Platelets, Vasoconstriction, and Nitroglycerin During Arterial Wall Injury
A New Antithrombotic Role for an Old Drug

Jules Y.T. Lam, MD, James H. Chesebro, MD, and Valentin Fuster, MD

Endothelial injury in vivo is associated with platelet deposition and a localized platelet-dependent vasoconstrictive response. To assess the influence of nitroglycerin on platelets and vasoconstriction, quantitative ß11In-labeled platelet deposition (no platelets x 10^9/cm^3) of the injured segment and the degree of angiographic vasoconstriction (percent diameter narrowing proximal and distal to the dilated segment) produced during injury by balloon angioplasty of the common carotid arteries were studied in heparinized normal pigs that were sacrificed immediately after the procedure. In deeply injured (injury extending through the internal elastic lamina) compared with mildly injured (deendothelialization only) arteries, there was both greater platelet deposition (63.8 vs. 6.9, p = 0.04) and more vasoconstriction (30% vs. 19%, p = 0.02). In the presence of deep arterial wall injury, nitroglycerin given intravenously at a dose sufficient to lower mean arterial pressure by 9±2% significantly decreased both platelet deposition (16.2 vs. 63.8, p<0.008) and the vasoconstrictive response (20 vs. 30%, p<0.02) relative to control. However, in the presence of mild arterial wall injury, nitroglycerin decreased vasoconstriction relative to control (10% vs. 19%, p<0.01) without causing a significant decrease in the already low level of platelet deposition (5.6 vs. 6.9, respectively; p = NS), suggesting a direct smooth muscle relaxant effect of nitroglycerin. This is the first reported in vivo effectiveness of nitroglycerin in the reduction of platelet deposition after deep arterial injury. (Circulation 1988;78:712–716)

Nitroglycerin has been used for over a century for the prevention and relief of myocardial ischemia. Its proven clinical efficacy is believed to be related to its vasodilatory effects on the coronary and systemic vascular beds.1–4 However, the exact mechanism by which nitroglycerin produces vasodilatation or relieves vasoconstriction remains to be elucidated. Recent in vitro studies have shown that nitrates can relax vascular smooth muscles through an endothelium-independent mechanism,5,6 which may explain its remarkable efficacy in relieving vasoconstriction that is often localized to the site of atherosclerosis where the endothelium may be injured or dysfunctional.7 Disruption of the endothelial barrier also leads to localized platelet deposition8 that may contribute to the vasoconstrictive response by releasing potent vasoconstrictive substances.9–13 Nitroglycerin inhibits platelet aggregation in vitro,14–16 and this antiplatelet effect may provide an additional mechanism for decreasing vasoconstriction that is localized to the site of arterial injury. However, it is unclear whether nitroglycerin can inhibit platelet function in vivo and thus alter the local platelet–arterial wall response to injury and inhibit injury-related and platelet-dependent vasoconstriction.9 This study assesses the efficacy of nitroglycerin in decreasing platelet deposition and the localized vasoconstriction after arterial wall injury in vivo.

Materials and Methods

Experimental Procedure
Twenty-one normal pigs 3–4 months old (weight, 28–35 kg) of the Babcock four-way cross stock (mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds) were sedated by intramuscular injection of 300 mg ketamine (Ketaset, Bristol, Evansville, Indiana), intubated after a small amount of ether (ether USP, J.T. Baker, Miami, Florida) was given by inhalation, and maintained in anesthesia by mechanical ventilation (Harvard respirator) with 0.5% halothane (Fluothane, Ayerst, New York, New York) in room air. The electrocardiogram and

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J.Y.T.L. is the recipient of a research fellowship from the Medical Research Council of Canada.
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intra-arterial pressure were continuously monitored throughout the procedure with a Honeywell multichannel recorder. Injury of the common carotid arteries was performed by balloon angioplasty as described. An 8F balloon dilatation catheter (Meditech polyethylene balloon, 8 mm × 3 cm) was inserted through a right femoral arteriotomy under full heparinization (single bolus, 100 USP units/kg i.v.). The catheter was then advanced into the right and left common carotid arteries under fluoroscopic guidance for initiation of angioplasty of the arterial segment located between the fourth and fifth vertebrae. Five inflations were performed, 30 seconds each at 6 atm (Meditech pressure manometer) with 60-second intervals between inflations. The angiographic lumen diameter before dilatation was 5–6 mm; during dilatation, the diameter of the inflated balloon within the artery was not more than 10% bigger than the original arterial lumen (measured on plain x-ray films taken during the procedure and compared with predilatation angiograms).

Quantitation of Vasoconstriction

To quantitate the severity of localized vasoconstriction immediately proximal and distal to the dilatation site, angiograms of the common carotid arteries were obtained in all pigs before and after the dilatation procedure; for these two selective intra-arterial injections at the beginning and end of the procedure, 6.0 ml meglumine diatrizoate (Renografin-76, Squibb, Princeton, New Jersey) was used with exposure factors of 90 kV, 200 mA, and 20 msec. The degree of vasoconstriction was expressed as the mean of the maximal narrowing of the lumen diameter measured with a caliper to the nearest 0.1 mm, just proximal and just distal to the dilated segment as measured on the postdilatation angiogram, and expressed as percentages of the respective arterial dimension before dilatation.

Therapy With Nitroglycerin

Eleven pigs randomly assigned for active drug treatment received nitroglycerin intravenously (Tridil, American Critical Care, McGaw Park, Illinois) starting 30 minutes before the angioplasty procedure, and administration was continued throughout the procedure until the animals were sacrificed. The nitroglycerin was prepared immediately before use in glass bottles and infused through polyethylene infusion sets. The therapeutic dose of nitroglycerin in pigs is unknown. Because an arbitrary dose may not be adequate or optimal in all pigs and because there may be no correlation between the rate of nitroglycerin infusion and plasma nitroglycerin concentration, nitroglycerin was individually titrated to produce a 10% fall in mean arterial pressure. This hemodynamic response, which has been shown to be associated with beneficial effects in both humans and experimental animals, required an infusion of 1,151 ± 235 μg/min nitroglycerin in the pigs. Although this rate may be higher than that usually infused in humans (and may reflect a different pharmacokinetic and pharmacodynamic profile in pigs as opposed to humans), doses up to 1,020 μg/min have been infused to achieve clinical benefit in patients. Ten pigs randomly assigned to nitroglycerin vehicle (n = 4, 2.4% alcohol, 2.4% propylene glycol) or 5% dextrose in water (n = 6) served as controls. Platelet deposition and vasoconstriction in the vehicle-treated group did not differ from the dextrose in water-treated group.

Tissue Preparation

At the completion of the angioplasty procedure, the animals were given a lethal dose of sodium pentobarbital and perfused antegradely with 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate (pH 7.25) at 100 mm Hg for 15 minutes to fix the arteries in situ. The carotid arteries were then removed, cleaned of all adventitia, and prepared for analysis.

The dilated portion of the fixed artery was easily identified because of the in situ fixation that showed the regions of vasoconstriction proximally and distally to the dilated area and from the spot films taken during and after the angioplasty. The dilated portion was divided into two equal segments, each about 1.5 cm; segments were also taken from the distal uninvolved artery.

Histopathology

From each arterial segment, two- or three-ring sections were removed and stained with hematoxylin and eosin and Lason’s elastin-Van Gieson stain. The histological sections were examined by two investigators to provide consensus evaluation of the presence of medial tears. An intimal tear extending through the internal elastic lamina into the media was defined as deep arterial wall injury; endothelial denudation without a tear through the internal elastic lamina was mild injury.

Quantitation of Platelet Deposition

Autologous platelets were labeled with 111In (tropolon)3 (300–400 μCi) and injected into the animal 18–24 hours before angioplasty. The quantitative platelet deposition on the arterial segments was calculated from the blood platelet counts, and the 111In activity on the arterial wall (arterial segments counted in a well counter) and in blood, as described. The number of platelets per unit area (×104/cm2) was then obtained by dividing the number of deposited platelets per arterial segment by the arterial wall surface area (area = π × d × l; d is diameter of segment and l is length of arterial segment).

Statistical Analysis

All values are expressed as mean ± SEM unless otherwise indicated. Differences between group means were assessed by a two-tailed Student’s group t test; p < 0.05 was considered significant.
Results

Localized vasoconstriction was observed immediately proximal and distal to the dilation site where there was endothelial denudation as previously described.9

Control Group

With deep arterial wall injury, the vasoconstrictive response (30±3%) and the platelet deposition (63.8±18.1) were significantly higher compared with mild arterial wall injury where the vasoconstrictive response (19±2%) and platelet deposition (6.9±0.5) were lower (p = 0.02 and p = 0.04, respectively). Neither platelet deposition nor vasoconstriction were observed in the distal uninjured portion of the artery with intact endothelium, as previously described.8,9

Effects of Nitroglycerin

In the presence of deep arterial wall injury, nitroglycerin significantly decreased platelet deposition relative to control (16.2±2.3 vs. 63.8±18.1, p = 0.008) and vasoconstriction relative to control (20±3%, n = 16 arteries, vs. 30±3%, n = 13 arteries, p = 0.02) (Table 1). In the presence of mild arterial wall injury, nitroglycerin did not decrease platelet deposition relative to control (5.6±0.9 vs. 6.9±0.5) but did significantly decrease the vasoconstriction relative to control (10±1%, n = 6 arteries, vs. 19±2%, n = 7 arteries, p = 0.01) (Table 1). During therapy with nitroglycerin, the vasoconstrictive response was also significantly greater in the arteries with deep injury compared with mild injury (20±3% vs. 10±1%, respectively, p<0.04) and was associated with greater residual platelet deposition in the deeply injured compared with the mildly injured group (16.2±2.3 vs. 5.6±0.9, respectively; p<0.01) even though the decrease in mean arterial pressure was the same in both groups (9±2%).

Discussion

This study demonstrates a new and unexpected finding for an old vasodilator drug, namely, that intravenous nitroglycerin can be a potent and effective platelet inhibitor agent in vivo; this supports its inhibition of platelet aggregation in vitro14–16 and its inhibition of whole blood platelet aggregation in vivo at therapeutic doses (titrated to a 10-mm Hg reduction in blood pressure) in humans when measured at the bedside within 60 seconds of sampling (but not after 30 minutes of incubation).22 As expected, it can also decrease vasoconstriction in the presence of deep arterial wall injury. However, the adherence of a single layer of platelets to the exposed subendothelium in the presence of mild injury appears resistant to inhibition by nitroglycerin as we have also observed with other platelet inhibitor therapies.9,23

Nitroglycerin also produced a significant decrease in the vasoconstrictive response associated with arterial wall injury. This is consistent with its potent endothelium-independent vasodilator properties.5,6 In this model, we have shown that there is also a platelet-dependent component to this vasoconstrictive response,9 and aspirin, an antiplatelet agent with no known vasodilator properties, can decrease this vasoconstrictive response by decreasing platelet deposition.9 Thus, in addition to its direct vascular-smooth muscle relaxant effect, nitroglycerin probably has a further vasodilating effect mediated by its antiplatelet properties, but in this study we had no way to determine how much of the vasodilation was endothelium-independent and how much was platelet-dependent. We can only infer that a platelet-dependent component was present based on the greater platelet deposition and vasoconstriction after deep compared with mild injury (with and without nitroglycerin) and our previous study with low-dose aspirin.9

The vasodilator response to nitroglycerin, not related to a decrease in platelet deposition in mild injury probably represents its direct action on vascular smooth muscle causing relaxation, an intrinsic property of nitroglycerin likely related to cyclic guanosine monophosphate (cGMP) generation.5,6 Thus, at least two mechanisms may contribute to the decrease in vasoconstriction at the site of arterial wall injury in vivo by nitroglycerin: first, a direct smooth muscle relaxant effect independent of the presence of the endothelium and independent of platelets and, second, a decrease in platelet deposition and the platelet-dependent vasoconstriction.

Localized vasoconstriction after endothelial injury in vivo may result from endothelial injury.24 This may reduce endothelium-derived relaxation factor(s),25 and prostacyclin (PGI2) production,26 or may promote platelet deposition and release of platelet-derived vasoconstrictive substances.7–13 The relative contribution of these components is unknown. Localized vasoconstriction is also influ-

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<th>Table 1. Change in Arterial Diameters (Vasoconstriction) Before and Immediately After Angioplasty</th>
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<td>Arterial diameters (± SEM) after and before angioplasty</td>
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enced by the vascular smooth muscle responsiveness because vasoconstriction does not occur in the dilated region where necrosis of smooth muscle cells results but does occur immediately proximal and distal to the dilated region where there is endothelial cell denudation but no necrosis of smooth muscle cells.8,9

Initial in vivo and in vitro studies showed that nitroglycerin can enhance endothelial prostacyclin synthesis and inhibit platelet thromboxane A2 production,14-16 but subsequent reports have failed to confirm an increased prostacyclin synthesis by endothelial cells.27 Blockade of prostaglandin production with indomethacin or aspirin has failed to attenuate the actions of nitroglycerin.28,29 Increasing evidence suggests that it is the increase in intracellular cGMP that may be responsible for the action of nitroglycerin both on the vascular smooth muscle5 and on platelets.30

The clinical relevance of the interaction between platelets, arterial injury, and vasomotor tone lies in the growing recognition that spontaneous arterial injury and platelet aggregates may contribute to such acute ischemic syndromes as unstable angina pectoris, acute myocardial infarction, and sudden ischemic death.31 In these conditions, thrombotic and vasospastic coronary arterial occlusions occur at the site of atherosclerosis where spontaneous endothelial and deep injury (plaque rupture) occur,31-33 and treatment with nitroglycerin has been beneficial.18-20 Human and experimental canine studies in arteries externally constricted with a plastic ring suggest that platelet vasoconstrictor substances that are released during episodes of unstable angina contribute, in part, to vasoconstriction and cyclic blood flow variations in the presence of a severe stenosis and in subendothelial or superficial injury.34-39; the cyclic flow variations and vasoconstriction can be reduced with thromboxane A2 and serotonin receptor inhibitors or aspirin but were not reduced by nitroglycerin (not titrated to lower mean arterial pressure by 10 mm Hg as in pigs and dogs) or diltiazem.35,38,39 Platelet–arterial wall interaction also occurs during deep arterial wall injury induced by balloon angioplasty or occurs spontaneously both in our porcine model and in humans, where platelet thrombi and vasospasm occur9 and nitroglycerin in dosages titrated to blood pressure may also be efficacious.40,41 Other drugs that decrease vasoconstriction such as verapamil, nifedipine, prostacyclin, and receptor blockade to thromboxane A2 (SQ 29548) and serotonin (ketanserin) do not decrease platelet deposition in the presence of deep arterial injury.42-45 Although the above experimental results appear to be at odds, the experimental models differ, and concepts from both may apply to the corresponding human situation (deep arterial injury compared with severe stenosis with associated subendothelial injury).

In this study, we have shown that nitroglycerin decreased both platelet deposition and localized vasoconstriction; this may explain, at least in part, its beneficial clinical effects. The antiplatelet effect of nitroglycerin in vivo may contribute to its vasodilator effect.

Acknowledgments

We gratefully acknowledge the technical assistance of Brenda I. Wendland, Holly B. Lamb, and James M. Bryne.

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KEY WORDS • nitroglycerin • platelet deposition • arterial wall injury
Platelets, vasoconstriction, and nitroglycerin during arterial wall injury. A new antithrombotic role for an old drug.
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Circulation. 1988;78:712-716
doi: 10.1161/01.CIR.78.3.712

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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