Is vasospasm related to platelet deposition?

Relationship in a porcine preparation of arterial injury in vivo

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ABSTRACT Although aggregating platelets can release potent vasoactive substances in vitro, the importance of platelets in mediating naturally occurring or provoked spasm in vivo is not clear. To investigate the possible role of platelets in arterial spasm following arterial injury induced by angioplasty, quantitative platelet deposition of the dilated arterial segment and the degree of vasoconstriction (average percent diameter narrowing just proximal and distal to the dilated segment) produced during angioplasty of the common carotid arteries were studied in 42 heparinized normal pigs that were killed immediately after the angioplasty procedure. Angiographic films of the carotid arteries were taken before and after the dilatation to assess the vasoconstriction. Vasoconstriction was greater (40% vs 19%, p < .002) when platelet deposition (×10⁶/cm²) was in excess of 10, and the severity of vasoconstriction in vivo had a close positive exponential correlation (r = .77, p < .001) with extent of platelet deposition in 24 untreated pigs. Platelet deposition and vasoconstriction were greater with severe arterial wall injury than with mild injury (58.8 versus 6.9, p < .0001; 37% vs 21%, p < .001, respectively). After severe injury in 18 pigs pretreated with 1 mg/kg/day aspirin, platelet deposition decreased (from 58.8 to 19.6, p < .02) and vasoconstriction decreased (from 37% to 21%, p < .003) relative to control. After mild injury, platelet deposition and vasoconstriction were mild and unchanged by aspirin. Thus, local vasoconstriction is influenced by the degree of platelet deposition. This is the first reported correlation in vivo between quantitative platelet deposition and localized vasoconstriction at the site of arterial injury and the reduction of vasoconstriction by platelet inhibitor therapy. Circulation 75, No. 1, 243–248, 1987.

BECAUSE platelets are rich in vasoactive prostanoid and nonprostanoid substances, they may modify vaso-motor tone by releasing these potent substances.1–6 Vasospasm often occurs in the region of atherosclerotic lesions,7, 8 where arterial injury and plaque rupture or ulceration may stimulate platelet deposition9–13 that could contribute to subsequent vasoconstriction. Arterial angioplasty causes both superficial and deep arterial wall injury associated with mild and severe platelet deposition, respectively.14 These platelets and their vasoactive products may contribute significantly to the localized vasoconstriction that may complicate an angioplasty procedure15 and that has been observed in an animal preparation of arterial angioplasty.14

If platelet deposition influences localized vasoconstriction, the degree of platelet deposition would be expected to correlate directly with the severity of vasoconstriction, and reduction in the former would be expected to reduce the latter. To test these hypotheses, the relationship between platelet deposition and vasoconstriction and the effect of platelet inhibitor therapy on vasoconstriction were assessed in vivo in a porcine preparation of angioplasty in which both vasoconstriction and platelet deposition in response to arterial injury could be quantitated.

Methods

Experimental procedure. Eighteen normal Yorkshire pigs, 3 to 4 months old (mean weight, 35 kg), were given three doses of 1 mg/kg/day aspirin by mouth before angioplasty; 24 similar
untreated pigs served as controls. The pigs treated with aspirin had a ferric chloride urine test positive for salicylate within 2 hr of administration of the drug. In all pigs, autologous platelet labeling with $^{111}$In was performed $^{16}$ 18-24 hr before angioplasty. For angioplasty the pigs were sedated with ketamine (300 mg im; Ketaset, Bristol) and anesthetized with 0.5% halothane (Fluothane, Ayerst) mixed with room air and administered through an endotracheal tube by a Harvard respirator. The electrocardiogram and intra-arterial pressure were continuously monitored (Honeywell multichannel recorder) throughout the procedure.

Angioplasty of the common carotid arteries was performed with an No. 8F Meditech polyethylene balloon (8 mm $\times$ 3 cm) dilatation catheter inserted through a right femoral arteriotomy. After intravenous injection of a single bolus of heparin (100 USP units/kg), the dilatation catheter was advanced under fluoroscopic guidance into the common carotid arteries. The right and left common carotid arterial segments between the fourth and fifth vertebrae were dilated by five 30 sec inflations to six atmospheres (Meditech pressure manometer), with 60 sec intervals between inflations. The angiographic luminal diameter before dilation was 5 to 6 mm; during dilation the diameter of the inflated balloon within the artery was not more than 10% greater than that of the original arterial lumen (measured from plain x-ray films taken during the procedure and compared with predilation 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 before and after the dilatation procedure (figure 1) were obtained in all pigs with selective intra-arterial injection of 6.0 ml of meglumine diatrizoate (Renografin-76, Squibb) diluted with 6.0 ml of saline. The degree of vasoconstriction was expressed as the mean of the greatest narrowing of the lumen (measured with a caliper) just proximal and distal to the dilated segment on the postdilatation angiogram, expressed as percentages of dimension before dilatation.

Histopathology. After the postdilatation angiography, the pigs were given an overdose of pentobarbital and perfused antegrade with 2% glutaraldehyde and 1% paraformaldehyde in 0.1M cacodylate (pH 7.25) at a pressure of 100 mm Hg for 15 min to allow fixation of the arteries in situ. The carotid arteries were then removed and cleaned of all adventitia. The dilated portion of the fixed artery was easily identified by the regions of vasoconstriction proximal and distal to it, and from the spot films taken during and after the angioplasty (Figure 1). The dilated portion was divided into two equal segments of about 1.5 cm each; segments were also taken from vasoconstricted regions and from proximal and distal uninvolved artery. From each arterial segment, two to three ring sections were removed and stained with hematoxylin-eosin and Lason’s elastin–Van Gieson stain. The histologic 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 severe arterial wall injury; endothelial denudation without a tear through the internal elastic lamina was considered mild injury. Scanning electron microscopy was used to document the presence or absence of endothelial denudation.

Quantitation of platelet deposition. The extent of platelet deposition on the arterial segments was quantitated by the method of Dewanjee et al. $^{17}$ with autologous $^{111}$In-labeled platelets. $^{16}$ Three samples of blood were obtained at the time the animals were killed for determination of mean radioactivity in counts per min (cpm) per weight of blood (microbalance). The radioactivity (cpm) in each arterial segment was also obtained from a gamma well counter (Beckman, gamma 8000) and corrected for radionuclide decay and the percentage of free $^{111}$In in plasma (unbound to platelets). The spectrometer of the counter was adjusted to include the photopeaks at 174, 247, and 421 keV (sum peak) of $^{111}$In radionuclide. With knowledge of the whole blood platelet count (Coulter counter), the number of platelets deposited on an arterial segment was calculated using the equation:

$$\text{No. deposited platelets} = \frac{(111\text{In rpm in arterial segment}) \times (\text{No. platelets/ml blood})}{(111\text{In-cpm/ml blood})}$$

The number of platelets per unit area was then obtained by dividing the number of deposited platelets per arterial segment

![FIGURE 1. Angiographic films showing the carotid artery before angioplasty (left), during angioplasty with the balloon inflated with contrast (middle), and after angioplasty (right) when the vasoconstricted regions (arrows) can be clearly seen.](image-url)
by the surface area of the arterial wall (area = \( \pi \times d \times \ell \), where \( d \) = diameter of segment; \( \ell \) = length of the arterial segment).

Initially, platelet deposition was calculated only from the dilated segment. After a relationship between platelet deposition and vasoconstriction was suggested, the platelet deposition within the actual vasoconstricted regions was subsequently obtained, with the use of a smaller number of control pigs (\( n = 15 \)), to assess whether there was also a relationship between platelet deposition in the actual vasoconstricted region and the vasoconstrictive response both proximally and distally.

**Statistics.** Results are expressed as mean ± SE. The statistical significance of differences between group means was evaluated by a two-tailed unpaired t test. The correlation of platelet deposition with vasoconstriction was assessed by regression analysis with a least squares method. A \( p \) value ≤.05 was considered indicative of a significant difference.

**Results**

Variable degrees of vasoconstriction localized to the arterial segments immediately proximal and distal to the dilated region (figure 1) were observed after arterial angioplasty. In these regions of vasoconstriction, there was a concentric decrease in luminal diameter and a concomitant circumferential thickening of the arterial wall (figure 2), consistent with muscular contraction. In the constricted segments the endothelium was denuded, the internal elastic lamina was intact, and no intraluminal thrombi or intramural hemorrhage was seen macroscopically or microscopically. The light and electron microscopic changes in the dilated segment included denuded endothelium and damage ranging from subendothelial or mild injury with an intact internal elastic lamina to severe injury with a tear through the internal elastic lamina into the media as previously observed.\(^4\) The blood pressure and heart rate did not change significantly during the angioplasty procedure.

**Relationships among vasoconstriction, platelet deposition, and arterial injury.** Vasoconstriction was significantly greater (40 ± 4% vs 19 ± 4%, \( p = .002 \)) when platelet deposition (\( \times 10^6/cm^2 \)) was in excess of 10. In the arterial segment found distal to both the site of angioplasty and vasoconstriction, platelet deposition was less than 0.5 and vasoconstriction was not present. Over the range of platelet deposition values within the dilated region, the degree of vasoconstriction was positively and directly correlated with the logarithm of the platelet deposition (\( r = .77, p < .001 \)); log platelet deposition = 1.21 + 0.06 vasoconstriction (figure 3, A). In addition, there was a positive correlation between the platelet deposition in the actual vasoconstricted region and the vasoconstrictive response both proximally (\( r = .56, p < .03 \), log platelet deposition = 0.85 + 0.04 vasoconstriction, figure 3, B) and distally (\( r = .72, p < .003 \), log platelet deposition = 0.26 + 0.02 vasoconstriction, figure 3, C).

Platelet deposition was higher when arterial wall injury was severe than when it was mild (58.8 ± 11.9 vs 6.9 ± 0.5, \( p = .0001 \)). Likewise, vasoconstriction

**FIGURE 2.** The carotid artery perfused and fixed in situ with indicated cross sections shows the balloon-injured segment (B) (angioplasty), the adjacent vasoconstricted regions (V) with thickened wall consistent with muscular contraction, and the proximal (P) and distal (D) uninvolved regions.
was not changed by aspirin (6.9 ± 0.5 vs 7.5 ± 0.5, p = NS); vasoconstriction also was not changed (21 ± 3% vs 22 ± 2%, p = NS, figure 4, B).

Discussion

This is the first reported correlation in vivo between quantitative platelet deposition and vasoconstriction at the site of arterial injury and the reduction of vasoconstriction by platelet inhibition. This association may be a consequence of the ability of platelets to release potent vasoconstricting substances that may also act synergistically. In intact mice, the time sequence from onset of platelet aggregation to vasoconstriction has been shown to be 30 sec or more. The exponential relationship is consistent with the growth rate of platelet thrombi. The importance of platelets as mediators of the vasoconstrictive response as opposed to its being solely a response to injury is supported by the ability of pretreatment with aspirin to significantly decrease platelet deposition and thus cause a milder vasoconstrictive response despite severe injury (figure 5). Aspirin produced no significant change in vasoconstriction in the presence of mild injury when platelet deposition was greater in severely injured vessels than in mildly injured vessels (37 ± 3% vs 21 ± 3%, p = .001).

Effects of aspirin. Aspirin significantly decreased platelet deposition, relative to control, in the presence of severe arterial wall injury (from 58.8 ± 11.9 to 19.6 ± 6.5, p = .02, figure 4, A). In addition, this antiplatelet agent, which has no known intrinsic vasodilator properties, also significantly decreased vasoconstriction relative to control (from 37 ± 3% to 21 ± 3%, p = .003, figure 4, A). In the presence of mild arterial wall injury, platelet deposition was low and

FIGURE 4. In the presence of severe arterial wall injury, aspirin (1 mg/kg) significantly decreased platelet deposition and vasoconstriction relative to control (A). However, in the presence of mild injury, platelet deposition and vasoconstriction were lower and were not changed by aspirin (B).
deposition was not decreased. This response to aspirin is consistent with its antiplatelet effect since it cannot eliminate a single layer of platelet deposition and has no known direct vasodilator effects; it has even been shown to cause vasoconstriction in vitro. A reflex sympathetic mechanism cannot be entirely ruled out but is unlikely because the pigs were anesthetized, denervation has not been shown to affect injury-mediated vasoconstriction in experimental animals, and spontaneous vasospasm can still occur in arteries of denervated autotransplanted hearts in humans. This association between platelet deposition and vasoconstriction may represent an extension of the physiologic mechanism of hemostatic plug formation by which a sharp puncture produces platelet deposition and spasm at the site of injury.

We observed vasoconstriction immediately proximal and distal to the dilatation site, but never within the actual dilated segment itself. This lack of response has been referred to as vessel paralysis, but it is probably related to severe injury to the smooth muscle cells in the dilated region, which results in histologic evidence of necrosis by 24 hr and thus an inability to respond.

When the platelet deposition within the actual vasoconstricted region was examined, there was also a positive correlation between deposition in those regions and the vasoconstrictive response (figure 3, B and C). However, compared with the dilated segment, the vasoconstricted segments showed a much greater change in vasoconstrictive response per unit change in platelet deposition (figure 3, C). This is not unexpected since these platelets are probably the ones that produce the greatest effects in view of their close proxim-

FIGURE 5. Schematic summarizing the interactions among arterial wall injury, platelet deposition, and vasoconstriction. Although the severity of arterial wall injury appears to determine the extent of platelet deposition, it is the magnitude of the deposition that appears to influence the vasoconstrictive response. Thus, even when arterial injury is severe, the vasoconstrictive response can be mild if the heavy platelet deposition is reduced by an effective platelet inhibitor (e.g., aspirin) that may not have intrinsic vasodilator properties.

ity to the vasoconstricted regions. In addition, the vasoconstrictive response per unit change in platelet deposition is greatest distally; this may reflect the additional effects of vasoconstrictive substances released more proximally and flowing antegrade to affect the distal vasoconstricted segment.

Localized vasoconstriction was also never observed in the undamaged regions of the artery distal to the site of dilatation, where scanning electron microscopy showed a normal intact endothelium and no focal platelet deposition. Thus, in this preparation, localized vasoconstriction appears to occur only at sites of platelet deposition where there is endothelial cell loss and an underlying media responsive to vasoconstricting stimuli. This is not unlike selective endothelial injury of the coronary arteries in vivo, in which there is an enhanced vasoconstrictive response localized to the site of injury.

Breach of the endothelium is a local stimulus for platelet deposition. This endothelial denudation may represent the primary inciting stimulus, without which both platelet deposition and the subsequent localized vasoconstriction do not occur. The magnitude of the resulting platelet deposition then determines the physiologic and functional consequences through thrombus formation and spasm; greater platelet deposition is associated with larger thrombus formation and greater vasoconstriction. By providing a potent stimulus for platelet deposition, plaque rupture or ulceration may explain the frequent occurrence of spasm; through the release of platelet-derived vasoconstrictor substances, the frequent occurrence of thrombosis, or both, at sites of atherosclerosis and thus may explain the possible mechanisms of myocardial ischemia or injury in the conversion from stable to the unstable coronary syndromes of unstable angina, myocardial infarction, and sudden death in which arterial injury (plaque rupture) and associated platelet deposition and thrombus formation have been observed. Arterial wall injury, plaque rupture, and platelet deposition also occur after coronary angioplasty, and may explain the subsequent but infrequent acute occlusion as a result of thrombosis and spasm.

Thus, the localized aggregation and release response of platelets at the site of arterial injury appears to be physiologically important in mediating the localized vasoconstriction. Whereas other mechanisms might be operative in concert with the platelets and platelet-derived substances in the modification of vasoconstriction, the platelet–arterial wall interaction appears to be very important. Recognition of this inter-

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action may promote understanding of the pathophysio-
logic mechanisms, which is important for future ra-
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