Inhibition of Platelet-Dependent Thrombus Formation by Human Activated Protein C in a Primate Model

By Andrés Gruber, John H. Griffin, Laurence A. Harker, and Stephen R. Hanson

The in vivo antithrombotic properties of human plasma activated protein C (APC), a natural anticoagulant enzyme, were investigated in a baboon model of thrombus formation on prosthetic vascular grafts. Infusion of 0.25 to 1.1 mg/kg/h purified, human, APC inhibited blood cloting, as measured by the activated partial thromboplastin time (APTT), and reduced vascular graft platelet deposition by 40% to 70%, as determined by the real-time scintillation camera imaging of 111In-labeled platelet deposition. APC infusion also preserved graft patency. Hemostatic plug formation remained normal, as measured by the template bleeding times. These results suggest that APC administration may produce immediate antithrombotic effects under arterial flow conditions.

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samples in 100 μL of this buffer per well were incubated (one hour, 37°C) for adsorption of PC antigen to the immobilized antibody. Subsequently, unbound constituents (eg, PC inhibitors) and the reversible inhibitor of APC (ie, benzamidine) were washed away and thus did not affect the C3-bound enzyme. The control wells were incubated with immunoaffinity-purified goat-polyclonal antihuman PC antibodies (1 mg/mL) for ten minutes to block the amidolytic activity of the C3-bound APC. Finally 100 μL of 0.7 mmol/L chromogenic substrate (S-2401 or S-2366, Kabi, Stockholm) for the APC was added to each well. Amidolytic activity was measured from the change of absorbance at 405 nm. The APC levels of the samples were calculated from the amidolytic activity of standards using dilutions of the reference human APC (0.005 to 5.0 μg/mL) in baboon plasma containing benzamidine and applied to the wells as described above. Standards prepared from dilutions of APC in blocking buffer gave similar values to plasma standards, suggesting that APC is protected from its plasma inhibitors under these conditions.

Other laboratory studies. APTT was determined within ten minutes from arterial blood sampling. Arterial blood was also collected into 1 mol/L EDTA, 1 mol/L benzamidine anticoagulant (100 parts blood, one part anticoagulant) before and 45 minutes after starting the infusion of APC into baboons. D-dimer antigen levels were determined using solid-phase radiometric assays (IRMA and RIA), while t-PA and plasminogen antigen levels were measured during and following infusions (1/1/1/1) of 0.25 mg/kg APC (A) and 1.10 mg/kg APC (B).

Inhibition of platelet deposition. Images of the vascular grafts were acquired and analyzed as described previously. Platelet deposition (PD) was calculated as PD = P1 × CPMf/CPMα, where platelet count (P1) is the number of platelets in 1 mL of circulating whole blood, CPM is the numeric value for the In radioactivity on the graft (CPMf) or in 1 mL of autologous blood (CPMα). Inhibition of platelet deposition was calculated as 1 – 100 × (1 – PDα/PDf × P1/P1), where α indicates an APC experiment and c indicates a control study. This equation was used because platelet deposition onto this type of Dacron graft has been shown to depend in a linear fashion on the circulating platelet count.

RESULTS AND DISCUSSION

APC was administered into the AV shunt proximal to the vascular graft as a bolus comprising approximately 25% to 30% of the total dose given immediately prior to insertion of the vascular graft, followed by continuous infusion throughout the subsequent 50 to 60 minutes. Four baboons received a total dose of 0.25 mg/kg APC (range 0.20 to 0.34 mg/kg). In these animals the circulating plasma APC levels averaged 0.48 ± 0.02 μg/mL (± SEM; range 0.27 to 0.75 μg/mL; n = 20) during five to 45 minutes of the infusion. Four other animals received 1.1 mg/kg APC, and the plasma levels averaged 1.58 ± 0.11 μg/mL (range 0.85 to 2.90 μg/mL; n = 20) during five to 45 minutes of the infusion. The measurements of APTT and APC amidolytic activity during the infusion period were comparable (Figs 1A and B), suggesting a direct relationship between the clotting time and the APC level in vivo. APTT and APC levels were unchanged in control animals infused with buffer or PC (data not shown).

In contrast to the enhanced in vitro lysis of exogenous thrombin-induced blood clots of dogs infused with bovine APC or cats with human APC, in agreement with findings in the squirrel monkey model, human APC did not appear to induce measurable fibrinolysis in baboons in vivo, since the plasma levels of D-dimer, plasminogen, and t-PA antigen did not change appreciably following APC infusion (Table 1, Student's t-test, baseline v 45 minutes, P > .05, n = 4 for all measurements).

Table 1. Fibrinolytic Assessment During APC Infusion

<table>
<thead>
<tr>
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<th>Baseline Value</th>
<th>45-Minute Value</th>
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<tbody>
<tr>
<td>APC Dose</td>
<td>0.25 mg/kg/h</td>
<td>1.10 mg/kg/h</td>
</tr>
<tr>
<td></td>
<td>0.45 ± 0.08</td>
<td>1.69 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>0.39 ± 0.08</td>
<td>1.33 ± 0.30</td>
</tr>
<tr>
<td>D-dimer</td>
<td>210 ± 13</td>
<td>217 ± 25</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>2.6 ± 0.3</td>
<td>4.3 ± 1.0</td>
</tr>
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In the control experiments, platelet deposition onto the vascular grafts averaged $14 \times 10^9$ platelets in the four of six grafts that were patent at 60 minutes, a value similar to results reported previously. In previous control studies we have also observed elevated plasma levels of fibrinopeptide A and the platelet-specific α-granule proteins, platelet factor 4 and β-thromboglobulin. Platelet thrombus formation associated with these highly thrombogenic vascular grafts was also unaffected by conventional antithrombotic therapies with heparin, aspirin, or the combination of these agents. In contrast, platelet accumulation in the vascular graft was decreased by infused APC in a dose-dependent manner in all experiments, and this reduction persisted for at least one hour after discontinuing the APC infusion (Fig. 2). The inhibition of platelet deposition between five and 45 minutes in response to APC was dose dependent ($P < .01$ by analysis of variance, $n = 20$). The difference for inhibition between the two doses of APC was significant as early as 30 minutes after initiating the infusion (Student’s $t$ test, $P < .02$, $n = 4$).

At 30 minutes platelet deposition was inhibited by 43% and 76% at the lower and higher APC doses, respectively, while at 45 minutes the respective values for inhibition of platelet deposition were 42% and 77%.

Whereas all of the grafts in untreated animals occluded between 30 and 80 minutes, none of the grafts in APC-treated animals occluded within two hours following graft insertion. No decrease in blood flow (range 100 to 200 mL/min; 13 to 26 cm/sec) was detected (data not shown).

The template bleeding times measured during the infusions of the lower and the higher APC doses were not significantly prolonged vs control preinfusion values: $8.4 \pm 1.7$ min ($n = 4$) vs $7.1 \pm 1.1$ min ($n = 7$) and $6.4 \pm 0.8$ min ($n = 4$) vs $4.9 \pm 0.8$ min ($n = 9$), respectively, ($mean \pm SEM$, $P > .05$ for all measurements by Student’s $t$ test). No adverse cardiovascular or hemorrhagic effects were observed during or after the infusion of APC.

Thus a potent antithrombotic effect was produced by human APC. APC has previously been shown to inhibit disseminated intravascular coagulation in rabbits, to reduce venous thrombosis in dogs, and to block the lethal effects of Escherichia coli infusions into baboons. Since APC is a physiologic antithrombotic protein that prevents thrombus formation under arterial flow conditions, it may also be therapeutically useful for immediate and transient inhibition of platelet-dependent arterial thrombosis in man (eg, for prevention of arterial reocclusion in patients who have undergone angioplasty, carotid endarterectomy, or successful coronary reperfusion by thrombolytic therapy).
addition, since APC did not compromise primary hemostasis, assessed here by bleeding time assays, it may contribute to antithrombotic protection for thromboembolic complications associated with other surgical procedures.

ACKNOWLEDGMENT

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REFERENCES

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