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CLINICAL INVESTIGATIONS

Nitroglycerin inhibits experimental thrombosis and reocclusion after thrombolysis

Nitroglycerin inhibits platelet aggregation in vitro, but its effect on thrombosis and platelet function in vivo is controversial. This study assessed the effect of nitroglycerin on primary thrombus formation in response to vessel wall injury and secondary thrombus formation, or rethrombosis, after lysis of an existing thrombus. In the first protocol the right carotid artery was instrumented with a flow probe, stenosis, an anodal electrode, and a proximal infusion line. A 300 μA anodal current was used to induce endothelial injury and subsequent thrombotic occlusion of the vessel. Anisoylated plasminogen streptokinase activator complex (APSAC; 0.05 U/kg intraarterially) was injected proximal to the thrombus 30 minutes after occlusion. After APSAC, nitroglycerin (1 μg/kg/min intraarterially, $n = 7$) or vehicle ($n = 6$) was infused proximal to the thrombus for 3 hours. Reocclusion occurred in two of seven nitroglycerin-treated dogs and six of six vehicle-treated dogs ($p < 0.05$). In the second protocol both carotid arteries were instrumented as described previously. Anodal current (300 μA, 180 minutes) was applied to the right carotid ($n = 12$) artery to determine control times to occlusion. The left carotid artery served as the test vessel, receiving either nitroglycerin (1 μg/kg/min intraarterially, $n = 6$) or trimethaphan (0.05 mg/kg/hr intraarterially, $n = 6$). Trimethaphan was used to produce controlled hypotension to match the approximately 10% decrease in mean arterial blood pressure that was observed during nitroglycerin infusion. Control arteries and those treated with trimethaphan formed occlusive thrombi in all instances. Nitroglycerin infusion resulted in a lower incidence of occlusion (1 of 6; $p < 0.05$ vs control value) and inhibited ex vivo platelet aggregation to adenosine diphosphate and arachidonic acid ($p < 0.05$). Local infusion of nitroglycerin reduced the formation of primary thrombi, independent of the hypotensive effect of the drug, and exerted systemic effects on platelet aggregation. Furthermore, platelet inhibition with nitroglycerin reduced the incidence of secondary thrombus formation (rethrombosis) after thrombolysis. The results suggest that a potential benefit of nitroglycerin therapy may be derived from its ability to inhibit thrombotic events in patients with unstable angina or myocardial infarction. (Am Heart J 1994;127:727-37.)

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Coronary thrombosis at sites of coronary stenosis or a ruptured atherosclerotic plaque plays a major role in the pathophysiology of unstable angina, acute myocardial infarction, or sudden death.[1, 2] Therefore thrombolytic drugs, anticoagulants, and antiplatelet drugs are mainstays of medical therapy in patients with coronary artery disease. Nevertheless, despite treatment with aspirin, patients with unstable angina may progress to acute myocardial infarction, and reocclusion can occur after succcessful thrombolytic therapy for acute myocardial infarction.

Nitroglycerin (NTG) is used widely to treat myocardial ischemia in patients with stable or unstable angina and acute myocardial infarction. Although relaxation of vascular smooth muscle is an important antiischemic property of nitrates, recent review arti-

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cles and editorials have emphasized the growing evidence that nitrates may exert a clinically important antiplatelet effect.[3,4] This view is supported by several studies that have demonstrated inhibition of ex vivo aggregation of platelets obtained from patients receiving an intravenous infusion of NTG.[5-7] There are conflicting experimental data, however, regarding the ability of NTG to inhibit platelet function and arterial thrombosis in vivo. NTG inhibited the deposition of platelets after deep arterial injury caused by balloon angioplasty[8] and the platelet-mediated cyclic flow variations in stenosed coronary arteries.[9] Other investigators, however, did not observe a beneficial effect of NTG in experimental models of coronary thrombosis and thrombolysis.[10-12] Therefore this study was performed to further characterize the effects of NTG on arterial thrombosis in vivo. Two experimental paradigms were employed. First, we investigated the effect of NTG on reocclusion after thrombolysis, which is associated with a marked increase in morbidity and mortality in patients with acute myocardial infarction.[13] Second, we examined the effect of NTG on primary thrombus formation caused by endothelial injury.

METHODS

Animal investigation. These studies conformed to the Position of the American Heart Association on Research Animal Use, adopted November 11, 1984, by the American Heart Association. The procedures followed in this study were in accordance with the guidelines of the University of Michigan (Ann Arbor) University Committee on the Use and Care of Animals. Veterinary care was provided by the University of Michigan Unit for Laboratory Animal Medicine. The University of Michigan is accredited by the American Association of Accreditation of Laboratory Animal Care, and the animal care and use program conforms to the standards in "The Guide for Care and Use of Laboratory Animals," Department of Health, Education, and Welfare Publication No. NIH 78-23.

Reagents. NTG was provided by Warner Lambert (Ann Arbor, Mich.). Trimethaphan camsylate (Arfonad) was provided by Hoffmann-La Roche Inc. (Nutley, N.J.). Anisoylated plasminogen streptokinase activator complex (APSAC) was provided by Smith Kline Beecham (King of Prussia, Pa.). Sodium citrate, adenosine diphosphate (ADP), arachidonic acid, epinephrine, and any reagents used in the laboratory but not mentioned specifically were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Surgical preparation. Male mongrel dogs (15 to 17 kg) were anesthetized with sodium pentobarbital (30 mg/kg intravenously), endotracheal tubes were placed, and the animals were allowed to breathe room air. One or both common carotid arteries and the right internal jugular vein were exposed. A catheter was inserted into the jugular vein for blood sampling and drug administration. Arterial blood pressure was monitored from the cannulated femoral artery with the use of a blood pressure transducer (Gould Inc., Cardiovascular Products, Oxnard, Calif.). Standard limb lead II of the ECG was recorded continuously.

The model used in this study is a modification of one developed by our laboratory for the study of experimentally induced coronary artery thrombosis.[14,15] The experimental procedure results in the formation of a platelet-rich intravascular thrombus at the site of an electrolytically induced endothelial lesion in proximity to a distal arterial stenosis. Electrolytic injury to the intimal surface of the carotid artery was accomplished with an intravascular electrode composed of a Teflon-insulated, silver-coated copper wire. Penetration of the vessel wall by the electrode was facilitated by attaching the tip of a 25-gauge hypodermic needle to the uninsulated part of the electrode. The electrode was positioned so that the uninsulated portion was in direct contact with the endothelial surface of the vessel. Proper positioning of the electrodes in each of the carotid arteries was confirmed by visual inspection at the end of each experiment. Each intraarterial electrode was connected to the positive pole (anode) of a dual-channel stimulator (Grass S88 stimulator and Grass constant current unit, model CCU1A, Grass Instrument Co., Quincy, Mass.). The cathode was connected to a distant subcutaneous site. The current delivered to each vessel was monitored continuously and maintained at 300 μA.

A Doppler flow probe (model 100, Triton Technology, San Diego, Calif.) was placed on each common carotid artery proximal to both the point of insertion of the intraarterial electrode and a mechanical constrictor. The mechanical constrictor was constructed of stainless steel in a C shape with a Teflon screw (2 mm diameter) that could be adjusted to control the circumference of the vessel and produce a regional stenosis. The constrictor was adjusted until the pulsatile flow pattern was reduced by 50% without altering the mean blood flow. Blood flow in the carotid vessels was monitored continuously. A 24-gauge needle was inserted in the carotid artery proximal to the electrode and flow probe to administer intraarterial infusions of APSAC, nitroglycerin, and trimethaphan.

Protocol 1: Prevention of rethrombosis after thrombolysis. The protocol designed to determine whether NTG prevents rethrombosis is shown in Fig. 1, *A*. The injury current was applied to the right carotid artery for a maximum of 3 hours and was terminated 10 minutes after blood flow in the vessel had remained stable at zero velocity to verify formation of a stable occlusive thrombus. APSAC (0.05 U/kg) was injected intraarterially proximal to the thrombus 30 minutes after occlusion. Previous studies have demonstrated that the selected dose of APSAC does not produce a systemic effect (unpublished data). After the injection of APSAC, an intraarterial infusion of NTG (1 μg/kg/min, $n = 7$) or vehicle (50% alcohol and 50% propylene glycol, $n = 6$) was begun. Reperfusion was defined as the restoration of flow to 20% of baseline values. After infusion of NTG or vehicle for 3 hours, the experiment was terminated.

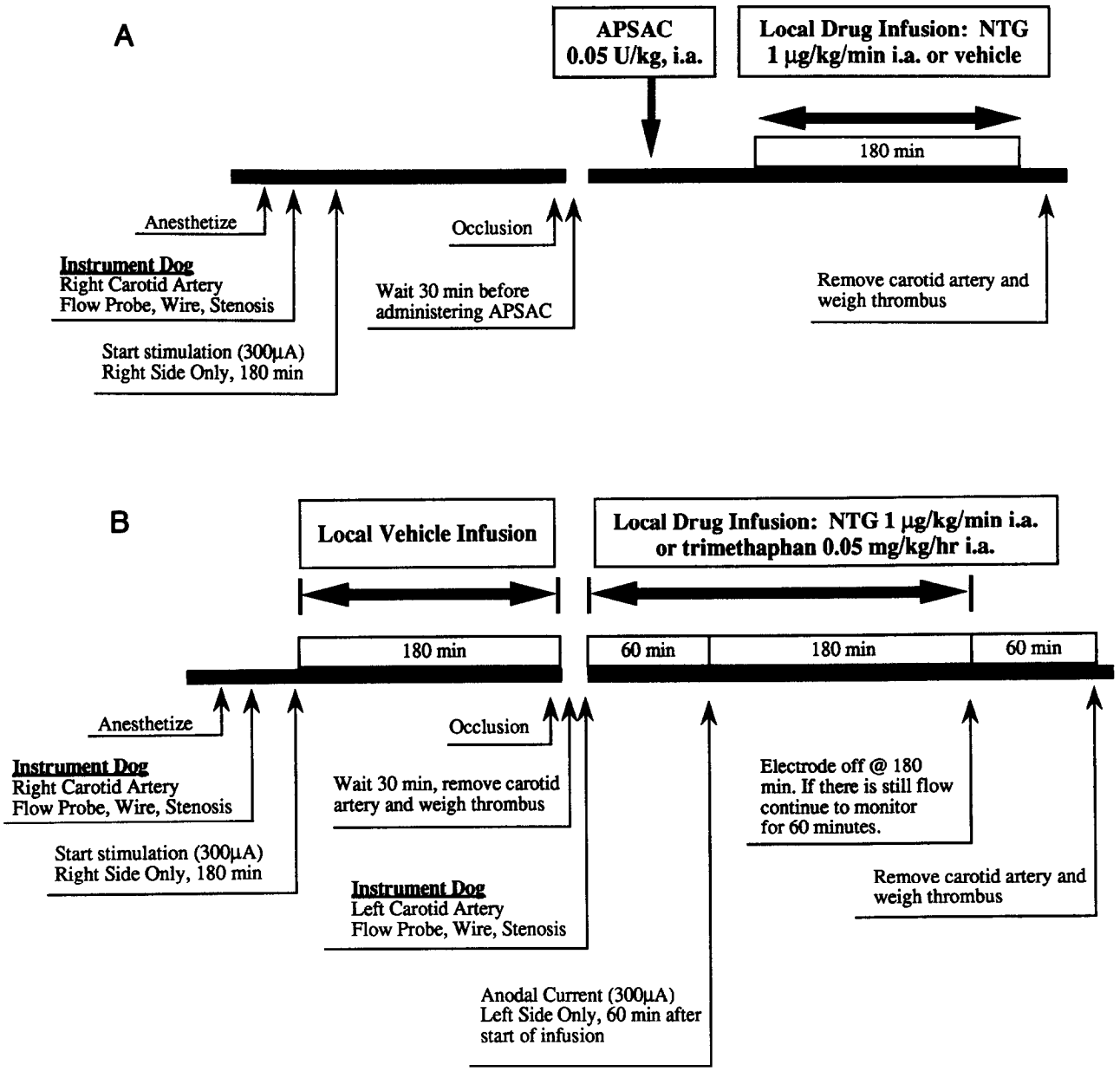

Fig. 1. A, Protocol used in first phase of these studies designed to evaluate effects of local infusion of NTG or vehicle on rethrombosis after lysis of existing occlusive carotid artery thrombus. **B,** Diagramatic representation of experimental protocol used in second phase of these studies designed to determine effects of NTG on prevention of carotid artery thrombus formation. Trimethaphan, a ganglionic blocking agent, was used to determine whether the hypotensive effect of NTG affected the thrombotic process. *NTG*, Nitroglycerin; *i.a.*, intraarterial; *APSAC*, anisoylated plasminogen streptokinase activator complex.

Protocol 2: Prevention of thrombus formation. The protocol designed to determine whether NTG prevents thrombus formation is shown in Fig. 1, *B*. Infusion of NTG resulted in mild hypotension in protocol 1. Therefore a ganglionic blocking agent (trimethaphan) was used as a control drug to evaluate the role that vasodilation plays in occlusive arterial thrombus formation. In each experiment the right carotid artery served as the control vessel, and the left carotid artery served as the test vessel (NTG or trimethaphan). Thirty minutes after thrombosis of the right carotid artery, the left carotid artery was instrumented in an identical manner. Either NTG (1 μg/kg/min intraarterially, $n = 6$) or trimethaphan (0.05 mg/kg/hr intraarterially, $n = 6$) was infused proximal to the electrode.

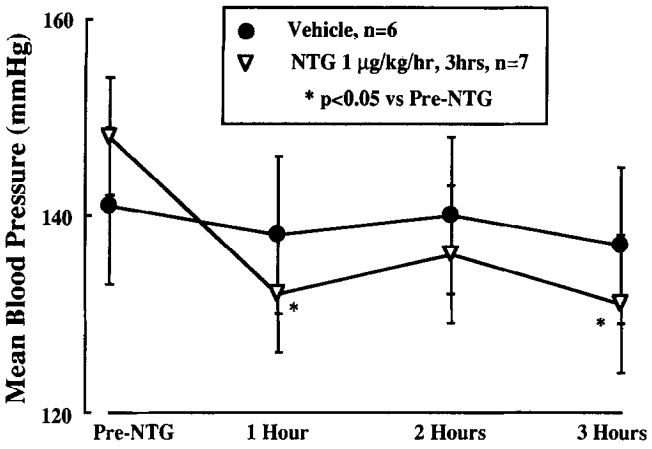

Fig. 2. Blood pressure data for reocclusion protocol.

Sixty minutes after the NTG or trimethaphan infusion was begun, the anodal current was applied and continued for a maximum of 3 hours.

Hematologic measurements. Blood (10 ml) was withdrawn for platelet studies from the jugular cannula into a plastic syringe containing 3.2% sodium citrate as the anticoagulant (1:10 citrate/blood [volume/volume]). Blood was taken for platelet aggregation and whole blood cell counts at baseline and 60, 180, and 240 minutes after the administration of NTG or trimethaphan. Whole blood cell counts, hematocrit, and hemoglobin were determined with an H-10 cell counter (Texas International Laboratories, Inc., Houston, Texas). Platelet-rich plasma (PRP), the supernate present after centrifugation of anticoagulated whole blood at 1000 rpm for 10 minutes (140 *g*), was diluted with platelet-poor plasma (PPP) to achieve a platelet count of 200,000/mm^3. PPP was prepared after the PRP was removed by centrifuging the remaining blood at 12,000 *g* for 10 minutes and discarding the bottom cellular layer. Ex vivo platelet aggregation was measured by established spectrophotometric methods with a four-channel aggregometer (BioData-PAP-4, BioData Corp., Hatboro, Pa.) by recording the increase in light transmission through a stirred suspension of PRP maintained at 37° C. Aggregation was induced with arachidonic acid (0.65 mmol/L) and ADP (20 μmol/L). A subaggregatory dose of epinephrine (550 nmol/L) was used to prime the platelets before stimulation.[15] Values were expressed as a percentage of aggregation, representing the percentage of light transmission standardized to PRP and PPP samples, yielding 0% and 100% light transmission, respectively.

Exclusion criteria. Animals were excluded from the study for any of the following reasons: (1) a circulating platelet count less than 100,000/mm^3; (2) failure of the platelets to aggregate in response to arachidonic acid before administration of NTG; (3) in protocol 2, failure of the right carotid artery (control vessel) to develop thrombotic occlusion within 4 hours of the onset of vessel wall injury by a 300 μA direct anodal current; or (4) presence of heartworms.[16]

Statistical analysis. The data are expressed as means ± SEM. Unpaired *t* tests were used for between-group comparisons of hemodynamic and platelet aggregation data. Repeated-measures analysis of variance was used for within-group analysis of hemodynamic data. The incidence of occlusion was compared by means of Fisher's exact test. Differences were considered significant at $p < 0.05$.

RESULTS

Protocol 1: Prevention of reocclusion after thrombolysis. Thirteen dogs were entered into protocol 1. As indicated in Fig. 2, blood pressure remained stable during infusion of vehicle. Infusion of NTG, however, caused a significant and sustained drop in mean arterial pressure. There were no significant differences in heart rate between groups or within either group compared with baseline values.

PRP was obtained from venous blood samples before and during infusion of NTG or vehicle. Platelet aggregation was induced by either arachidonic acid or ADP. As illustrated in Fig. 3, there was a significant inhibition of ex vivo platelet aggregation during infusion of NTG. Platelet aggregation remained unchanged during infusion of vehicle.

Infusion of NTG significantly reduced the incidence of arterial reocclusion after thrombolysis induced by APSAC. Reocclusion occurred in each of the six dogs treated with vehicle compared with two of seven dogs ($p < 0.05$ vs vehicle) that received an infusion of NTG proximal to the thrombus. The mean time to reocclusion was 61 minutes for the control group and 210 minutes for the NTG group ($p < 0.01$ vs control group).

Protocol 2: Prevention of thrombus formation. Based on the results obtained in protocol 1, additional experiments were performed to determine whether NTG can inhibit primary thrombus formation. Thirteen dogs were entered into protocol 2. One experiment was terminated after the right (control) carotid artery failed to occlude during electrical stimulation. Red cell counts, platelet counts, hematocrit and hemoglobin were all within normal limits and were unaffected by treatment with NTG, or trimethaphan (Table I).

The hemodynamic effects of both agents are shown in Fig. 4. NTG and trimethaphan caused a similar hypotensive effect, reducing mean arterial blood pressure approximately 10% to 15% from baseline values. Mean heart rate, however, was unaffected by either drug. As observed in protocol 1, infusion of NTG significantly inhibited platelet aggregation in response to both ADP and arachidonic acid (Table II). Administration of the ganglionic blocker trimethaphan, however, did not inhibit ex vivo platelet aggregation.

Carotid artery blood flow velocity at baseline and 4 hours after vessel wall injury is depicted in Fig. 5. Control vessels all occluded within the 3-hour period

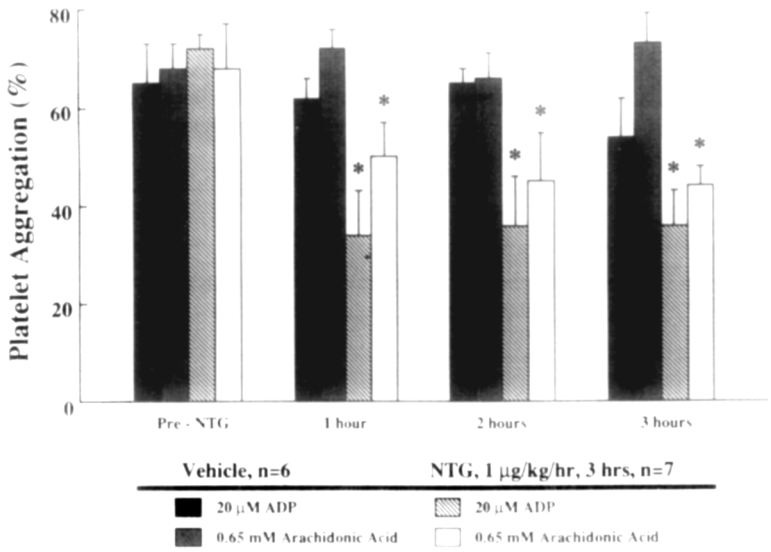

Fig. 3. Platelet aggregation data for reocclusion protocol. $*p < 0.05$ versus pre-NTG values.

of electrolytic injury. Similarly each of the vessels treated with trimethaphan occluded within the 3-hour period of anodal current injury. Only one of six vessels occluded during infusion of NTG ($p < 0.05$ vs control value). The single artery that did occlude in the NTG group closed 244 minutes after the start of the anodal current application, whereas the mean time to occlusion was 134.6 ± 10.4 minutes in the control arteries and 154.0 ± 14.0 minutes in the arteries treated with trimethaphan.

DISCUSSION

The principal findings of this study are that NTG reduced both the incidence of reocclusion after thrombolysis and the frequency of thrombosis after vessel wall injury. Although administration of NTG caused a mild reduction in arterial blood pressure, an equivalent reduction in blood pressure by trimethaphan was not associated with a reduction in the incidence of thrombotic occlusion after intimal injury. In addition, platelet aggregation ex vivo was significantly inhibited by treatment with NTG but not by trimethaphan. Thus the results of this study support the hypothesis that treatment with NTG may inhibit thrombosis in vivo by inhibiting platelet function.

Although previous studies have demonstrated that NTG inhibits platelet aggregation, in vivo studies of the effects of NTG on platelet function and arterial thrombosis have yielded conflicting results.[8-12, 17] Comparison of positive and negative reports suggests that differences in dosage and route of administration may be the principal reasons for the discordant results. Golino et al.[17] reported that intravenous NTG at a dose of 5 μg/kg/min did not inhibit cyclic flow

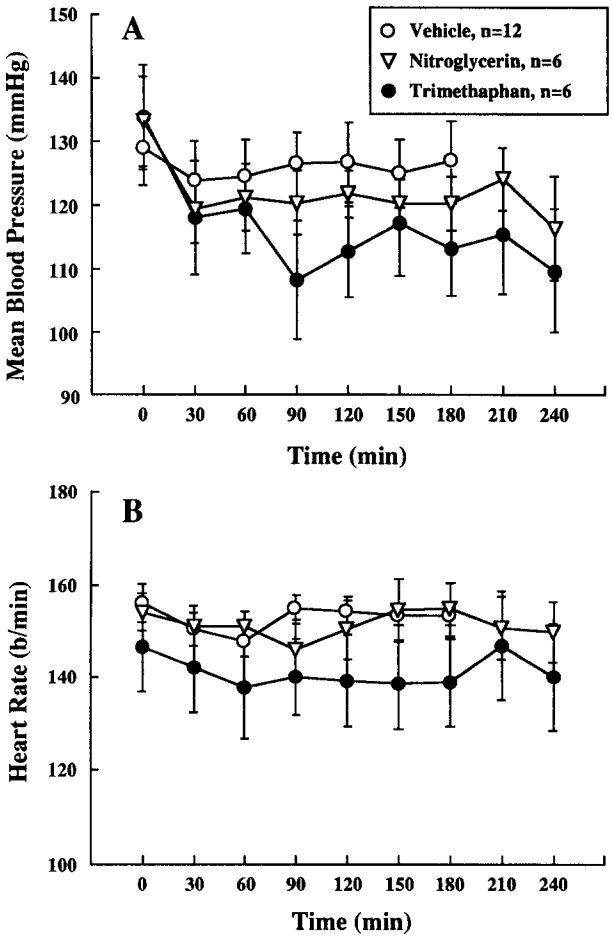

Fig. 4. A, Effect of treatment with NTG (1 μg/kg/min intraarterially) and trimethaphan (0.05 mg/kg/hr intraarterially) on mean arterial blood pressure versus vehicle treatment. Similar reductions in blood pressure (≈10% to 15% decrease) resulted from treatment with either agent. **B,** Mean heart rate over time in the three groups.

Table I. Effects of treatment (nitroglycerin or trimethaphan) on whole blood cell counts in dogs

Time	*Red cell count* ($\times 10^6/mm^3$)	*White cell count* ($\times 10^3/mm^3$)	*Platelet count* ($\times 10^3/mm^3$)	*Hematocrit* (%)	*Hemoglobin* (gm/dl)
Dogs treated with NTG (240 min infusion; 1.0 μg/kg/min intraarterially)					
Baseline	5.1 ± 0.2	12.3 ± 2.4	222 ± 17	27.8 ± 2.2	11.7 ± 1.5
60 min	5.3 ± 0.6	12.6 ± 2.3	205 ± 10	31.3 ± 3.2	10.4 ± 1.3
180 min	5.1 ± 0.2	15.7 ± 2.3	210 ± 11	30.2 ± 1.5	10.9 ± 1.1
240 min	5.3 ± 0.2	14.8 ± 2.7	186 ± 13	30.9 ± 1.3	10.9 ± 1.1
Dogs treated with trimethaphan (240 min infusion; 0.05 mg/kg/min intraarterially)					
Baseline	4.7 ± 0.3	10.8 ± 1.9	207 ± 26	25.4 ± 2.0	18.4 ± 2.2
60 min	5.0 ± 0.2	10.0 ± 2.7	188 ± 25	27.5 ± 1.1	19.9 ± 3.2
180 min	5.1 ± 0.2	12.2 ± 2.1	185 ± 33	28.3 ± 1.1	20.1 ± 3.1
240 min	5.1 ± 0.2	10.7 ± 1.4	190 ± 27	24.7 ± 0.8	20.3 ± 3.0

Each value represents mean ± SEM, $n = 6$ in each group.
Normal ranges (canine): red cell count, 4.4 to 6.4; white cell count, 5.8 to 17.8; platelet count, 175 to 300; hematocrit, 26.0 to 42.0; hemoglobin, 10.0 to 24.6.

Table II. Effects of treatment (nitroglycerin or trimethaphan) on ex vivo platelet aggregations from dogs in protocol 2

Time	*ADP* (20 μmol/L)	*Arachidonic acid* (650 μmol/L)
Dogs treated with NTG (240 min infusion; 1.0 μg/kg/min intraarterially)		
Baseline	75.8 ± 4.0	70.7 ± 3.9
240 min	48.5 ± 9.8*	52.3 ± 5.4*
Dogs treated with with trimethaphan (240 min infusion; 0.05 mg/kg/min intraarterially)		
Baseline	59.0 ± 7.9	65.4 ± 6.2
240 min	58.0 ± 11.8	72.0 ± 9.5

ADP, Adenosine diphosphate.
Values are percentages of aggregation representing percentage of light transmission of sample standardized to 0% for platelet-rich plasma and 100% for platelet-poor plasma. Each value represents mean ± SEM, $n = 6$ in each group.
*$p < 0.05$ versus baseline value. All aggregations were performed with preaddition of a subaggregatory priming dose of epinephrine (550 nmol/L).

variations in the "Folts" model of coronary artery stenosis. By use of the same experimental preparation, Folts et al.[9] also found that NTG was ineffective at a dose of 5 μg/kg/min, but there was significant inhibition at doses of 10 and 15 μg/kg/min. Martorana et al.[10] reported that intravenous NTG did not prevent coronary artery occlusion caused by electrical stimulation, but the maximum dose studied was 5 μg/kg/min. Nicolini et al.[11] and Mehta et al.[12] investigated the effect of NTG on thrombolysis induced by tissue-type plasminogen activator (TPA). The dose of NTG was 125 μg/min and the average weight of the dogs was 21 kg, yielding an average dose of 6 μg/kg/min. Platelet aggregation measured ex vivo decreased markedly after administration of TPA alone but not after treatment with the combination of TPA and NTG. NTG did not prevent reocclusion after thrombolysis, and when NTG was given with TPA, the time to reperfusion was longer and the duration of reperfusion shorter.

The present study differs from previous ones in several respects. Nicolini et al.[11] and Mehta et al.[12] used TPA in their studies and provided evidence that NTG may decrease the thrombolytic potency of TPA by accelerating its clearance by the liver. APSAC, which has a prolonged plasma half-life,[18] was used in the present study. In addition, NTG was administered intravenously in previous studies and immediately proximal to the arterial thrombus in the present study. Plasma concentrations of NTG were not measured in this study; neither were they reported in the experimental studies cited previously. Previous research, however, has shown that NTG is extracted in the systemic and pulmonary capillary beds.[19] Thus the concentration of NTG at the site of an arterial thrombus would be greater during an intraarterial infusion than during an intravenous infusion. Although NTG is usually administered intravenously, intracoronary nitrates are often used during coronary angioplasty to relieve coronary artery spasm, and they have been used as an adjunct to thrombolytic therapy.[20, 21] Hackett et al.[21] reported that intracoronary injection of isosorbide dinitrate reestablished coronary artery patency in 11 of 16 patients with reocclusion during intracoronary infusion of streptokinase. Although the effect was attributed to relief of vasoconstriction, an antiplatelet effect cannot be dismissed. There is a markedly increased risk of complete occlusion after balloon angioplasty of coronary arteries with visible thrombi and of old saphenous vein bypass grafts.[22, 23] Intracoronary infusion of a thrombolytic agent has been advocated as a means of reducing the risk of thrombotic occlusion after angioplasty.[24] The results of this study suggest

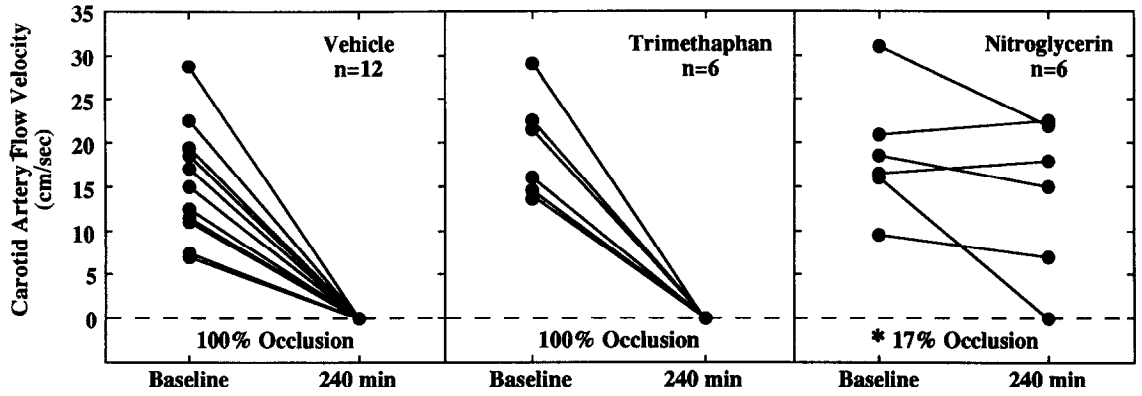

Fig. 5. Coronary artery blood flow velocity for each animal in each group measured before initiation of anodal current application and again 240 minutes later. Control arteries and those treated with trimethaphan exhibited 100% incidence of occlusive thrombus formation, whereas arteries treated with NTG resisted occlusion. $*p < 0.05$ versus vehicle or trimethaphan, Fisher's exact test.

that concomitant infusion of NTG might be worth investigating.

Although the inhibitory effect of NTG on platelet aggregation was reported in 1967,[25] nitrates have been viewed primarily as vasodilators rather than antiplatelet agents.[3, 26] Recently, however, it has been demonstrated that NTG and other organic nitrates cause relaxation of vascular smooth muscle and inhibit platelet function by the same mechanism, involving an increased formation of cyclic guanosine monophosphate (GMP).[3] Johnstone et al.[27] conducted a series of experiments to determine whether inhibition of cyclic GMP formation would block the antithrombic effect of NTG. Pigs were treated with either intravenous NTG alone or NTG and methylene blue, an inhibitor of guanylate cyclase. The blood of pigs treated with NTG alone inhibited the deposition of platelets on aortic media in an ex vivo perfusion chamber. Infusion of methylene blue blocked the antiplatelet effect of NTG, supporting in vitro evidence that NTG inhibits platelet function by increasing platelet cyclic GMP.

There are several implications of the antiplatelet mechanism of NTG that may have clinical relevance. One is that both NTG and nitric oxide (NO), which is believed to be the principal endothelial-derived relaxing factor, inhibit platelet aggregation by increasing platelet cyclic GMP.[3, 28, 29] Experimental studies have indicated that increased platelet cyclic GMP, but not increased cyclie adenosine monophosphate, inhibits thrombin-induced adhesion of platelets to endothelium, suggesting that endothelial production of NO rather than prostacyclin may be the primary endothelial defense against platelet adhesion.[30] Thus stimulation of endothelial production of NO inhibits the adhesion of platelets, and inhibition of endothelial NO production enhances platelet adhesion in vitro.[30-32] Several experimental studies have attempted to demonstrate that vascular NO synthesis inhibits platelet activation in vivo, but the results are ambiguous because platelets also synthesize NO, and inhibitors of platelet NO formation such as N^G-monomethyl-L-arginine (L-NMMA) enhance platelet aggregation.[33-35] Thus it is likely that inhibition of platelet rather than endothelial NO synthesis explains the ability of L-NMMA to promote cyclic flow variations in coronary arteries with endothelial injury and stenosis.[33] Several interesting studies have examined platelet-endothelial interactions after administration of acetylcholine, which stimulates endothelial formation of NO but does not affect platelet formation of NO or platelet aggregation.[33, 34, 36] The addition of acetylcholine to the cardiac perfusate increased the cyclic GMP content of platelets that had passed through the coronary vascular bed.[36] An intraaortic infusion of acetylcholine or an infusion of exogenous NO proximal to a carotid artery stenosis completely inhibited cyclic flow reductions at the site of stenosis.[34] There is extensive experimental and clinical evidence that hypertension, hypercholesterolemia, and atherosclerosis impair endothelial formation or release of NO.[37, 38] Therefore NTG or other exogenous stimulators of platelet guanylate cyclase may be useful for limiting platelet aggregation and deposition at sites of endothelial dysfunction.

A second implication of the mechanism of action NTG is that it may act synergistically with other antiplatelet compounds that do not act via cyclic GMP, for example, aspirin or agents that stimulate the formation of platelet cyclic AMP. In vitro experiments

demonstrated synergistic disaggregation of platelets by NTG, by prostaglandin E_1, which increases platelet cyclic adenosine monophosphate, and by TPA.[39] Treatment with prostaglandin E_1 and isosorbide dinitrate was reported to exert a synergistic effect on platelet function in patients with peripheral vascular disease.[40] A recent study of human subjects treated with aspirin and NTG showed additive effects on platelet aggregation ex vivo.[41]

Despite experimental evidence that NTG has significant antithrombotic effects, previous experience has demonstrated that results of experimental studies may not be predictive of the effect of a drug on patients. Low-dose aspirin, for example, did not prevent reocclusion after thrombolysis in dogs,[42] but treatment with aspirin reduced mortality in the ISIS-2 myocardial infarction trial,[43] and a meta-analysis of thrombolytic trials concluded that aspirin may reduce the frequency of reocclusion after thrombolytic therapy.[44] One limitation of experimental studies is that unforeseen drug interactions may occur. Prostacyclin, for example, augmented thrombolysis induced by streptokinase in a canine model of coronary thrombosis.[45] Subsequently a pilot clinical study and an experimental study suggested that prostacyclin may interfere with the thrombolytic efficacy of TPA, possibly by accelerating the hepatic clearance of TPA.[46, 47] It has been suggested that NTG also may accelerate clearance of TPA by the liver, because the plasma concentrations of TPA were lower when TPA was administered concurrently with NTG compared with administration of TPA alone.[12] Thus the decreased rate of reocclusion observed in the present study may be specific for thrombolytic drugs with a prolonged plasma half-life. In addition, preliminary clinical studies have suggested that intravenous NTG may induce resistance to heparin, which might affect the tendency toward reocclusion after thrombolytic therapy or coronary angioplasty.[48]

Additional limitations of the present study were the duration of NTG treatment, which was only 4 hours, and the fact that NTG was infused immediately proximal to the thrombus rather than intravenously. Hypotension and development of tolerance might limit the antithrombotic potency of prolonged treatment with either intravenous NTG or oral nitrates. Recent publications, however, suggest that previous studies may have overestimated the concentration of NTG required to inhibit platelet aggregation. Chirkov et al.[49] demonstrated that platelet sensitivity to NO increases after the onset of aggregation and that low concentrations of NTG cause reversal of platelet aggregation in vitro. Salvemini et al.[50] found that the concentration of NTG required to inhibit thrombin-induced platelet aggregation is reduced in the presence of endothelial or smooth muscle cells, which convert NTG to NO. Several clinical studies examined the effect of sublingual or intravenous NTG on bleeding time and ex vivo platelet aggregation.[51, 52] Both studies reported that bleeding time was prolonged by NTG despite the absence of a detectable change in ex vivo platelet aggregation. Thus simple ex vivo measurement of platelet aggregation after administration of NTG does not reflect enhancement by the vascular bed of the antiplatelet effect of NTG.

Both platelets and the vasculature exhibit tolerance after exposure to NTG.[50, 53, 54] Preincubation of platelets with NTG caused a ninefold increase in the IC_{50} (inhibitory concentration of 50%) for the inhibition of ADP-induced aggregation by NTG.[53] Exposure to NTG for 18 hours attenuated the ability of endothelial or smooth muscle cells to potentiate the antiplatelet effect of NTG.[50] Pretreatment of isolated human coronary arteries with NTG reduced the cyclic GMP concentration and the relaxation observed during the subsequent addition of either NTG or an endothelial-dependent vasodilator.[54] There are several approaches that might be used to circumvent the limitations of NTG as an antiplatelet agent. First, sulfhydryl agents such as *N*-acetylcysteine potentiate the antiplatelet effects of both NTG and NO, reducing the concentration required to inhibit aggregation in vitro[28, 50, 55, 56] and the NTG dose needed to inhibit cyclic flow variations caused by platelet aggregation in the Folts model of coronary artery stenosis.[9] The hemodynamic effects of NTG are also potentiated by *N*-acetylcysteine, which might limit its clinical utility.[57] The ability of sulfhydryl agents to prevent or reverse nitrate tolerance is somewhat controversial. The addition of *N*-acetylcysteine prevented the development of tolerance during incubation of platelets with NTG.[53] *N*-acetylcysteine restored the ability of nitrate-tolerant endothelial or smooth muscle cells to potentiate the antiplatelet activity of NTG.[50] Experimental and clinical studies have produced conflicting data with regard to the ability of *N*-acetylcysteine to prevent tolerance to the hemodynamic effects of NTG.[58-61] There are several alternative approaches to circumventing the limitations of NTG. One is the use of an inhibitor of cyclic GMP phosphodiesterase, which has been shown to reverse vascular tolerance to NTG.[62] Another option is the development of S-nitroso compounds, which stimulate guanylate cyclase directly and do not require a pool of sulfhydryl donors.[3, 55] Finally SIN-1, which is believed to act as a donor of NO, has been shown to inhibit platelet aggregation and thrombosis

in experimental models of coronary stenosis and balloon angioplasty.[63-65]

Treatment with NTG may exert an unrecognized but important antiplatelet effect under several common clinical circumstances such as coronary angioplasty, unstable angina, and acute myocardial infarction. Lam et al.[8] reported that NTG inhibited the deposition of platelets after deep arterial injury caused by balloon angioplasty of pig carotid arteries. Thus both a direct effect on coronary smooth muscle and a reduction in the accumulation of platelets that release several vasoconstrictive mediators may explain the fact that intravenous NTG prevented the coronary vasoconstriction that occurs in patients after percutaneous transluminal coronary angioplasty.[66]

Coronary thrombosis has been demonstrated by coronary angioscopy in the majority of patients with unstable angina,[67] and "reactivation" of unstable angina has been observed after cessation of intravenous heparin or argatroban, a synthetic thrombin inhibitor.[68, 69] Some patients with unstable angina exhibit cyclic variations in coronary blood flow that are analogous to those caused by platelet deposition and inhibited by NTG in the Folts model of coronary stenosis.[9, 70] Thus both antiplatelet and hemodynamic effects may underlie the therapeutic mechanism of NTG in patients with unstable angina.

A meta-analysis of clinical trials conducted before the widespread use of thrombolytic therapy for acute myocardial infarction concluded that treatment with NTG improves survival.[71] One postulated mechanism of action of NTG is limitation of infarct expansion as a result of afterload reduction.[26] Thus several ongoing clinical trials are comparing nitrates with angiotensin converting enzyme (ACE) inhibitors, another class of vasodilators, as adjunctive therapy in patients treated with thrombolytic therapy.[72] Although ACE inhibitors do not directly inhibit platelets, they may influence platelet function indirectly because ACE inhibition may induce NO formation by endothelial cells, and sulfhydryl-containing ACE inhibitors may potentiate the inhibition of platelet aggregation by NO.[56, 73] Therefore it will be interesting to compare the incidence of recurrent ischemia in the nitrate and ACE inhibitor groups in the GISSI-3 and ISIS-4 trials.

Platelet activation is believed to play an integral role in coronary thrombosis after coronary angioplasty and after spontaneous rupture of atherosclerotic plaques.[1, 2] Treatment with aspirin and heparin reduces but does not eliminate the risk of coronary thrombosis after coronary angioplasty and in patients with unstable angina. Thrombolytic agents can cause both inhibition and activation of platelets,[74, 75] which may be one of the reasons why some patients with acute myocardial infarction experience reocclusion or failure of reperfusion despite treatment with aspirin, heparin, and a thrombolytic drug.[13] Reocclusion after thrombolysis is associated with increased morbidity and mortality,[13] and immediate angioplasty after successful thrombolytic therapy does not decrease the risk of reocclusion.[76] Thus more potent platelet antagonists and thrombin inhibitors are needed, and after favorable effects were observed in experimental studies similar to this one, several have been entered into clinical trials, e.g., argatroban, hirudin, and antibodies to the platelet glycoprotein IIb/IIIa receptor.[15, 69, 77-80] Currently clinical trials are testing the efficacy of glycoprotein IIb/IIIa receptor antagonists and thrombin inhibitors as therapy for unstable angina and adjuncts to coronary angioplasty and thrombolytic therapy. In view of the evidence discussed in this article, an additional antithrombotic strategy may merit further basic and clinical investigation, that is, increasing the platelet concentration of cyclic GMP.

Conclusions. The results of this study demonstrate that intraarterial infusion of NTG reduced the incidence of thrombosis after vessel wall injury and reocclusion after thrombolysis in an experimental animal. It remains uncertain, however, whether intravenous infusion of NTG inhibits coronary thrombosis in patients with coronary artery disease. Therefore further research should be conducted to evaluate the antithrombotic effects of local and systemic infusions of NTG in patients, for example, during balloon angioplasty of coronary artery arteries or bypass grafts at increased risk of thrombosis after angioplasty.

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