A new primate model for the study of intravenous thrombotic potential and its modification

Norman A. Shoenfeld, MD, Anson Yeager, MD, Raymond Connolly, PhD, Karen Ramberg, MS, Laureen Forgione, BS, Albert Giorgio, BS, C. Robert Valeri, MD, and Allan D. Callow, MD, PhD, Boston, Mass.

Advances in venous reconstruction have been limited by inherent venous thrombogenicity and the absence of a suitable prosthetic material for use in the venous system. We have designed an in vivo experimental model to evaluate early blood-material interactions within the venous system and to quantitate drug efficacy in the alteration of platelet function and fibrin deposition in the baboon. An 8F catheter was placed percutaneously in the femoral vein of an adult male baboon. Indium 111-labeled autogenous platelets or iodine 125-labeled human fibrinogen was infused before the introduction, into the inferior vena cava, of a linear array of 5 x 15 mm alternating Dacron and polytetrafluoroethylene samples attached to a benzalkonium-heparin-treated guide wire. At 60 or 120 minutes the samples were removed and a 1 ml aliquot of blood was drawn. The materials and blood samples were counted in a gamma well counter, and the material counts were normalized to the circulating label present in the 1 ml blood sample. The experiment was repeated after pretreatment with heparin, aspirin, or dextran. Whole blood clotting times and bleeding times were monitored. The results showed decreased platelet and fibrin deposition on polytetrafluoroethylene when compared with Dacron in the venous system. Aspirin, heparin, and dextran were all found to decrease platelet and fibrin deposition onto intravenously placed graft material samples (p < 0.05, Student's t test). The data confirm the ability of the model to evaluate quantitatively anticoagulants, antiplatelet agents, and prospective graft materials for use in venous reconstructions. (J Vasc Surg 1988;8:49-54.)

In the past decade, major advances have been made in the surgical treatment of chronic venous valvular insufficiency and obstruction. The experience in Vietnam emphasized the importance of venous repair in the treatment of complex vascular traumatic injuries of the lower extremity.1 However, reconstructive procedures on the venous system remain limited by inherent venous thrombogenicity and the absence of a suitable prosthetic material for use in the venous system. Current efforts to inhibit thrombosis in venous reconstructions involve the neutralization of Virchow's triad: vessel thrombogenicity is minimized by meticulous reconstructions with autogenous tissue; venous stasis is prevented by use of the distal arteriovenous fistula3,4 or intermittent pneumatic calf compression5,6; and anticoagulant or antiplatelet medication is used to protect against early thrombosis and allow time for the repair of peri-anastomotic endothelial injury. In published series of venous reconstructions, adjuvant pharmacologic therapy varies widely from no treatment to combinations of heparin, low molecular weight dextran, fibrinolytic agents, and aspirin. This study was undertaken in an attempt to develop a model in which in vivo blood-material interactions in the venous system could be monitored and in which the effect of antiplatelet and anticoagulant medication could be quantitated within the more hostile venous system.
The goal was a model to identify prosthetic materials compatible with the venous system and drugs that may serve as adjunctive therapy to prevent thrombotic complications in venous reconstructive surgery.

MATERIAL AND METHODS

Adult male baboons (*Papio papio*, weighing 20 to 30 kg) were used in this study. The baboon is our preferred animal model because its platelets and coagulation system closely resemble those of humans. The animals were sedated with intramuscular ketamine (250 mg) and maintained under anesthesia with intravenous pentobarbital (50 mg) as required.

**Platelet preparation.** Forty-five milliliters of baboon blood was drawn into a syringe containing 9 ml of acid citrate dextrose anticoagulant. The blood was centrifuged at 160 g for 15 minutes to obtain platelet-rich plasma (PRP). Centrifugation of the PRP was repeated at 1000 g for 15 minutes to obtain a platelet pellet, which was washed once, then resuspended in normal saline solution with 0.1% dextrose. The platelets were then incubated with approximately 150 μCi indium 111 (111In) oxine (Amersham Corp., Arlington Heights, Ill.). The labeled platelets were centrifuged to remove unbound 111In, then resuspended in platelet-poor plasma and infused back into the baboon at approximately 100 μCi per dose. This is a modification of a technique described by Thakur et al., which gave viable functional platelets.

In an alternate series of experiments, commercially available iodine 125 (125I)-labeled human fibrinogen (Amersham Corp.), in a dose of approximately 100 μCi, was infused instead of the labeled platelets.

**Material preparation and experimental design.**

A linear array of alternating 3 × 15 mm samples of expanded polytetrafluoroethylene (PTFE) (W. L. Gore & Associates, Inc., Flagstaff, Ariz.) and knitted Dacron (C. R. Bard, Inc., Billerica, Mass.) was constructed with benzalkonium-heparin-treated 4-0 polypropylene (Prolene) suture material and attached at both ends to a benzalkonium-heparin-treated stainless steel guide wire (Fig. 1). Each array consisted of four PTFE samples and three Dacron samples. An 8F catheter was placed percutaneously into a femoral vein of the baboon with the Seldinger technique.

The guide wire with attached materials was passed up through the catheter into the inferior vena cava and left in place for a predetermined amount of time (60 or 120 minutes). Material placement in the inferior vena cava proximal to the vena cava bifurcation was confirmed in several studies by location of the guide wire on x-ray film, and the guide wire was passed to the same distance in all experiments. The materials were then removed through the 8F introducing catheter and a 1 ml blood sample was drawn. The material samples and the 1 ml blood sample were individually counted in a gamma well counter to quantitate labeled platelet deposition and circulating labeled platelets, respectively. The data were analyzed by normalization of the material counts to the counts present in the 1 ml blood sample. This corrected for variations in the 111In dose and decay. The same procedure was followed with 125I-labeled fibrinogen to study fibrin deposition. After the study was completed, the cannula was removed and hemostasis was achieved by external compression of the femoral vein.

**Aspirin experiments.** After a control array of PTFE-Dacron was left in place for 2 hours, the baboon was given 5 mg/kg of aspirin by nasogastric tube. The 2-hour time point was chosen because a statistically significant effect of aspirin on platelet deposition in an ex vivo baboon shunt was first seen after the graft material had been exposed to flow for 2 hours, and not before. Two hours was allowed for absorption, and a therapeutic effect was verified by the prolongation of the template bleeding time.
New primate model for the study of venous thrombotic potential

A second array of PTFE-Dacron was advanced into the inferior vena cava and similarly left in place for 2 hours. This and all other experiments were performed with labeled platelets and labeled fibrinogen.

**Heparin experiments.** A control study was performed with PTFE-Dacron segments maintained within the inferior vena cava for 1 hour. Heparin in a dose of 100 U/kg was given intravenously, and a therapeutic effect was verified by the prolongation of the whole blood clotting time. A repeat material array was placed for 1 hour and then removed and counted.

**Dextran experiments.** A control array of PTFE-Dacron was placed in the inferior vena cava for 1 hour. Low molecular weight dextran (average molecular weight 40,000 Pharcacia Inc., Piscataway, N.J.) was infused at a dose of 5 ml/kg (0.5 gm/kg). After this, a repeat material array was placed in the inferior vena cava for 1 hour, then removed and counted.

**Morphologic studies.** Graft specimens were processed for examination under the scanning electron microscope, after placement in the inferior vena cava for 2 hours. This consisted of rinsing in saline solution, fixation in 2.5% glutaraldehyde/0.1 mol/L sodium cacodylate buffer, dehydration in graded alcohols, CO₂ critical-point drying, and coating with gold-palladium.

Each series of experiments was performed in a minimum of three different baboons. All data were statistically evaluated by means of Student's t test. The experiments were well tolerated by the baboons; there were no significant complications. Animal care complied with the “Principles of Laboratory Animal Care” and the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 80-23, revised 1978).

**RESULTS**

Forty-nine control samples and 49 posttreatment material samples were tested in the aspirin series of experiments. The therapeutic effect of aspirin was confirmed by a prolongation of the template bleeding time in all experiments, from 4.3 ± 0.4 to 7.6 ± 0.5 minutes (p < 0.01). The results are seen in Figs. 2 and 3. As before, PTFE accumulated less platelets and fibrin than Dacron in these 1-hour control studies. Heparin led to a decrease in the labeled fibrin deposition on both materials. There is a difference in platelet and fibrin deposition between the control PTFE and control Dacron material sample. All these findings were significant at the p < 0.02 level.

In the heparin series of experiments, 84 control and 84 posttreatment material samples were tested. The therapeutic effect of heparin was confirmed by an increase in the whole blood clotting time from 285 ± 22 to 4360 ± 1450 seconds (p < 0.01). The results of these experiments are seen in Figs. 4 and 5. As before, PTFE accumulated less platelets and fibrin than Dacron in these 1-hour control studies. Heparin led to a marked decrease in both platelet and fibrinogen deposition on PTFE and Dacron (p < 0.01).

In the dextran series of experiments, 68 control
Fig. 4. Intravenous $^{111}$In-labeled platelet deposition: the effect of heparin.

Fig. 5. Intravenous $^{125}$I-labeled fibrinogen deposition: the effect of heparin.

and 68 posttreatment material samples were tested. The administration of dextran in this dosage resulted in a prolongation of the bleeding time from $4.0 \pm 1.0$ to $7.5 \pm 1.8$ minutes ($p < 0.05$), with no change in the whole blood clotting time. The results of these experiments are seen in Figs. 6 and 7. Dextran significantly reduced platelet and fibrin deposition on PTFE and Dacron in the venous system ($p < 0.05$). Scanning electron micrographs of materials in the control group confirmed platelet aggregation and the formation of a fibrin network on the graft materials in this intravenous model (Fig. 8).

**DISCUSSION**

Previous experimental studies have examined ways to improve patency of venous reconstructions. The advantage of autogenous tissue reconstruction compared with available prosthetic materials has been demonstrated, particularly in the inferior vena cava.\(^2\) Arteriovenous fistula in the canine model has been found to decrease thrombus formation and improve patency in venous reconstructions.\(^6,7\) The use of intermittent pneumatic calf compression to increase flow has been shown to improve patency rates.\(^5\) Heparin and low molecular weight dextran have improved patency in canine venous bypasses.\(^10,12\) Platelet inhibition has also been shown to be effective.\(^13,14\) Experiments in the baboon ex vivo arteriovenous shunt model have confirmed the reduced platelet deposition with aspirin, heparin, and dextran.\(^9,15\)

The primate model described is specific to the low pressure venous system. As an in vivo test, it avoids many of the problems inherent to in vitro testing schemes. Control data demonstrate no baseline interference with platelet function or the coagulation cascade induced by this model. The test is well tolerated by the experimental animal, with no significant complications experienced in more than 100 studies with this technique.

A minimum of three different animals were used in each of the six separate experiments. Significant interanimal variability was present, as shown by the difference in the control values between the heparin and dextran experiments. The experimental design is such that each animal serves as its own control, which adjusts for this interanimal variability. There are other sources of variability in this model. There is no doubt that the flow characteristics in such a system are not constant, with changes in flow in the inferior vena cava occurring with changes in the distance from the wall and respiration. In addition, the removal of the material samples through the catheter introduces variation into the results. This is balanced by the large number of replicates that can be tested in this system. The standard error of the mean reported with the data encompasses all sources of variability and appears to be acceptable for an in vivo experimental model.

Aspirin, through the irreversible acetylation of cyclooxygenase, inhibits the synthesis of thromboxane A$_2$, a potent platelet aggregator and vasoconstrictor.\(^9\) The aspirin-induced reduction in platelet deposition on the graft materials in this study demonstrates the presence of platelet aggregation in this model and the ability of the model to delineate changes in platelet function.

The antiplatelet effect of heparin may be a result of the inhibition of the formation of the potent platelet activator thrombin from prothrombin. Heparin, through this mechanism, interferes with thrombin
cleavage of fibrinogen and activation of factor XIII and thus alters fibrin polymerization, interfering with platelet-platelet attachment and platelet plug formation. The demonstrated reduction of platelet deposition by heparin confirms the interaction of platelets and the coagulation system in this in vivo model.

The mechanism of the reduction in platelet and fibrin deposition with dextran is multifactorial. Dextran alters the electronegativity of platelets and causes a reduction in factor VIII activity. It acts as a plasminogen activator and inhibits $\alpha_2$-antiplasmin, thereby accelerating plasma clot lysis. The fibrin polymerized in the presence of dextran is structurally modified, becoming more easily lysed and less platelet reactive. In addition, this dose of dextran in baboons has been found to increase plasma volume by 36%. This may in turn reduce venous stasis and in so doing inhibit thrombosis.

Of interest is the variation present in the control data between the heparin and dextran experiments. The latter experiments were performed on a different set of baboons than used for the heparin experiments, and the quantitative difference in the results may represent interanimal variation, with some baboons having a more reactive platelet-coagulation cascade than others. The model does use each animal as its own control and thereby corrects for these differences.

Fig. 6. Intravenous $^{111}$In-labeled platelet deposition: the effect of dextran.

Fig. 7. Intravenous $^{125}$I-labeled fibrinogen deposition: the effect of dextran.

Fig. 8. Scanning electron photomicrograph reveals platelet aggregates and formed fibrin network on intravenous Dacron graft at 1 hour.
A new in vivo primate model to evaluate short-term blood-material interactions in the venous system has been described. Heparin, low molecular weight dextran, and aspirin all significantly reduced platelet and fibrin deposition onto thrombotic stimuli within the venous system. The model may serve to evaluate the potential role of additional antiplatelet, anticoagulant, or fibrinolytic medications in reducing thrombosis after venous reconstruction and to identify prosthetic graft materials suitable for venous applications.

We thank W. L. Gore & Associates, Flagstaff, Arizona and C. R. Bard Inc., Billerica, Massachusetts for providing graft materials. The technical and administrative assistance of E. Samm, M. Burke, S. Laliberte, and D. Caton is gratefully acknowledged.

REFERENCES