CONCISE REPORT

Antithrombotic Effect of a Monoclonal Antibody to the Platelet Glycoprotein IIb/IIIa Receptor in an Experimental Animal Model

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A murine monoclonal antibody directed at the platelet glycoprotein IIb/IIIa complex, which blocks platelet aggregation ex vivo, was tested for its antithrombotic effects in an established animal model of acute platelet thrombus formation in partially stenosed arteries. Infusion of 0.7 to 0.8 mg/kg of the F(ab')2 fragment of the antibody completely blocked new thrombus formation despite multiple provocations, making it the most potent antithrombotic agent tested in this model.© 1986 Grune & Stratton, Inc.

PLATELETS HAVE BEEN IMPLICATED IN a variety of serious vaso-occlusive and thromboembolic disorders that together constitute the most common cause of death in the United States. Although some studies with available antiplatelet agents have been encouraging, overall these drugs have shown only modest clinical efficacy, perhaps in part because none of them is capable of completely inhibiting platelet function at tolerable dosages. We have recently prepared and characterized murine monoclonal antibodies to the platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa) that, ex vivo, completely block platelet aggregation induced by the agonists thought to operate in vivo (adenosine diphosphate [ADP], epinephrine, thrombin, and collagen). They produce this inhibition by blocking the binding of fibrinogen (and perhaps von Willebrand factor, fibronectin, and thrombospondin) to the GPIIb/IIIa receptor. More recently we showed that the infusion into dogs of F(ab')2 fragments of one of these antibodies could produce nearly total inhibition of platelet aggregation measured ex vivo, and that this correlated with nearly quantitative blockade of the GPIIb/IIIa receptors. The current study was designed to assess the antithrombotic effect of this antibody in a well-defined animal model of platelet thrombus formation in partially stenosed arteries.

MATERIALS AND METHODS

The production of antibody 7E3, its radiolabeling with 125I, and use in a platelet binding assay have been described in detail before. For the present studies, antibody was purified from ascites by affinity chromatography on protein A-Sepharose, fragmented into F(ab')2 with 2% pepsin, dialyzed against 0.15 mol/L NaCl, and frozen at −20 °C. The absence of residual intact 7E3 antibody was confirmed by polyacrylamide gel electrophoresis. Since preliminary studies showed that the off rate of 7E3 F(ab')2 fragments, like that of the intact antibody, is very slow, it was possible to assess the number of F(ab')2 fragments that bind to platelets in vivo. Thus, the difference in ex vivo binding of excess [125I]-7E3 to platelets obtained before and after infusion of the F(ab')2 fragments represented the number of F(ab')2 molecules bound in vivo. Platelet aggregation in response to adenosine diphosphate (ADP) was also measured before and after infusion as previously reported. The cyclical blood flow reduction model has previously been described in detail. Briefly, either the circumflex coronary artery of an anesthetized dog or the carotid artery of an anesthetized monkey is dissected out and an electromagnetic probe is placed on it to measure blood flow. The artery is stenosed approximately 70% by placing a 3- to 4-mm long encircling plastic cylinder of the appropriate internal diameter around the outside of the artery. Cyclical reductions in blood flow due to platelet thrombus formation then commence either spontaneously or after additional intimal damage is produced by brief (−1 second) clamping of the artery with a hemostat. Within several minutes, blood flow in the vessel decreases to a critical level, after which the flow is abruptly restored to its original level either by spontaneous embolization of the platelet thrombus or by embolization induced by gently shaking the cylinder. Once established, the cyclical flow reductions persist if no intervention is made. The frequency and amplitude of the flow reductions can be temporarily enhanced by infusing 0.5 μg/kg/min of epinephrine intravenously for ten minutes. The experimental protocol consisted of obtaining samples of blood anticoagulated with either 0.01 volume 40% sodium citrate (for platelet aggregation and 125I-7E3 binding), or 0.037 volume 269 mmol/L EDTA (for platelet counts), and then performing the surgery. After cyclical flow reductions were established, another set of blood samples was obtained and the antibody (0.7 to 0.8 mg/kg) was infused intravenously as a bolus. A final set of samples was obtained 30 minutes after infusing the antibody. Platelet-rich plasma was prepared as previously described, platelet counts on the platelet-rich plasma were performed by microscopy after dilution and lysis of erythrocytes (Unopette, Becton-Dickinson, Rutherford, NJ), and whole blood platelet counts were performed by an electronic resistive particle counter (S + IV, Coulter Electronics, Hialeah, Fla.). Unfortunately, in preliminary studies we were unable to obtain reproducible results with both the template and cuticle bleeding time methods; we are now trying to develop modified techniques for assessing this important hemostatic parameter in the experimental animals.

RESULTS

Cyclical reductions in blood flow were obtained in four dogs and four monkeys. In all four dogs and four monkeys,
infusion of the F(ab')2 fragments resulted in complete cessation of the cyclical flow reductions and restoration of the control flow rate in ten minutes or less (Fig 1). In the dogs, restoration of coronary flow correlated with the disappearance of ST segment deviations on the electrocardiograms, indicating the reversal of myocardial ischemia. The cyclical flow reductions could not be restored with epinephrine infusions of 0.5 or 1 μg/kg/min for ten minutes, increasing the intimal damage by repeated brief clamping of the vessel with a hemostat, passing current (1 to 2 mA) through the cylinder, or a combination of these provocations (Fig 1). Platelet aggregation in response to ADP (≥25 amol/L) was virtually abolished by the antibody infusion in all eight animals, although the shape change response remained intact (Fig 2). 125I-7E3 antibody binding studies were performed on one dog and one monkey. In both animals 84% of the GPIIb/IIIa receptors were blocked by the F(ab')2 fragments (in the dog, 34,300 molecules of 125I-7E3 bound per platelet before infusion and 5,500 after; in the monkey, 49,700 before and 8,100 after), a value nearly identical to
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Fig 2. Inhibition of ADP-induced platelet aggregation by infusion of the F(ab')2 fragment of 7E3. The platelets obtained before the infusion aggregated briskly in response to 25 μmol/L ADP, whereas the sample obtained 30 minutes after infusion showed an early increase in optical density reflecting shape change, but no aggregation. In the parentheses are the number of molecules of 125I-7E3 that bound per platelet ex vivo, indicating that the F(ab')2 fragments occupied 84% of the GPIIb/IIIa receptors.

that observed at this dose in the pilot study. Platelet counts decreased by less than 15% in the three animals tested. None of the animals demonstrated a significant change in heart rate or blood pressure upon antibody infusion. There was no clear evidence of excessive hemorrhage from the operative sites after antibody infusion, although no objective data on blood loss were obtained.

DISCUSSION

These studies indicate that the F(ab')2 fragment of antibody 7E3 is a potent antithrombotic agent in this model system. Considerable evidence accumulated by Folts et al. indicates that the reductions in blood flow in this model are due to interactions of platelets with the subendothelial surface in the area of stenosis, with increasing aggregation of platelets leading to vaso-occlusion. The central role of platelet adhesion and aggregation in this model has been independently confirmed by several groups. Although aspirin and other nonsteroidal antiinflammatory drugs inhibit the cyclical reductions in blood flow, infusion of 0.5 μg/kg/min epinephrine for ten minutes consistently restores the blood flow reductions with all of these agents. This augmentation of platelet thrombus formation by epinephrine may be important in understanding the effects of stress and cigarette smoking on cardiovascular disease, since both can elevate epinephrine levels. Even prostacyclin does not completely abolish the cyclical reductions in flow after epinephrine infusion and this is consistent with the ex vivo evidence that subaggregating doses of epinephrine can prevent inhibition of human platelet aggregation by prostacyclin. Thus, since the inhibition of flow reductions by the F(ab')2 fragment of antibody 7E3 was not reversed by epinephrine, it appears to be more potent than any of these antiplatelet agents. Only chlorpromazine has consistently protected against the augmented cyclical blood flow reductions produced by infusion of 0.5 μg/kg/min of epinephrine, but preliminary evidence suggests that even this drug may not protect against cyclical reductions in blood flow produced by the 1 μg/kg/min dose of epinephrine also tested in this study. Moreover, the antibody fragment is considerably more potent than chlorpromazine in inhibiting platelet aggregation ex vivo.

We chose to use F(ab')2 fragments instead of intact antibody to avoid inducing thrombocytopenia or reticuloendothelial cell blockade in case the Fc portion of the murine monoclonal antibody is recognized by the animal's splenic macrophages. No significant reductions in platelet counts occurred during these acute experiments or with the more chronic experiments in the pilot study.

Despite the profound inhibition of thrombus formation and ex vivo platelet aggregation, none of the animals developed spontaneous bleeding or clear evidence of excessive hemorrhage from their operative wounds. This paradoxical lack of dramatic bleeding despite total blockade of GPIIb/IIIa-mediated platelet aggregation measured ex vivo may be explained by the antibody leaving intact other platelet functions, including the interaction of von Willebrand factor with glycoprotein Ib, the interaction of collagen with another platelet receptor, and the ability to facilitate fibrin formation. Alternatively, the small number of residual unblocked GPIIb/IIIa receptors may have contributed to the animals' hemostatic integrity.

The efficacy of the monoclonal antibody fragment in this model can be ascribed to a fortuitous combination of factors, including the free access of the antibody to platelets in the circulation, the high affinity and specificity of the antibody for GPIIb/IIIa receptors, the absence of competing antigen in the plasma, the inability of platelets to undergo antigenic modulation, the maintenance of the platelet count, and the maintenance of sufficient qualitative platelet function to achieve hemostasis. The immunogenicity of murine monoclonal antibodies may, however, preclude their use as chronic therapeutic agents in humans, and there is no reason to believe that antiplatelet therapy would have a beneficial effect on established thrombi. Nonetheless, platelets have been implicated in the pathogenesis of a variety of ongoing acute phenomena, including preinfarction angina, transient ischemic attacks, acute occlusion of coronary arteries after thrombolysis or angioplasty, and acute occlusion of vascular grafts and anastomoses, that might potentially benefit from single-dose therapy with such antibodies. In fact, our preliminary data indicate that in a dog model, 7E3 F(ab')2 fragments can prevent reocclusion of coronary arteries after thrombolysis with recombinant tissue plasminogen activator. Additional studies will be required to establish whether such therapy would be safe in humans, and whether it has advantages over conventional antiplatelet agents.

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