Antithrombotic Therapy for Deep Arterial Injury by Angioplasty

Efficacy of Common Platelet Inhibition Compared With Thrombin Inhibition in Pigs

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Background. Platelet-thrombus formation is a complication of arterial wall deep injury by balloon angioplasty that may lead to acute arterial occlusion and may contribute to restenosis.

Methods and Results. Because common platelet-inhibitor drugs with a heparin bolus (100 units/kg) may be effective in inhibiting platelet-thrombus formation after arterial angioplasty, these were compared with a bolus of hirudin alone (control), the specific thrombin inhibitor hirudin (1.0 mg/kg), and saline (hirudin control) in normal pigs after angioplasty of the common carotid arteries. In the presence of deep arterial wall injury (injury exposing the media), indium-111-labeled platelet deposition ($\times 10^6$/cm$^2$) was 68.8$\pm$12.3 and 48.1$\pm$16.9 in the control animals. This was significantly reduced by pretreatment with low-dose aspirin (1 mg/kg/day), by high-dose aspirin (20 mg/kg/day) plus dipyridamole, and especially by thrombin inhibition with hirudin. Treatment regimens with aspirin alone (20 mg/kg/day), dipyridamole alone, or sulfinpyrazone were ineffective. Likewise, the incidence of mural thrombosis was 75% and 80% in deeply injured arteries of controls and was significantly reduced to 46% with aspirin plus dipyridamole, 25% with low-dose aspirin, and 0% with hirudin. The incidence of mural thrombosis was unchanged with high-dose aspirin (69%), dipyridamole (90%), or sulfinpyrazone (92%). This mural thrombosis could not be identified by angiography. In the presence of mild injury (deendothelialization), platelet deposition was low ($<10^6$/cm$^2$, a single layer) and was not changed by any therapy, including hirudin.

Conclusions. These therapies do not affect platelet adhesion to deeply or mildly injured artery. These data suggest a greater role for thrombin inhibition than with thromboxane or cyclooxygenase inhibition in the pathogenesis of platelet-rich mural thrombosis after deep injury during angioplasty. Antithrombotic therapy for arterial thrombosis by thrombin inhibition appears promising. (Circulation 1991;84:814–820)

Histological1–5 and angioscopic6–8 studies of arteries after experimental and clinical angioplasty have demonstrated the extensive injury to the arterial wall involving endothelial denudation, medial dissection, plaque fracture, and a high incidence of platelet-rich mural thrombosis.1–8 Although the exposure of the subendothelium is a stimulus for platelet adhesion and activation, it is the exposure of tissue factor, medial collagen (types I and III), smooth muscle cells and elastic tissue by deep injury, and atherosclerotic fatty gruel by plaque fracture that appear to have a major influence on the formation of mural thrombi.9–11 Thus, both collagen exposure and thrombin generation contribute to the initiation and progression of thrombosis.2,9–11 We previously showed the initial role of thrombin as a mediator of thrombus formation after balloon angioplasty.12–14 Thus, a dose-dependent decrease in mural thrombosis was observed with incremental doses of heparin.12,13 And, more importantly, the use of a direct and specific antithrombin-III–independent thrombin inhibitor, hirudin,13 completely prevented...
mural thrombosis. Although this inhibition of thrombin activity is very effective in decreasing mural thrombosis, it is unclear whether therapy directed against platelets, through the use of platelet inhibitors, will be as effective.

Because of the potential deleterious effects of platelet thrombi on the outcome of angioplasty, platelet-inhibitor drugs have been used empirically to prevent occlusion and thrombi since their introduction in 1977. However, it is not clear whether platelet mural and occlusive thrombi occurring in the presence of exposed collagen and thrombin after angioplasty can, indeed, be prevented (and to what degree) by platelet-inhibitor therapy, whether one platelet-inhibitor regimen is better than another, and whether their efficacy in comparison with hirudin is better. Thus, short-term platelet-inhibitor and thrombin-inhibitor therapy were evaluated by quantitative indium-111-labeled platelet deposition and mural thrombus formation at the site of arterial injury by balloon dilation in pigs.

Methods

Normal 3–4-month-old pigs (average weight, 35 kg) were of the Babcock four-way cross stock (mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds). They were housed at the Mayo Institute Hills Farm and were fed a normal chow diet. In all pigs, autologous platelets were labeled with 300–400 μCi 111In-tropolone 18–24 hours before angioplasty.

Drug Therapy

Three daily doses of platelet inhibitors were administered starting 2 days before and on the day of angioplasty. In 104 of the 130 pigs, heparin was administered as a single bolus (100 units/kg) immediately before angioplasty; 19 received low-dose aspirin (1 mg/kg/day p.o.); 12 received high-dose aspirin (20 mg/kg/day p.o.); 22 received high-dose aspirin (20 mg/kg/day p.o.) plus dipyridamole (100 mg i.v. during 90 minutes); eight received sulfonpyrazone (400 mg/day p.o.); six received dipyridamole (200 mg i.v. during 1 hour); and 37, serving as controls, received no platelet-active drug (only the bolus of heparin). Pigs receiving dipyridamole also received 100 mg i.v. b.i.d. dipyridamole for the 2 days before angioplasty. The last oral dose of drug was given 1 hour before angioplasty, and intravenous administration of drugs was initiated 30 minutes before angioplasty. Another 10 pigs received 1.0 mg/kg i.v. recombinant desulfato hirudin (CGP 39393; sequence of hirudin variant I but lacks sulfate on tyrosine 63; specific activity, 11,496 ATU/mg; referred to as hirudin in the text), and 16 pigs received intravenous 0.9% saline. The active drug animals or matching-saline controls were given a 1.0-mg/kg bolus followed immediately by a continuous infusion of the same dose per hour; data for these groups were, in part, previously reported. Although oral and intravenous administration of drugs during angioplasty was not performed in a blind fashion, all subsequent tissue and sample analyses were performed by investigators who were unaware of the treatment administered.

Experimental Protocol

The pigs were sedated with 300 mg i.m. ketamine (Ketaset, Bristol, Evansville, Ind.). After inhalation of ether (ether USP, J.T. Baker, Miami, Fla.), the pigs were intubated, mechanically ventilated with room air by a respirator (Harvard Apparatus, South Natick, Mass.) and maintained anesthetized with 0.5% halothane (Fluothane, Ayerst Laboratories, New York). The electrocardiogram and intra-arterial pressure were continuously monitored throughout the procedure. Immediately after catheter insertion, a single bolus of heparin (100 USP units/kg i.v.) was administered to animals receiving platelet-inhibitor therapy and their matching controls, corresponding to a dose less than 3.1 units/kg/min. Heparin was not administered to the 10 animals receiving hirudin or their 16 matching controls that received intravenous saline only.

An 8F balloon dilation catheter (polyethylene balloon, size 8 mm × 3 cm, Medi-Tech, Inc., Watertown, Mass.) was advanced under fluoroscopic control through a right femoral cut-down into the left and right common carotid arterial segment between the fifth and the fourth vertebrae. Five inflations were performed, 30 seconds each at 6 atm (pressure manometer, Medi-Tech, Inc.), with 60 seconds between inflations. The angiographic lumen diameter before dilatation ranged from 5 to 6 mm; during dilatation, the diameter of the inflated balloon within the artery was not more than 10% greater than the original arterial lumen (measured on plain radiographic films obtained during the procedure and compared with predilatation angiograms after injection of 6.0 ml meglumine diatrizoate; Renografin-76, Squibb & Sons, Princeton, N.J.). Postdilatation spot films were obtained in all pigs during injection of contrast.

Histopathological Study

Next, the pigs were given an overdose of pentobarbital and perfused with 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate (pH 7.25) at physiological pressure for 15 minutes to fix the arteries in situ. The carotid arteries were then removed, cleaned, and prepared for analysis. The location of the dilated portion of the fixed artery was easily identified after the in situ tissue fixation, which showed regions of vasoconstriction proximal and distal to the dilated area, and from spot films obtained during and after angioplasty. The dilated portion of the fixed carotid artery was divided into two equal segments, and a similar-sized segment was taken from the adjacent proximal and distal ends. A twofold magnifying lens (Sunnex Laboratories, Inc., Needham, Mass.) was then used to examine for the presence of mural thrombus. This macroscopic thrombus has the potential of being physiologically and clinically relevant by embolizing, enlarging, obstructing blood flow, or contributing to more severe
We previously demonstrated that macroscopic mural thrombosis is usually associated with at least 20×10⁶ platelets/cm².²

Histopathological examination was used to confirm a tear or thrombus because angiography is insensitive. Thus, from each arterial segment, two or three ring sections were stained with hematoxylin-eosin and with van Gieson’s elastic stain. The histological sections were examined for the presence or absence of deep injury (tears through the internal elastic lamina into the media) at the site of dilation. The consensus results of two observers were recorded. Endothelial denudation without a tear through the internal elastic lamina was considered mild or subendothelial injury.¹² Two carotid arteries from each control group could not be analyzed because of technical problems.

Ring-sized specimens were cut from arterial segments subjected to different treatments, coated with carbon and gold-palladium alloy, and examined with a scanning electron microscope (Autoscan, ETEC Corp., Hayward, Calif.) and a transmission electron microscope (model 300, Phillips Electronics Co., Manwah, N.J.). Representative areas were photographed and examined by two investigators.

Quantification of Platelet Deposition

The platelet deposition on each dilated artery (in millions per square centimeter) was calculated from platelet counts and ¹¹¹In activity on the arterial wall and in the blood as previously described.¹²,¹⁷ Three samples of blood obtained at the time of animal death were counted in a gamma well counter (Gamma 8000, Beckman Instruments, Fullerton, Calif.), and the radioactivity in counts per minute per unit weight of each blood sample and per arterial segment (each one of which was measured for size) was obtained. The spectrometer of the counter was adjusted to include the peaks at 174, 247, and 421 keV (sum peak) of the ¹¹¹In radionuclide. The ¹¹¹In counts per minute per gram of blood were transformed into counts per minute per milliliter of blood. The percentage of radioactivity bound to platelets was then determined, and the number of platelets per counts per minute was calculated from the known blood platelet count (Coulter counter, Coulter Electronics, Hialeah, Fla.).² The number of platelets deposited on the arterial segments per square centimeter was then calculated by dividing the arterial segment counts per minute by both the number of platelets per counts per minute and the arterial surface area.

Statistical Analysis

Results were expressed as mean±SEM. The statistical significance of the difference between group means was evaluated by an analysis of variance, and when significant, Student’s t test was used to assess intergroup differences. A χ² test was used for analyzing discrete variables.

Results

At the site of balloon injury, platelet deposition was related to the severity of arterial wall injury with deep arterial injury producing extensive platelet deposition (Figure 1) and mild or subendothelial injury producing a very low level of platelet deposition (<10×10⁶/cm²) (Figure 2) that consisted of a single layer of platelets as previously shown.² In the nondilated, normal, uninjured distal areas of the artery, platelet deposition was even lower, averaging less than 0.5×10⁶/cm².

In the platelet-inhibitor controls, which received a single bolus of heparin (100 units/kg) just before angioplasty, platelet deposition was 68.8±12.2×10⁶/cm² in the presence of deep arterial wall injury and was not different (48.1±16.9×10⁶/cm²) in the hirudin controls. Platelet deposition was significantly reduced by pretreatment with aspirin (1 mg/kg/day) to 18.1±5.7×10⁶/cm² and by the combination of aspirin (20 mg/kg/day) and dipyridamole to 28.9±4.9×10⁶/cm². Treatment with aspirin (20 mg/kg/day) alone,
dipyridamole alone, or sulfinpyrazone did not significantly alter platelet deposition relative to platelet-inhibitor controls (Figure 1). Platelet deposition in the hirudin group was significantly reduced to $5.5 \pm 0.1 \times 10^6$/cm$^2$ compared with hirudin controls (Figure 1).

In the presence of mild injury, the low level of platelet deposition observed in controls was not significantly altered by any of the drug regimens, including hirudin (all < $10^6$/cm$^2$) (Figure 2).

**Thrombus Formation**

Macroscopic mural thrombus was not observed overlying mildly injured arteries in the controls or active-drug animals. However, in the presence of a deep injury, mural thrombus overlying the area of exposed media was seen (and later confirmed histologically by light microscopy) in 80% of arteries (31 of 39 deeply injured arteries) from the heparin controls and in 75% of arteries from saline controls. This was significantly reduced ($p \leq 0.01$) by pretreatment with 1 mg/kg aspirin or 20 mg/kg aspirin plus dipyridamole, and it was abolished with hirudin treatment. The other drug regimens did not significantly ($p > 0.05$) alter mural thrombosis (Figure 3). Despite the high incidence of mural thrombosis after deep arterial injury, none was evident on spot films obtained during contrast injection after injury and immediately before death.

**Discussion**

Transluminal coronary angioplasty is widely used in the management of patients with coronary artery disease. Early reocclusion and restenosis are major

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**Figure 2.** Bar graph of platelet deposition in arteries with mild injury as a function of drug treatment. Platelet deposition was extremely low and equivalent to a monolayer or less of platelet deposition and was not significantly decreased by any antithrombotic therapy. Values are mean ($\times 10^6$/cm$^2$). T bars, SEM. There was no significant difference between control groups. ASA, aspirin; Dip, dipyridamole; Sul, sulfinpyrazone; HIR, hirudin. n, Number of mildly injured arteries.

**Figure 3.** Bar graph of percentage of deeply injured arteries with a mural thrombus as a function of drug treatment. Incidence of mural thrombosis is significantly ($p \leq 0.01$) decreased by aspirin (1 mg/kg), aspirin plus dipyridamole, and hirudin relative to controls. ASA, aspirin; Dip, dipyridamole; Sul, sulfinpyrazone; HIR, hirudin. There was no significant difference between control groups. n, Number of deeply injured arteries. Corresponding numerical values are above each bar.
limitations of the procedure and are the cause of significant morbidity.\textsuperscript{18–21} Angioscopic studies in humans after coronary angioplasty show an incidence of platelet-rich mural thrombus of more than 75% despite platelet-inhibitor therapy.\textsuperscript{7,8} Experimental and clinical evidence suggest that platelets play an important role in both the pathogenesis of acute occlusion and probably are a major contributor to the later intimal proliferation that leads to restenosis.\textsuperscript{9,10,22,23} Because platelet deposition at the site of angioplasty is important in the pathogenesis of these complications, its quantification is important in determining the efficacy of pharmacological therapy aimed at inhibiting platelet deposition. The results of this study show that platelet deposition and platelet thrombus formation can be significantly decreased by the platelet inhibitors, low-dose aspirin (1 mg/kg) and dipyridamole plus aspirin (20 mg/kg) but not by dipyridamole alone, sulfipyrazone alone, or high-dose aspirin (20 mg/kg) administered within 2 days of the arterial injury. However, even the beneficial effect of the former two platelet-inhibitor therapies is modest and incomplete for the inhibition of mural thrombus (predominantly platelets) compared with the results obtained with the thrombin inhibitor, hirudin. The saline control group shows that a single bolus of 100 units/kg heparin has no significant antiplatelet effects as expected.\textsuperscript{12} Neither the platelet-inhibitor nor the thrombin-inhibitor therapy was effective in preventing platelet adhesion to the mildly injured arterial wall.

Although the beneficial effect of aspirin may be related to cyclooxygenase inhibition, it is not clear why the higher dose of aspirin was not effective in this study. This same finding has been reported by others; furthermore, experimental studies have suggested that high-dose aspirin may even be thromboxane\textsuperscript{24} Inhibition of vessel wall prostacyclin synthesis by higher aspirin dosage may, in part, contribute to the effect.\textsuperscript{24,25} Combined dipyridamole and aspirin is attractive as a regimen because each affects a different prostaglandin metabolic pathway (i.e., inhibition of phosphodiesterase and cyclooxygenase\textsuperscript{26}), thereby increasing platelet cyclic AMP and decreasing thromboxane A\textsubscript{2}, respectively. Thus, this combination has been effective in reducing aortocoronary vein graft occlusion both experimentally and clinically.\textsuperscript{27–29} However, this combination was also as effective as low-dose aspirin alone in this model of angioplasty. Although sulfipyrazone and dipyridamole can also inhibit platelet function,\textsuperscript{30} they appear more effective against platelet deposition on prosthetic materials than on biological vascular surfaces\textsuperscript{30–32}; thus, the incidence of mural thrombosis on an injured biological surface was not significantly better than that in the controls. Platelet deposition for sulfipyrazone appears more severe than that in control with a large standard error of the mean because of one outlier, a pig with a much larger area of deep arterial injury. However, these platelet-inhibitors that affect the prostaglandin pathway of platelet aggregation do not appear as effective as the thrombin inhibitor, hirudin. This suggests that the predominant mechanism underlying platelet deposition at the site of arterial injury in vivo is more thrombin dependent than prostaglandin dependent, although the latter may contribute if thrombin is not fully inhibited.

Agents that inhibit platelet function may not necessarily possess antithrombotic properties for any or all thrombotic problems or stimuli. Thus, the potential antithrombotic usefulness of an agent for a particular thrombotic condition needs to be tested in vivo. In the presence of deep arterial injury created by balloon angioplasty, where the stimulus for thrombus formation is great,\textsuperscript{1,2,5,6} even the beneficial effects of aspirin or aspirin plus dipyridamole appear to be limited, reducing thrombus formation to no better than 31% of control (or 25% of deeply injured arteries); this may be insufficient to prevent complications such as restenosis that appear related to thrombosis and intimal proliferation.\textsuperscript{9,10,22,23} A newer approach may be the use of the antithrombin III–dependent thrombin inhibitors, such as hirudin, which are relatively more potent than the platelet inhibitors in reducing thrombus formation.

Even though this model of arterial injury involves a normal rather than an atherosclerotic artery, the arterial substrates in the media (except for lipids) are similar to those in plaques, and the incidence of platelet-rich mural thrombosis anchored to regions of deep injury are similar to atherosclerotic arteries in experimental animals and humans.\textsuperscript{1,6–8,10,33,34} Pigs appear to be a good model for the study of thrombosis and atherosclerosis. The incidence of mural thrombus with deep type III injury (70–80%) and platelet response in pigs approximates that in humans.\textsuperscript{1,6–8,10,35} Fibrocellular atherosclerotic plaques occur naturally without dietary manipulation or in an accelerated fashion with dietary manipulation or stents in pigs, and they closely resemble those in humans.\textsuperscript{36–38} Intimal proliferative lesions in pigs are similar to those in humans.\textsuperscript{38}

In the presence of mild or type II injury (de-endothelialization), platelet deposition is a monolayer and quantitatively very low, and mural thrombosis has not occurred, as previously reported.\textsuperscript{1,2,10,12–16} Platelet adhesion to the subendothelium was not affected by any of the drugs used in this or previous studies.\textsuperscript{12–16} This single layer of platelets may play an important role in the intimal proliferation after endothelial injury through the release of mitogenic substances.\textsuperscript{9,10,22} Thus, although aspirin or aspirin plus dipyridamole may decrease mural thrombosis and acute thrombotic occlusion, these agents will probably not affect smooth muscle cell proliferation after mild arterial injury or restenosis because of this mechanism of intimal proliferation. The effect of specific thrombin inhibitors on cellular proliferation requires further study because thrombin appears to be a mediator of the proliferative responses, which can be inhibited in certain animal models.\textsuperscript{10,39,40}
Although recommendations about the therapeutic use of specific platelet-inhibitor drugs for angioplasty can only be derived from results of clinical trials, our study suggests that prior in vivo quantitative evaluation of antithrombotic efficacy for angioplasty may help screen for potentially suitable drugs for clinical trials. The demonstration that aspirin (1 mg/kg) and the combination of aspirin and dipyridamole can decrease quantitative platelet deposition and mural thrombosis is consistent with the recent clinical findings that aspirin and dipyridamole can decrease the incidence of acute complications after angioplasty but not the incidence of restenosis. This may relate to the incomplete antithrombotic effects of these drugs, the negligible effect on platelet adhesion and release at the injured vessel wall, or both. Both mechanisms may contribute to restenosis. It remains to be seen whether more complete suppression of mural thrombosis as can be obtained with specific thrombin inhibitors can have an effect on restenosis (this may also demonstrate which of the two mechanisms may be more important for restenosis) or whether additional antiproliferative therapy may be necessary for the monolayer of platelet deposition. The intensity (dose), duration, and route of administration (intravenous or local delivery) of specific thrombin inhibition to prevent later occurrence of mural thrombosis needs experimental evaluation before such therapy can be implemented in clinical trials of angioplasty.

Conclusion

Although there is a rationale for using platelet-inhibitor drugs to inhibit platelet deposition and thrombus formation so that the complications of angioplasty will be decreased, not all drugs that affect platelet function in vitro exhibit potent antithrombotic properties in vivo. Aspirin at 1 mg/kg/day or the combination of aspirin and dipyridamole decreases, but does not eliminate, mural thrombosis during angioplasty. Thus, it is not surprising that this modest effect can decrease the incidence of acute occlusion, clinically, but may insufficiently reduce subtotal mural thrombosis, cellular proliferation, or both to prevent restenosis. Newer antithrombotic therapies, such as specific thrombin inhibition with hirudin, are far more effective than platelet-inhibitor therapy and can totally prevent mural thrombus. Platelet glycoprotein membrane receptor inhibitors may provide another approach toward pharmacological inhibition of mural thrombosis. However, the type, appropriate dosage, effect at different shear rates, methods of laboratory monitoring, and effect on cellular proliferation require further study.

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References


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