparations were used interchangeably for platelet aggregation studies. Recombinant tissue-type plasminogen activator (rt-PA, Actilyse) was provided by Boehringer Ingelheim, Ingelheim, FRG.; urokinase (Abbokinase) was purchased from Abbott Pharmaceutica, Chicago, Ill., and acylated plasminogen–SK activator complex (APSAC, Eminase) was purchased from Beecham Pharmaceuticals, Brussels. Tranexamic acid (Exacyl) was from Laboratoires Choay, Paris; 6-aminohexanoic acid (6-AHA) was from BDH Pharmaceuticals, Poole, U.K.; aprotinin (Trasylol) was from Bayer Pharmaceutica, Brussels; prostaglandin El (PGE1) was from The Upjohn Co., Kalamazoo, Mich.; and aspirin (Aspetic) was from Synthelabo Benelux, Brussels. Silicon oil was from Janssen Pharmaceutica, Beerse, Belgium; and Sepharose 2B, protein A–Sepharose, and Sephadex G25 were from Pharmacia, Upsala, Sweden. Na125I was from IRE, Fleurus, Belgium; goat immunoglobulin G (IgG) raised against human IgG and conjugated with horseradish peroxidase was from Sigma Chemical; o-phenylenediamine was from Fluka, Buchs, Switzerland; and luciferin-luciferase (Chromolume 250, Chrono-log Corp., Havertown, Pa.) was obtained from Pierce Chemical Co., Rockford, Ill. The recombinant human plasminogen mutant Ala740 was produced by site-directed mutagenesis of the active site serine to alanine, as described elsewhere.

Collection and Preparation of Human Platelets

Venous blood was collected into 0.11 M trisodium citrate to a final dilution of 9:1 (blood:citrate). Blood was obtained from 100 healthy volunteers and from 13 patients with acute myocardial infarction before and 7 days after infusion of 1.5 million units SK over 60 minutes.

Platelet-rich plasma (PRP) was prepared by centrifugation at 100g for 10 minutes at room temperature. The top two thirds of the PRP was removed for aggregation experiments and kept for up to 1 hour at room temperature before use. Platelet counts were determined either by a cell counter (model 810, Baker Instruments, Altentown, Pa.) or by microscope. Platelets were treated with aspirin by incubation of PRP for 30 minutes with 1 mM aspirin at 37°C. Platelets were separated from plasma by gel filtration of PRP on a column of Sepharose 2B, equilibrated with HEPES balanced salt buffer at a flow rate of 2 ml/min at room temperature.

Platelet Aggregation

To determine the prevalence of SK-induced platelet aggregation in the normal population, fresh PRP from each of the 100 volunteers was used. Platelet aggregation was monitored at 37°C with stirring using a dual-chamber aggregometer after the addition of SK (1,000, 5,000, or 10,000 units/ml), urokinase (5,000 units/ml), or rt-PA (2,500 units/ml) to 250 µl PRP. In four individuals with SK-induced aggregation, platelet aggregation was also measured after the addition of 0.14 units APSAC. The SK, urokinase, and APSAC units indicated on the labels were used, and rt-PA was converted from the weight indicated on the label to units, assuming a specific activity of 500,000 units/mg. The effect of aprotinin (500 units/ml), PGE1 (9 µM), tranexamic acid (1 mM), or 6-AHA (1 mM) on SK-induced platelet aggregation was studied using PRP from reactive donors. The inhibitors were added to PRP 2 minutes before the addition of SK (5,000 units/ml).

The secretion of adenosine triphosphate (ATP) during platelet aggregation was measured using the firefly luminescence method in a Lumaggregometer (Chrono-Log Corp., Havertown, Pa.). For the measurement of thromboxane B2, samples of PRP from nonresponsive donors were mixed 1:1 with plasma from previously identified donors that either supported platelet aggregation (responders) or that did not support platelet aggregation (nonresponders) after the addition of SK (5,000 units/ml) to PRP. SK or tissue-type plasminogen activator (t-PA) was added to the platelet suspensions, incubated for 4 minutes at 37°C with stirring, and then centrifuged at 10,000g for 3 minutes. The amount of thromboxane B2 in the plasma supernatant was measured by a standard radioimmunoassay as previously described.

Titration of Anti-SK Antibodies and SK Neutralizing Activity

Antibody titers to SK in plasma were measured using a one-site noncompetitive ELISA according to the method of Engvall and Perlman. Wells of micro-ELISA plates were incubated with a 200-µl solution of albumin-free SK (1,000 units/ml) in phosphate buffered saline (PBS) for 24 hours at 4°C. The wells were then treated with PBS containing 1% bovine serum albumin and washed three times with PBS containing 0.002% Tween 80 (PBS-Tween). Plasma (100 µl) in appropriate twofold dilutions was added to the wells and incubated for 2 hours at room temperature. The wells were then washed three times with PBS-Tween and incubated for 1 hour with the horseradish peroxidase–conjugated goat IgG raised against human IgG. After three additional washes with PBS-Tween, the peroxidase reaction was performed by addition of 200 µg/ml o-phenylenediamine and 0.003% hydrogen peroxide in 0.1 M citrate and 0.2 M sodium phosphate buffer, pH 5.0. After 1 hour, the reactions were arrested by addition of 50 µl of 4 M sulphuric acid, and the absorbance was read at 492 nm.

Antibodies neutralizing SK-induced clot lysis were titrated by the method of Deutsch and Fisher using kits obtained from Biomerieux (Charbonneries les Bains, France) and serial fourfold diluted plasma...
samples (1/10, 1/40, and 1/160). The anti-SK antibody titers and the SK-neutralizing activity were expressed in units by comparison with the control serum supplied with the Biomerieux kit (lot 102338A), which had an assayed anti-SK activity of 80 units/ml. In the micro-ELISA, 0.005 units/ml of this standard produced an absorbancy at 492 nm of 0.49±0.02; therefore, the titer of plasma dilution producing an absorbance closest to 0.5 was assumed to contain 0.005 units/ml. In the clot lysis inhibition assay, the units were determined as the reciprocal of the highest plasma dilution inhibiting lysis with 1 unit of SK. The assays were performed by a technician who was blinded with respect to the aggregatory response of the plasma toward SK.

**Immunoglobulin G Preparation**

Serum from one individual with an SK-induced platelet aggregation, an anti-SK antibody titer of 25 units/ml, and an SK-neutralizing activity titer of 40 units/ml was used for the purification of IgG. Serum (5 ml) was applied to a 0.9×15-cm column of protein A-Sepharose at room temperature, a flow rate of 12 ml/hr was used, and the eluate was collected in 2-ml fractions. IgG-depleted serum was collected, pooled, and stored at −20°C. The column was then washed with 30 ml of 0.1 M Tris-HCl buffer, pH 8.1, and eluted with 0.1 M glycine-HCl buffer, pH 2.8. IgG containing eluates were pooled, the pH was neutralized with 1 M Tris-HCl buffer, pH 9.0, and the solution was dialyzed against PBS. IgG from a non-responsive donor was also purified in an identical manner. Protein concentrations were determined spectrophotometrically at 280 nm using an absorbance coefficient (A1 cm=13.5); the purity of the IgG preparation was confirmed by sodium dodecyl sulfate gel electrophoresis.17

**Protein Iodination**

SK was radiolabeled with Na<sup>125</sup>I using Iodo-Beads. Two Iodo-Beads were added to a mixture of 1 ml SK (1 mg/ml dissolved in PBS) and 1 mCi Na<sup>125</sup>I and incubated for 30 minutes at room temperature. Unbound radiiodine was removed by passing the labeled protein over a Sephadex G25 column equilibrated with 0.15 M NaCl and 0.05 M Tris-HCl buffer, pH 7.4. Protein labeled by this method had a specific activity of approximately 6.0×10<sup>5</sup> cpm/μg. Greater than 96% of the radioactivity was precipitable by 13% trichloroacetic acid.

**Platelet Binding Assay**

In binding assays, 425 μl gel-filtered human platelets (1.5×10<sup>8</sup> platelets/μl) in HEPES balanced salt buffer were incubated with 125<sup>I</sup>-SK in the absence or presence of plasminogen (0.5–2.5 μM). Platelet activation was performed by the addition of thrombin (0.01 NIH units/ml) before addition of SK and plasminogen, and additional buffer was added to a final volume of 675 μl. After a 30-minute incubation period, bound ligand was separated from free by layering duplicate 325-μl aliquots over 250 μl silicon oil (specific gravity, 1.04) in 1.5-ml conical polypropylene tubes. These samples were then centrifuged for 5 minutes in a microcentrifuge. The supernatant was decanted by inversion, and the tips of the tubes (containing the platelet pellet) were amputated and counted in a scintillation counter. All experiments were performed in triplicate. Bound 125<sup>I</sup>-SK was expressed as molecules bound per platelet, based on the specific activity of 125<sup>I</sup>-SK, a molecular mass of SK of 47,000 Da, and the platelet count.

**Statistical Analysis**

Data are presented as mean±SEM unless otherwise indicated. The statistical significance of differences within or between groups was determined using Student’s t test for paired or unpaired values, respectively. Comparison of categorical variables was performed by χ<sup>2</sup> analysis, and linear regression analyses were performed using a statistical software package (PRIMER) on an IBM personal computer. Values of p<0.05 were considered statistically significant.

**Results**

**Prevalence of SK-Induced Platelet Aggregation**

Venous blood was collected from 100 normal volunteers, and PRP was generated as described above. All of the subjects were healthy blood donors, and none had previously received SK. Forty-one percent of the donors were women, and the age of the donors was 39±13 (mean±SD) years. Fourteen of the samples tested showed a pronounced platelet aggregation response after the addition of SK to PRP to a final concentration of 5,000 units/ml (Table 1). There were nine men and five women in this group, with an age distribution of 35±12 years. None of these 14 samples of PRP aggregated with 1,000 units SK/ml, whereas only eight of 14 aggregated with 10,000 units SK/ml. Urokinase and rt-PA did not initiate platelet aggregation in any sample. APSAC also initiated platelet aggregation in PRP from four of four (100%) selected individuals with an aggregatory response to SK. Fifteen individuals were retested between 2 weeks and 2 months after their first screening; 10 individuals tested negative on both occasions, and

<table>
<thead>
<tr>
<th>SK (units/ml)</th>
<th>N</th>
<th>Platelet aggregation (n)</th>
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</thead>
<tbody>
<tr>
<td>1,000</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5,000</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>10,000</td>
<td>100</td>
<td>8</td>
</tr>
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</table>

N, number of samples tested; n, number of samples in which streptokinase (SK) induced (+) or did not induce (−) aggregation. The samples in which aggregation was obtained at a SK concentration of 10,000 units/ml constitute a subgroup of the 14 samples in which aggregation was also demonstrated at a SK concentration of 5,000 units/ml.
five individuals tested positive on both occasions. The frequency distribution of anti-SK antibody titer and SK-neutralizing activity is depicted in Figures 1A and 1B, respectively. Platelet aggregation was observed in 13 of 34 subjects with a titer of 0.8 units/ml or more and in one of 66 with a titer of less than 0.8 units/ml ($p<0.001$). The median titer in the subjects with aggregation was 13 units/ml; in the subjects without aggregation, it was 0.4 units/ml. Platelet aggregation occurred in 12 of 28 subjects with a SK-neutralizing activity of more than 10 units/ml and in two of 72 subjects with SK-neutralizing activity of less than 10 units/ml ($p<0.001$). Linear regression analysis of the SK-neutralizing activity versus the anti-SK antibody titers yielded a correlation coefficient of 0.7.

Table 2 summarizes the antibody titers for the group divided on the basis of the aggregatory response to SK in vitro. Both anti-SK antibody titers and SK neutralizing activity were significantly higher in the subjects with SK-induced platelet aggregation than in the nonresponsive group.

Venous blood samples were also obtained from 13 patients before and 1 week after treatment with SK for acute myocardial infarction. Platelet aggregation induced with 5,000 units SK was measured using either PRP from the individual patients or by adding 125 µl of the patient’s plasma to 125 µl fresh PRP from a known nonreactive donor. Before treatment with SK, none of the 13 patients had SK-induced platelet aggregation, whereas five of these 13 patients were positive 1 week after treatment. During this 1-week period, the titer of anti-SK antibodies increased in this group from 5.3±2.0 to 52±16 units/ml ($p<0.01$).

### Mechanism of SK-Induced Platelet Aggregation

SK-induced platelet aggregation was accompanied by ATP secretion, which is indicative of platelet activation and secretion (Figure 2). Thromboxane B2 levels were measured in plasma after the addition of SK to samples of plasma from five positive and five negative donors mixed 1:1 with fresh PRP from a known nonreactive donor to final concentrations of 100, 1,000, or 5,000 units/ml and after the addition of rt-PA to a final concentration of 1 or 50 µg/ml (Table 3). rt-PA did not cause a significant release of thromboxane B2, whereas SK induced a concentration-dependent release of thromboxane B2. In the responders, a significant thromboxane B2 release was observed after the addition of 1,000 units/ml SK, a concentration that did not cause visible platelet aggregation.

The effects of inhibitors of fibrinolysis or of platelet function on platelet aggregation induced with 5,000 units SK/ml are summarized in Table 4. Tranexamic acid (1 mM), 6-AHA (1 mM), and PGE1 (9 µM) completely abolished SK-induced aggregation, whereas aprotinin (500 kallikrein inhibitor units/ml) and aspirin (1 mM) only partially inhibited SK-induced aggregation.

Figure 3 illustrates the dependence of SK-induced platelet aggregation on specific IgG. Aggregation was monitored after the addition of 5,000 units/ml SK to PRP preincubated with serum or purified IgG from

### Table 2. Correlation Between Platelet Aggregation Induced With 5,000 units/ml Streptokinase and Anti-Streptokinase Antibody Titer or Streptokinase-Neutralizing Activity

<table>
<thead>
<tr>
<th>Aggregation</th>
<th>Anti-SK antibody (units/ml)</th>
<th>SK-neutralizing activity (units/ml)</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25±6</td>
<td>44±10</td>
</tr>
<tr>
<td>Negative</td>
<td>3.2±1.3</td>
<td>16±2</td>
</tr>
<tr>
<td>$p$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

Values are mean±SEM. $n$, Number of samples in which streptokinase (SK) induced (positive) or did not induce (negative) platelet aggregation.

### Figure 1. Bar graphs showing frequency distribution of anti-streptokinase (anti-SK) antibody titer (panel A) and streptokinase (SK)-neutralizing activity (panel B) in 100 control subjects. Cross-hatched areas represent donors with SK-induced platelet aggregation.
either a responsive or a nonresponsive donor. Serum and IgG from the responsive donor supported SK-induced aggregation, whereas IgG-depleted serum from the same donor did not. Aggregation did not occur after the addition of SK to PRP mixed with either serum or IgG from a nonresponsive donor. The requirement of plasminogen in SK-induced platelet aggregation is demonstrated in Figure 4. Gel-filtered human platelets (final concentration 1.5×10⁶/ml) were resuspended in plasminogen-depleted plasma from a nonresponsive and a responsive donor. The mixtures were incubated with SK (5,000 units/ml) before the addition of plasminogen (150 μg/ml). Platelet aggregation was observed immediately after plasminogen was reintroduced to the mixture of platelets, SK, and plasma from the responsive donor. In a similar set of experiments, addition of the inactive plasminogen mutant (Ser⁷⁰→Ala⁷⁰⁴) induced aggregation up to 80% of that obtained by addition of native plasminogen.

**SK Binding to Platelets**

The results of binding experiments of ¹²⁵I-SK to gel-filtered platelets are summarized in Table 5. Resting platelets incubated with 50 nM ¹²⁵I-SK bound approximately 6,000 molecules of SK per platelet in the absence of plasminogen. This binding was less than 50% reversible by the addition of excess unlabeled SK. Addition of plasminogen resulted in a concentration-dependent increase in binding, which was largely reversible with excess SK or with 1 mM tranexamic acid. In the presence of plasminogen, thrombin-activated platelets bound twofold to fivefold more SK molecules per platelet than did resting platelets; this binding was partially reversible with excess unlabeled SK or tranexamic acid. The addition of IgG (20 μM) from a responsive donor did not enhance the binding of SK to resting platelets in the absence of plasminogen. However, in the presence of plasminogen (0.5 μM), IgG from a responsive donor markedly enhanced the binding of SK to platelets.

**Discussion**

Anti-SK antibodies and SK-neutralizing activity in plasma are ubiquitous in the adult population and increase in titer after infection with β-hemolytic streptococci or treatment with SK.18–20 This widespread occurrence has played a role in determining the therapeutic dose of SK¹⁹ and in the generalized use of adjunctive therapy with steroids. We⁵ previously reported a specific instance in which SK-specific antibodies supported platelet aggregation in vitro and were circumstantially related to an episode of clot propagation during intracoronary infusion of SK.

In the present study, we found a relatively high prevalence of SK-induced platelet aggregation in PRP from a normal population of healthy blood donors (14% with 5,000 units/ml SK). A highly significant statistical correlation was found between this in vitro aggregatory response and the anti-SK antibody titer, measured either by SK-neutralizing activity or by ELISA techniques. In some instances, however, a high titer of anti-SK antibodies did not correspond with in vitro aggregation to SK, suggest-
TABLE 4. Effects of Inhibitors of Fibrinolysis or of Platelet Function on Platelet Aggregation Induced With 5,000 units/ml Streptokinase

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Inhibition of SK-induced aggregation (%)</th>
</tr>
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<tbody>
<tr>
<td>Aprotinin</td>
<td>500 KIU/ml</td>
<td>60±14</td>
</tr>
<tr>
<td>6-AHA</td>
<td>1 mM</td>
<td>100</td>
</tr>
<tr>
<td>Tranexamic acid</td>
<td>1 mM</td>
<td>100</td>
</tr>
<tr>
<td>PGE₁</td>
<td>9 μM</td>
<td>100</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1 mM</td>
<td>75±16</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, Number of donors tested; SK, streptokinase; KIU, kallikrein inhibitor units; 6-AHA, 6-aminohexanoic acid; PGE₁, prostaglandin E₁. Compounds were incubated with platelet-rich plasma for 2 minutes before addition of 5,000 units/ml SK except for aspirin, which was incubated for 30 minutes at 37°C. Percent inhibition was determined by comparisons with platelet-rich plasma from the same donor used on the same day. All experiments were performed in duplicate.

ing that the platelet aggregating antibodies constitute a distinct qualitative subset of anti-SK antibodies.

Platelet aggregation was not observed with urokinase or rt-PA but did occur with APSAC, which contains immunoreactive SK. These observations indicate that SK-induced platelet aggregation is specific for SK and not dependent on the conversion of plasminogen to plasmin. It is important to note that thrombolytic therapy with t-PA and with SK has been associated with platelet activation, as evidenced by increased thromboxane B₂ production through an undefined mechanism, and hyperplasminemia itself has been shown to activate platelets and to stimulate thrombin activation, which is another potent agonist of platelet activation.

Plasminogen does appear to play a role in SK-induced platelet aggregation, since its introduction in a mixture of platelets, SK, and plasminogen-depleted plasma from a responsive donor promptly led to platelet aggregation. Furthermore, the addition of an inactive plasminogen mutant in an identical experiment also initiated platelet aggregation. However, aprotinin partially inhibited SK-induced platelet aggregation, suggesting a role for plasmin activity as well. Taken together, these findings can be interpreted as indicative of an interaction between two independent mechanisms in promoting platelet aggregation in the presence of SK: the first mechanism is immunologically based and requires plasminogen but not plasmin activity, and the second is related to the more generic platelet-activating effects of plasmin.

The role of specific anti-SK antibodies in SK-induced platelet aggregation is supported by mixing experiments using IgG, serum, and platelets. The combination of fresh human platelets from a nonresponsive donor, SK, and serum or IgG isolated from a responsive donor promoted platelet aggregation in vitro, whereas aggregation was not observed in identical experiments in which serum or IgG from a nonresponsive donor was used. Furthermore, in binding experiments using ¹²⁵I-SK, IgG does not appear to be a requirement for, nor does it appear to

FIGURE 4. Graph showing plasminogen dependence of streptokinase-induced platelet aggregation. Gel-filtered human plasma (50 μl of 7.5×10⁹/μl) were suspended in 200 μl plasminogen-depleted plasma obtained from a nonresponsive donor (A) and a responsive donor (B). Mixtures were incubated with streptokinase (5,000 units/ml), and plasminogen (1.5 μM) was added at times noted with arrow.

FIGURE 3. Graphs showing platelet aggregation after the addition of streptokinase (5,000 units/ml) to platelet-rich plasma from a nonresponsive donor (panel A), platelet-rich plasma from the same donor mixed 1:1 with serum from a responsive donor (panel B), the same platelet-rich plasma mixed with immunoglobulin G (final concentration, 2 mg/ml) from the responsive donor (panel C), platelet-rich plasma mixed with immunoglobulin G (final concentration, 2 mg/ml) from a nonresponsive donor (panel D), platelet-rich plasma mixed with immunoglobulin G–depleted serum from a responsive donor (panel E), and platelet-rich plasma mixed with serum from a nonresponsive donor (panel F). Arrows indicate time of addition of streptokinase.
TABLE 5. Binding of 125I-Streptokinase to Gel-Filtered Human Platelets

<table>
<thead>
<tr>
<th>Plasminogen (μM)</th>
<th>Competitor</th>
<th>Molecules bound per platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resting platelets</td>
</tr>
<tr>
<td>0</td>
<td>...</td>
<td>5,900±800</td>
</tr>
<tr>
<td>0.5</td>
<td>...</td>
<td>9,500±600</td>
</tr>
<tr>
<td>2.5</td>
<td>...</td>
<td>12,000±900</td>
</tr>
<tr>
<td>0</td>
<td>SK (3 μM)</td>
<td>3,400±400</td>
</tr>
<tr>
<td>0.5</td>
<td>SK (3 μM)</td>
<td>5,400±400</td>
</tr>
<tr>
<td>0.5</td>
<td>Tranexamic acid (1 mM)</td>
<td>5,600±500</td>
</tr>
<tr>
<td>0</td>
<td>IgG (20 μM)</td>
<td>5,600±600</td>
</tr>
<tr>
<td>0.5</td>
<td>IgG (20 μM)</td>
<td>49,000±3,800</td>
</tr>
</tbody>
</table>

Values are mean±SEM of three experiments performed in duplicate. SK, streptokinase; IgG, immunoglobulin G; ND, not done. Gel-filtered human platelets (425 μl at 1.5×10^5/μl) were incubated with 125I-SK (50 nM) in the presence of aprotinin (500 units/ml) and/or plasminogen, tranexamic acid, or excess unlabeled SK in the concentrations noted. Resting platelets were kept in buffer containing prostaglandin E_1 (9 μM) and theophylline (450 μg/ml). Platelets were activated by the addition of thrombin (0.01 NIH units/ml) 5 minutes before addition of potential ligands.

enhance, the direct binding of SK to platelets in the absence of plasminogen. Our observations indicate that SK interacts with platelets via plasminogen, which is known to bind directly to platelets. Plasminogen binding to platelets is markedly enhanced by thrombin stimulation and inhibited by lysine-binding analogues, such as tranexamic acid. In agreement with these findings, we observed that SK binding to platelets in the presence of plasminogen was markedly enhanced by thrombin stimulation and at least partially inhibited by tranexamic acid. These observations, taken together, strongly suggest that SK interacts with platelets via plasminogen and that anti-SK antibodies are not absolutely necessary for this binding to occur. However, in the presence of plasminogen, SK-specific antibodies markedly enhance the binding of SK to platelets. These findings provide the basis for a mechanism, albeit speculative, for SK-induced platelet aggregation, whereby SK-specific antibodies react with SK bound to platelets via plasminogen, which in turn leads to platelet activation, aggregation, and secretion.

Whether or not SK-specific platelet aggregatory antibodies promote thromboembolism during treatment with SK or diminish the therapeutic efficacy of the drug is not known. Induction of platelet aggregation in vitro did not occur with 1,000 units/ml SK, whereas it was readily obtained with 5,000 units/ml, which is substantially higher than circulating levels of SK during intravenous administration of 1.5 million units SK for 1 hour. However, in the responsive group, a small but significant release of thromboxane B_2 was observed with subaggregatory concentrations of SK (i.e., 1,000 units/ml). These findings suggest that a critical threshold concentration of SK must be present on the platelet surface before antibody-induced platelet aggregation can efficiently proceed but also that platelet activation occurs to some extent in the absence of overt aggregation. Furthermore, only eight of 14 positive samples aggregated after the addition of an even higher concentration of SK (i.e., 10,000 units/ml), which supports the concept that the ratio of antigen to antibody is also important in determining whether or not aggregation will proceed. When SK is delivered into a thrombosed peripheral or coronary artery, its local concentration may become high enough to induce platelet aggregation. Indeed, the first patient with SK-induced platelet aggregation was identified by clot propagation in a coronary artery coincidental with local infusion of SK. Others have also described a paradoxical occurrence of thrombosis during therapy with SK, whereas Fitzgerald et al reported a marked production of thromboxane during treatment with SK. The propensity of SK to activate platelets may play a role in primary resistance and in acute reocclusive phenomena after the administration of SK for acute myocardial infarction. In our studies, aspirin partially attenuated SK-induced platelet aggregation in vitro, which suggests that the additive effect of aspirin and SK with respect to clinical outcome in patients with acute myocardial infarction may be due, at least in part, to the effect of aspirin on SK-induced platelet aggregation.

In summary, specific, qualitatively distinct anti-SK antibodies are capable of promoting SK-induced platelet aggregation. This phenomenon occurs after anti-SK antibodies react with SK bound to the platelet surface via plasminogen, which in turn, triggers platelet activation and aggregation. SK-induced platelet aggregation appears to be related to the titer of anti-SK antibodies but in vitro requires the presence of relatively high concentrations of SK. Lower concentrations of SK promote platelet activation and thromboxane production in sensitized individuals, which may be partially mitigated by the simultaneous administration of aspirin. The influence of these antibodies on patient response and outcome during treatment with SK likely merits further investigation.

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obtaining samples from patients treated with SK, and B. Verheyden for her superb secretarial assistance.

References

Key Words • plasminogen activator, recombinant tissue-type • platelet aggregation • streptokinase • immunoglobulin G • thrombolysis
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