Inhibition of Platelet Function
In Vivo or In Vitro by
Organic Nitrates*

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Organic nitrates have been used to relieve angina since Brunton (1) first described their use in 1867. The assumption has been that nitrates decrease myocardial oxygen demand by reducing preload and afterload. In the last 20 years it has been established that unstable angina and possibly silent ischemia at rest are often due to platelet aggregates forming in stenosed coronary arteries. This platelet thrombus reduces coronary flow until the thrombus either disaggregates or embolizes distally, thereby restoring coronary flow. Several studies (2,3) have clearly demonstrated that aspirin reduces myocardial ischemic events in patients with unstable angina.

There is considerable interest in being able to measure platelet function in vivo and in vitro to determine which substances—drugs or hormones—increase or decrease platelet activity. Until 1962 the only available means of measuring platelet function was clot retraction or bleeding time. In 1962 Born (4) developed the platelet aggregometer, a device in which light is passed through platelet-rich plasma placed in a clear glass cuvette. When a platelet agonist, such as collagen or adenosine diphosphate, is injected into the platelet-rich plasma, the platelets aggregate, making clumps of platelets that then fall to the bottom of the cuvette. As the platelets aggregate, the solution clears and the light transmittance increases. The degree to which the platelet-rich plasma solution clears is proportional to the functional activity of the platelets. Although this technique is useful, it takes approximately 90 min from the time blood is drawn from a patient or animal until the platelets can be studied. Thus, transient changes in platelet activity, such as those produced by cigarette smoking, can be missed (5).

The present study. Stamler and Loscalzo (6) in this issue of the Journal carefully review the considerable evidence that suggests that part of the antiangiial effect of nitro compounds given intravenously over time may well be due to the antiplatelet effect. The two main methods used to determine the effects of nitrates on platelet function were 1) in vitro or ex vivo studies using the platelet aggregometer, and 2) the established “Folts model of coronary platelet thrombosis,” as recently reviewed by Bush and Shebuski (7). In the past, ex vivo testing of platelets obtained from patients receiving nitroglycerin intravenously produced confusing results, in part because of depletion of reduced thiols during phlebotomy and preparation of platelet-rich plasma. Stamler et al. (8) have clearly shown that replenishing the stores of reduced thiols with N-acetylcysteine promotes platelet inhibition with intravenous administration of nitroglycerin to patients.

Some confusion also arose using the Folts model of coronary thrombosis to study the in vivo effects of organic nitrates. This model studies periodic acute platelet thrombus formation in stenosed canine coronary arteries with intimal damage. The acute platelet thrombus formation followed by embolization produces cyclical flow reductions. These cyclical flow reductions will continue for many hours if no effective platelet inhibitor is given (7,9). In 1981 Kowey et al. (10) showed that intracoronary infusions of 80 μg/min of nitroglycerin for 10 min produced a 15 mm Hg fall in arterial pressure and significantly decreased the cyclical flow reductions. In 1982 Folts et al. (11), using this model, observed that nitroglycerin given sublingually or topically applied to the area of stenosis for 10 or 15 min had no effect on cyclical flow reductions even though arterial blood pressure declined significantly, indicating that the nitroglycerin was absorbed and circulating. In 1990, Golino et al. (12), using the same coronary platelet thrombus model, were unable to show decreases in cyclical flow reductions with intravenous administration of 5 μg/kg per min of nitroglycerin for 30 min. They did show that 21 μg/kg per min of intracoronary nitroglycerin given for an unspecified length of time produced a small decrease in the size and frequency of the cyclical flow reductions. However, as Stamler and Loscalzo (6) clearly point out in their review in this issue of the Journal, the effects of organic nitrates are dose and time dependent. Recently it was shown (13) in the Folts model that infusions of nitroglycerin, (10 to 15 μg/kg per min for 1 h) did indeed inhibit in vivo platelet activity and abolished acute platelet thrombus formation and the cyclical flow reductions. In addition, pretreating the dog with 100 mg/kg of N-acetylcysteine, which provides additional reduced thiols, produced abolition of cyclical flow reductions with only 5 μg/kg per min of nitroglycerin within 45 min (13). Finally, in a pilot study with this model (unpublished observations), we found that sodium nitroprusside given intravenously at 8 to 10 μg/kg per min abolished acute platelet thrombus formation and cyclical flow reductions within only 10 to 15 min after the onset of infusion.

Recently Ovize et al. (14) demonstrated in the Folts dog model that molsidomine and its metabolite SIN-1 (3 morpholinosydnonimine), a donor of nitric acid, significantly reduced acute platelet thrombus formation and cyclical flow...
reductions. These investigators (14) concluded that the anti-ischemic properties of SIN-1 may well be due in part to its antiplatelet effects.

**Mechanism of platelet activity inhibition.** Stamler and Loscalzo (6) in their extensive review show that the mechanism by which organic nitrates inhibit platelet activity appears to be mediated by release of nitric oxide with the subsequent elevation of platelet cyclic guanosine monophosphate (GMP) and that this can be enhanced by providing reduced thiols such as N-acetylcysteine. They also show that the effects of organic nitrates on platelets are time dependent. Thus, one would not expect to see significant platelet inhibition from periodic sublingual application of nitrates, and we did not see it when giving nitroglycerin by this route in our animal model (11). It remains to be seen whether transdermal application of nitrates will have a significant platelet-inhibitory effect.

**Potential problems.** Two potential problems may occur with the use of intravenous nitrates as pharmacologic agents to inhibit platelet activity and acute platelet thrombus formation. The first is the potential for producing tolerance, which is seen with the hemodynamic effects of nitrates clinically (15) and may also occur with the antiplatelet effects. The second is the possibility that when blood pressure is decreased by nitrates, there may be an increased release of catecholamines that act synergistically with platelet-activating factor, collagen and adenosine diphosphate (16). It has been shown (17–19) that the platelet inhibitory effect of aspirin, for example, is significantly diminished by elevated plasma epinephrine and norepinephrine levels.

These two potential problems need to be addressed in future studies to delineate the role of organic nitrates as platelet inhibitors and to determine if any of the nitrates will provide adequate protection when plasma catecholamine levels are elevated.

**References**