Intravenous Nitroglycerin Infusion Inhibits Cyclic Blood Flow Responses Caused by Periodic Platelet Thrombus Formation in Stenosed Canine Coronary Arteries

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Background. Nitroglycerin and other clinically relevant organic nitrate derivatives have been shown to inhibit platelet aggregation in vitro. This antithrombotic effect of nitrates is potentiated by reduced thiol. To determine the potential relevance of this mechanism of action in vivo, we examined the effect of intravenous nitroglycerin infusion on periodic platelet thrombus formation in a canine model of coronary artery stenosis.

Methods and Results. We used a canine model of coronary artery stenosis associated with cyclic reductions in coronary blood flow that have been shown to depend on periodic platelet thrombus formation. We quantified the frequency of cycles per 40-minute observation period and monitored the effect of a continuous infusion of intravenous nitroglycerin on the cycle frequency. The administration of 10–15 μg/kg/min nitroglycerin reduced cyclic platelet thrombus formation significantly and did so without a significant change in coronary artery blood flow. Pretreatment with the reduced thiol, N-acetylcysteine (100 mg/kg during 30 minutes), led to inhibition of cyclic platelet thrombus formation by intravenous nitroglycerin at doses that alone had no such effect (5 μg/kg/min).

Conclusion. These data suggest that one mechanism by which intravenous nitroglycerin improves ischemia in acute coronary artery disease syndromes may be by inhibition of platelet thrombus formation and may highlight the potential importance of reduced thiol in this mechanism. (Circulation 1991;83:2122–2127)

Organic nitrate vasodilators, particularly nitroglycerin, have been used for many years in the treatment of ischemic heart disease.1 The mechanism by which nitroglycerin ameliorates ischemia at the physiological and biochemical level is controversial.2 The antianginal effects of intravenous nitroglycerin in patients with coronary artery disease are usually attributed to a decrease in preload and to coronary vasodilatation.2

The role of platelets in mediating coronary ischemia and unstable angina is well established.3,4 Clinical data demonstrating the efficacy of aspirin in these syndromes support by inference the role of platelets in the pathophysiology of these disorders.5,6 Although nitroglycerin has been shown to inhibit platelet aggregation in vitro, this effect is not believed to occur in vivo because it requires suprapharmacological concentrations not achievable in humans.7,8 Recently, nitroglycerin intravenously administered to patients produced significant platelet inhibition as manifested ex vivo.9 The antiplatelet effect of nitroglycerin has been shown to be associated with an increase in platelet cyclic GMP levels and is potentiated by adequate stores of intracellular reduced thiols.10 In addition, by way of detailing the mechanism, we recently showed that these increases in platelet cyclic GMP inhibit intraplatelet calcium flux and subsequent fibrinogen binding.11

We have an established in vivo model of mechanically stenosed coronary arteries in dogs in which
acute platelet thrombi form periodically, causing cyclic reductions in coronary blood flow.\textsuperscript{12–14} These cyclic reductions in coronary flow (CFRs) are determined primarily by in vivo platelet activity, and the size and frequency of CFRS can be increased by infusing a platelet agonist such as epinephrine\textsuperscript{15} or by ventilating the dog with cigarette smoke,\textsuperscript{16} or the CFRS may be diminished with platelet antagonists, such as aspirin\textsuperscript{13} and prostacyclin.\textsuperscript{17} We have also demonstrated that these CFRS are not due to vasospasm.\textsuperscript{18} Thus, the model provides a minute-by-minute measure of in vivo platelet activity. To test the in vivo relevance of the antiplatelet effects of nitroglycerin, we used this model of platelet-dependent cyclic CFRS to study 1) the effect of intravenous nitroglycerin on acute platelet thrombus formation in the coronary artery, and 2) the influence of pretreatment with a reduced thiol, N-acetylcysteine, on the antiplatelet effects of nitroglycerin.

**Methods**

**Experimental Preparation and Protocol**

Twenty-seven healthy adult mongrel dogs (weight, 23–27 kg) of either sex were premedicated with 3 mg/kg morphine sulphate followed by 20 mg/kg sodium pentobarbital. Supplemental pentobarbital was given as needed to maintain clinical signs of general anesthesia. The dogs were intubated and ventilated with room air, after which the chest was opened and the heart exposed. The left circumflex coronary artery was dissected free and an electromagnetic flow probe (Gould-Statham, Oxnard, Calif.) placed on it to measure coronary blood flow continuously. A flow probe was also placed around the left anterior descending coronary artery. The circumflex coronary artery was then clamped several times with a vascular clamp in the area to be stenosed to produce moderate intimal damage. At this site distal to the flow probe, a plastic encircling cylinder 4 mm in length was placed externally around the coronary artery to produce a 70–75% mechanical reduction in arterial diameter.\textsuperscript{12–14} With this degree of stenosis, coronary blood flow does not decline from control unstenosed levels, but the reactive hyperemic response to complete occlusion for 20 seconds is eliminated.\textsuperscript{19} Coupled with moderate intimal damage, this stenosis promotes the formation of acute platelet thrombi in the stenosed lumen, causing a decline in coronary blood flow and electrocardiographic signs of myocardial ischemia. When the thrombus embolizes distally, blood flow is abruptly restored, thus producing cyclical CFRS. A CFR due to acute platelet thrombus formation is defined as an abrupt flow change of greater than 10 ml/min. Any changes less than 10 ml/min are considered normal flow variations within the accuracy of electromagnetic flow meter (EMF) measurements. In all 15 dogs, CFRS occurred and were recorded for 40 minutes. Reductions in cyclic blood flow are quantified by counting their frequency (number of cycles per 40-minute period of observation), although the peak amplitude (i.e., maximal blood flow achieved per cycle) and the rate of decline of blood flow are both attenuated, as well, with platelet inhibitors. The dogs were then divided into three groups: Group A (n=5) was given 5 \(\mu\)g/kg/min nitroglycerin for 40 minutes; group B (n=5) was given 10 \(\mu\)g/kg/min for 40 minutes, and group C (n=5) was given 15 \(\mu\)g/kg/min for 40 minutes, all by continuous intravenous infusion. Heart rate, arterial blood pressure, and the

| Table 1. Hemodynamic and Antiplatelet Effects of Intravenous Nitroglycerin in Dogs |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
|                                 | Peak circumflex blood flow (ml/min) | CFRs/40 min | LAD blood flow (ml/min) | Mean arterial blood pressure (mm Hg) | Heart rate (beats/min) |
| Group A (n=5)                   |                               |               |                            |                                   |                     |
| Control                         | 43±12                         | NS            | 34±8                        | 113±10                           | 140±12             |
| After 5 \(\mu\)g/kg/min NTG for 40 min | 41±11 | 7±4             | 39±9                        | 103±5                            | 143±10             |
| Group B (n=5)                   |                               |               |                            |                                   |                     |
| Control                         | 51±10                         | NS            | 29±8                        | 117±13                           | 137±14             |
| After 10 \(\mu\)g/kg/min NTG for 40 min | 46±8 | 3±2*             | 33±7                        | 100±12                           | 156±9              |
| Group C (n=5)                   |                               |               |                            |                                   |                     |
| Control                         | 40±9                          | NS            | 41±9                        | 119±12                           | 132±17             |
| After 15 \(\mu\)g/kg/min NTG for 40 min | 38±7 | 2†               | 56±10                       | 92±11                            | 168±18             |

Data are mean±SD.

CFRS, cyclic coronary flow reductions; LAD, left anterior descending coronary artery; NTG, nitroglycerin.

Statistical comparisons by analysis of variance and Newman-Keuls methods.

*CFRS abolished in two dogs after 28±11 minutes and diminished in three dogs.

†CFRS abolished in four dogs after 23±9 minutes in four dogs and decreased to two very small CFRS in the fifth dog.
amplitude and frequency of the CFRs were continuously recorded.

In 12 additional dogs (group D), nitroglycerin was infused intravenously at 5 μg/kg/min for 40 minutes. After the infusion, the dogs were given 30 minutes to recover, and then 100 mg/kg N-acetylcysteine was infused for 30 minutes. Thirty minutes after the N-acetylcysteine infusion, an infusion of nitroglycerin at 5 μg/kg/min for 40 minutes was repeated. Continuous monitoring of heart rate, mean arterial blood pressure, and coronary blood flow in the circumflex and left anterior descending coronary arteries was maintained throughout the experiment.

Statistical Analysis

Data were analyzed by one-way analysis of variance with Newman-Keuls comparisons. A p value less than 0.05 was considered statistically significant.

Results

Effects of Nitroglycerin Infusion on CFRs

Group A (n=5). All dogs in group A showed CFRs (8±3 during the 40-minute control period) (Table 1). Throughout the 40-minute infusion of nitroglycerin at 5 μg/kg/min, the CFRs continued unabated (Figure 1), and the mean arterial blood pressure declined by an average of 10 mm Hg without a significant change in heart rate.

Group B (n=5). Dogs in group B were treated with 10 μg/kg/min nitroglycerin. CFRs were abolished in two dogs after 28±11 minutes of infusion, and the
CFRs were decreased in size and frequency in three dogs (Figure 2, Table 1). Forty minutes after the nitroglycerin infusion began, mean arterial blood pressure declined by an average of 17 mm Hg, and heart rate increased by 19 beats/min (Table 1).

Group C (n=5). Dogs in group C received 15 
µg/kg/min nitroglycerin. CFRs were abolished in four dogs after 23±9 minutes of infusion and were significantly decreased in size and frequency in the fifth dog. Mean arterial blood pressure declined by 34±11 mm Hg after 10 minutes of infusion; however, after 40 minutes of infusion, the mean arterial blood pressure stabilized at 27 mm Hg less than that of control (Table 1). The heart rate increased by an average of 36 beats/min (Table 1).

Blood flow in the normal, unstenosed left anterior descending coronary artery did not change significantly during the nitroglycerin infusions at any of the doses tested. In addition, there was no significant change in blood flow in the left anterior descending coronary artery despite decreases in mean arterial blood pressure and increases in heart rate with the higher doses of nitroglycerin. This may be due to statistically insignificant changes in the calculated rate-pressure product (data not shown).

Effect of N-Acetylcysteine on Reduction in CFRs by Nitroglycerin

Group D (n=12). In dogs of group D, infusion of 5 
µg/kg/min nitroglycerin first produced a decrease in mean arterial pressure from 115±10 to 108±4 mm Hg but did not reduce the frequency of CFRs (in 11 of 12 dogs) as shown in Figures 3 and 4 and Table 2. The infusion of 100 mg/kg N-acetylcysteine alone had no significant hemodynamic effects and also did not alter the frequency of the CFRs. On repeating the infusion of 5 
µg/kg/min nitroglycerin (after the N-acetylcysteine infusion), however, CFRs were

<table>
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<td><strong>Peak circumflex blood flow (ml/min)</strong></td>
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<td><strong>Group D (n=15)</strong></td>
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| After 5 
µg/kg/min              |                |                |                 |                 |
| NTG for 30 min                | 47±11          | 7±5*           | 40±7            | 108±4           | 160±14          |
| After NAC (100 mg/kg)          | 52±10          | 9±3            | 42±6            | 112±8           | 144±16          |
|                                | NS             | p<0.01         | NS              | p<0.05          | p<0.05          |
| After NTG (5 
µg/kg/min)         | 46±8           | 4±2†           | 44±7            | 99±11           | 166±12          |

Data are mean±SD.
CFRs, cyclic coronary flow reductions; LAD, left anterior descending coronary artery; NTG, nitroglycerin; NAC, N-acetylcysteine.
Statistical comparisons by analysis of variance and Newman-Keuls methods.
NTG at 5 
µg/kg/min abolished CFRs temporarily in one dog.
abolished in nine dogs and significantly diminished in two dogs after an average infusion time of 32±8 minutes (Figure 5).

Interestingly, by 60 minutes after the second nitroglycerin infusion, the CFRs returned to control frequency in 11 of 12 dogs, suggesting reversibility of the nitroglycerin effect on platelets and the CFRs produced by them. Again, there was no significant increase in blood flow in the normal, unstenosed left anterior descending coronary artery in any dog and only a modest reduction in pressure, suggesting that intravenous nitroglycerin produced only mild arteriolar vasodilation in the normal coronary artery.

**Discussion**

In these experiments, we elected to test three constant infusion rates of nitroglycerin rather than titrate the infusion to a given blood pressure decline as is occasionally done in patients with coronary artery disease. Because there may not be a good correlation between infusion rate and plasma nitroglycerin levels,\(^{20}\) we believed it was reasonable to test three constant infusion rates to simplify our analysis. We found that an infusion rate of 10–15 μg/kg/min nitroglycerin significantly reduced acute platelet thrombus formation and CFRs 20–40 minutes after the start of infusion.

We previously tested topical applications of nitroglycerin (0.5 mg) over the stenotic area of the circumflex coronary artery to determine whether the CFRs were due to coronary vasospasm.\(^{18}\) In these earlier experiments, there was no change in size or frequency of the CFRs, although a transient reduction of arterial blood pressure occurred.\(^{18}\) We have given bolus injections of 0.4 mg nitroglycerin dissolved in saline and have also placed several 0.4-mg nitroglycerin tablets on the buccal mucosa with periodic rubbing of the tablets. Both techniques produced a transient decline in arterial blood pressure of 10–15 mm Hg lasting up to 20 minutes but had no effect on CFRs (unpublished observations).

Our data, thus, suggest that a more-prolonged exposure to nitroglycerin is necessary for its antiplatelet effects to become manifest. We and others using this model have observed that agents such as aspirin,\(^{13,15}\) prostacyclin,\(^{17}\) or the monoclonal antibody (7E3) to the GlIb-IIIa platelet receptor\(^{21}\) abolishes CFRs within 3–5 minutes. Interestingly, continuous infusion of nitroglycerin requires 20–40 minutes to become effective.

Recent studies suggest that platelet inhibition by organic nitrates also requires the presence of reduced thiol stores,\(^{22}\) is caused by the synthesis of S-nitrosothiols,\(^{10}\) and is preceded by guanylate cyclase stimulation and cyclic GMP formation.\(^{10,23}\) This process may take time in vivo and may account for the slow onset of action of nitroglycerin in these experiments. Alternatively, a sufficiently high level of circulating nitroglycerin may be required to inhibit platelet activity, and this may be achieved only with a longer continuous infusion, particularly in view of the fact that the drug was not administered with a loading dose.

A low dose of nitroglycerin (5 μg/kg/min for 30 minutes) appears to be a subthreshold dose that does not significantly alter in vivo platelet activity or CFRs.
N-Acetylcysteine by itself does not alter platelet activity or CFRs in standard pharmacological doses. However, a subthreshold dose of nitroglycerin given after N-acetylcysteine significantly inhibits in vivo platelet activity, intracoronary thrombus formation, and CFRs and probably does so by potentiating the guanylate cyclase–stimulating effect of nitroglycerin.10

In our model of coronary artery stenosis with periodic acute platelet thrombus formation, nitroglycerin used in high, but clinically relevant, doses and administered intravenously for 20–40 minutes abolished CFRs and acute platelet thrombus formation. This is a transient effect because the CFRs returned 30–60 minutes after the discontinuation of the nitroglycerin. These data suggest that some of the antianginal effect of continuous administration of intravenous nitroglycerin may be due to inhibition of the periodic formation of occlusive coronary thrombosis mediated by platelet aggregation. The potential clinical relevance of these data is supported by recent observations documenting the occurrence of CFRs in the coronary circulation in humans.24

References

Key Words • nitroglycerin • N-acetylcysteine • platelet aggregation • thiol • coronary artery
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