Effect of Atherosclerosis on Endothelium-Dependent Inhibition of Platelet Activation in Humans

Jean G. Diodati, MD; Nader Dakak, MD; David M. Gilligan, MD; Arshed A. Quyyumi, MD

Background—We investigated whether luminal release of nitric oxide (NO) contributes to inhibition of platelet activation and whether these effects are reduced in patients with atherosclerosis.

Methods and Results—Femoral blood flow velocity and ex vivo whole blood platelet aggregation by impedance aggregometry were measured in femoral venous blood during femoral arterial infusion of acetylcholine (ACh; 30 μg/min) in 30 patients, 19 of whom had angiographic atherosclerosis. Measurements were repeated with sodium nitroprusside (40 μg/min), L-arginine (160 μmol/min), and Nω-monomethyl-L-arginine (L-NMMA; 16 μmol/min). There was significant inhibition of collagen-induced platelet aggregation with ACh (45±9.5% lower, P<0.001), and this inhibition was greater in patients without atherosclerosis (68.7±10.4% reduction) than in those with atherosclerosis (32.5±8.1%, P=0.04). The magnitude of inhibition correlated with vasodilation with ACh, indicating an association between the smooth muscle and antiplatelet effects of endothelium-dependent stimulation. Neither L-NMMA nor sodium nitroprusside altered platelet aggregation. L-Arginine inhibited platelet aggregation equally in vitro (34±8% reduction, P<0.01) and in vivo (37±13% reduction, P<0.01).

Conclusions—Stimulation of NO release into the vascular lumen with ACh inhibits platelet aggregation, an effect that is attenuated in patients with atherosclerosis and endothelial dysfunction. Basal NO release does not appear to contribute to platelet passivation in vivo. L-Arginine inhibited platelet aggregation by its direct action on platelets. These findings provide a pathophysiological basis for the observed increase in thrombotic events in atherosclerosis. Use of L-arginine and other strategies to improve endothelial NO activity may impact favorably on thrombotic events in atherosclerosis.

(Circulation. 1998;98:17-24.)

Key Words: endothelium • atherosclerosis • blood flow • nitric oxide • platelets

The vascular endothelium profoundly influences smooth muscle tone by the abluminal release of endothelium-derived relaxing factors. The predominant relaxing factor is nitric oxide (NO), a byproduct of L-arginine metabolism, which not only diffuses into the vascular smooth muscle layer but is also released luminally.1–4 In vitro, NO inhibits platelet adhesion and, to a lesser extent, platelet aggregation by increasing cytosolic levels of soluble cGMP.5–14 In whole blood, in which the half-life of NO is attenuated by hemoglobin and other oxidants,15–17 the platelet inhibitory effects of NO are less easily demonstrated and may be insignificant.18

Subjects with risk factors or established atherosclerosis have depressed vasodilator responses to endothelium-dependent agonists, which are attributed largely to decreased abluminal availability of NO.19–25 Human studies examining the in vivo effects of luminally released NO on platelets are sparse, and it is unknown whether any inhibitory action of the vascular wall on platelets is attenuated in patients with endothelial dysfunction.19,24,25 Therefore, in the present study, we investigated whether basal or stimulated release of endothelium-derived relaxing factors from the vascular endothelium inhibits platelet aggregation. If this proved true, we hypothesized that endothelial dysfunction accompanying atherosclerosis would be associated with a reduction in the platelet inhibitory effects of the vessel wall and that L-arginine, the substrate for NO synthesis in platelets and vascular endothelium, would improve the inhibitory effects of acetylcholine (ACh) on platelet aggregation in patients with atherosclerosis. For this purpose, we studied the effects of ACh, L-arginine, and Nω-monomethyl-L-arginine (L-NMMA) on agonist-stimulated ex vivo whole blood platelet aggregation in the human peripheral circulation.

Methods

Patients

ACh Study

ACh studies were performed in 30 patients aged 57±2 years (19 men) undergoing cardiac catheterization for diagnosis of chest pain. Nineteen patients had atherosclerosis involving the coronary or femoral circulations, and the remaining 11 had no angiographic evidence of atherosclerosis (Table). Patients with atherosclerosis had lower HDL levels, were older, and were more often male. None had
significant stenosis in the iliofemoral circulation. Patients with unstable angina or a myocardial infarction within 2 months of the study were excluded. All cardiac medications were withdrawn for ≥48 hours, patients refrained from smoking and intake of caffeine for ≥12 hours, and aspirin and other agents known to alter platelet function were discontinued ≥10 days before the study. Because of the known circadian variation in platelet aggregation, we ensured that the time at which the study was performed was not significantly different among patients and that all individuals remained supine for ≥1 hour before blood sampling for assessment of platelet aggregation. Informed consent was obtained from all patients, and the study was approved by the Investigational Review Board of the National Heart, Lung, and Blood Institute, Bethesda, Md.

**L-Arginine and L-NMMA Studies**

Nine patients with atherosclerosis aged 61 ± 3.6 years (6 men) underwent the L-arginine study. Another group of 15 patients, 10 with atherosclerosis and 5 with normal coronary arteries, were recruited for the L-NMMA study. Inclusion and exclusion criteria were as described above.

**Protocol**

A 6F right Judkins or multipurpose A2 (Cordis, Inc) catheter was introduced 1 cm beyond the end of a 7F angiographic sheath that was inserted into the right femoral artery, and femoral angiograms were obtained to exclude obstructive femoral atherosclerosis. A 6F sheath was also introduced into the right femoral vein. A 3F Doppler catheter (Millar, Inc) was introduced 1 cm beyond the tip of the catheter in patients undergoing the ACh and L-arginine studies, and a 0.018-in Doppler flow wire (Cardiometrics Inc) was used in patients in the L-NMMA study to measure femoral blood flow velocity. All patients received 5000 U of heparin intravenously before diagnostic cardiac catheterization, and the study commenced 2 hours later. We determined infusion rates for the agonists by estimating femoral blood flow to be 150 mL/min.

**ACh Study**

In 30 patients, baseline measurements were made during infusions of dextrose 5% and repeated after intrafemoral arterial infusion of ACh at 30 μg/min (estimated in vivo concentration 10−7 mol/L) for 2 minutes and again 10 minutes after discontinuation of the ACh infusion. These measurements included arterial and venous whole blood platelet aggregation in response to collagen (n = 29) and ADP (n = 24), Doppler flow velocity (n = 25), and oxygen saturation in arterial and venous blood (n = 30) by use of an Oxicom 2000 whole blood oximeter (Waters Instruments). Recovery platelet aggregation studies with collagen were performed in 26 patients and with ADP in 19.

**Sodium Nitroprusside Study**

In a subgroup of 13 patients who underwent the ACh study above, 5 with and 8 without atherosclerosis, the study was continued, and after a 15-minute recovery period, 40 μg/min of sodium nitroprusside was infused into the femoral artery for 3 minutes, and blood flow velocity, arterial and venous oxygen saturation, and platelet aggregation were measured.

**L-NMMA Study**

In 15 patients, ACh was infused at 150 μg/min for 2 minutes, and platelet aggregation and flow velocity were measured. Twenty minutes later, L-NMMA was administered at 16 μmol/min for 5 minutes, and collagen- and ADP-induced ex vivo whole blood platelet aggregation, flow velocity, and oxygen saturation in arterial and venous blood were measured.

**Ex Vivo Whole-Blood Platelet Aggregation**

Platelet aggregation was measured by use of a mobile, 4-channel impedance aggregometer (Chrono-Log Corporation), which allowed measurement of aggregation beginning 1 minute after collection of the sample from the patient.26–29 Blood (2.25 mL) was collected in preheated plastic syringes containing 0.25 mL of sodium citrate (3.8%), pH 7.4. All materials in contact with blood were strictly kept at 37°C. Platelet aggregation was tested at 37°C in whole blood diluted 1:1 in sterile physiological saline solution. Samples were never in contact with glass. Aggregation was initiated by addition of 2 to 10 μL of aggregating agents: (1) collagen 2 to 5 μL (to a final concentration of 2 to 5 μg/mL) and (2) ADP 2.5 to 10 μL (to a final concentration of 5 to 20 μmol/L). Aggregation was quantified as (1) the area under the curve relating electric impedance to time (Ω·s), (2) maximal amplitude (Ω), and (3) maximal rate of rise (Ω/s) 5 minutes after addition of the aggregating agent. Results were similar when these 3 methods were used; therefore, only area under the curve measurements are reported. Using ADP and thrombin as platelet-aggregating agents in our previous study,27 we demonstrated that with repeated measurements, whole blood platelet aggregation values were within 10% of the previous readings. A standard screen on the initial blood sample was performed in each patient, with both agents at 2 concentrations (collagen 2 and 5 μg/mL; ADP 5 and 20 μmol/L) to obtain a concentration for each agent that would give an intermediate response. Any change in aggregation with further intervention could thus be easily measured.

**In Vitro Effects of ACh, L-Arginine, and L-NMMA on Platelet Aggregation**

To establish whether the observed in vivo effects on platelet function were due to a direct action of the agents on platelets in the lumen or to an effect via the vascular endothelium, we performed in vitro studies with ACh, l-arginine, and L-NMMA in whole blood using impedance aggregometry. We estimated the dose of each agent used in the in vitro study using the delivered intra-arterial infusion rate and assuming mean resting femoral arterial blood flow of 100 mL/min.

Blood was collected from adult, male, normal volunteers; subjects had refrained from ingesting aspirin or any other agent known to affect platelet function for 14 days. Blood was drawn with a 2-syringe technique; the first 2 mL was discarded, then 36 mL was withdrawn into 40-mL plastic syringes containing 4 mL of 3.8% sodium citrate at pH 7.4. All studies were performed within 2 hours of blood collection.

Platelet aggregation was assessed by impedance aggregometry in whole blood that was diluted 1:1 with normal saline solution. Collagen (1 to 5 μg/mL) and ADP (2.5 to 20 μmol/L) were used as aggregating agents. All specimens were kept at 22°C and rewarmed in the 4-channel impedance aggregometer at 37°C for 2 minutes before testing.
Statistical Analysis

Data are expressed as mean ± SEM. Differences between means were compared by paired or unpaired Student’s t test, as appropriate. P values are 2-tailed, and a value of ≤0.05 was considered to be statistically significant. Values at rest, after ACh, and at recovery in each subset of patients were compared by ANOVA for repeated measures. Correlations were tested by use of Pearson’s coefficient. Univariate and multivariate stepwise regression analyses were performed to test whether the magnitude of change in collagen-induced platelet aggregation with ACh was related to patient demographics such as age; presence of hypertension, diabetes, or cigarette use; cholesterol level; presence or absence of angiographic atherosclerosis; total number of risk factors; flow velocity change; and venous oxygen saturation level with ACh. Risk factors were defined as presence of hypertension, cholesterol >200 mg/dL, diabetes, smoking in the previous year, age >60 years, and male sex.

Results

Effect of ACh

Significant femoral microvascular dilation with ACh (30 μg/min) was evident from an increase in flow velocity of 117 ± 18% (from 4.6 to 9.6 cm/s, P < 0.0001) and a simultaneous increase in femoral venous oxygen saturation by 18.3 ± 2.2% (from 68.7% to 80.8%, P < 0.001). There was no change in systolic blood pressure or heart rate during ACh infusion.

There was no baseline arteriovenous difference in platelet aggregation; however, significant inhibition of agonist-stimulated whole blood platelet aggregation was observed in femoral venous blood during arterial infusion of ACh. Compared with baseline, platelet aggregation was 45% ± 9.5% (P < 0.001) lower with collagen and 28.5 ± 12.8% (P = 0.003) lower with ADP (Figure 1). Platelet aggregation returned toward baseline 10 minutes after discontinuation of ACh infusion. There was no significant alteration in systemic arterial platelet aggregation with ACh (Figure 1).

Atherosclerosis and Effect of ACh

The response to ACh in 19 patients with coronary atherosclerosis was compared with the response in 11 patients without atherosclerosis (Figure 2). Although there was significant attenuation of collagen-induced platelet aggregation in both groups, the magnitude of inhibition was lower in patients with atherosclerosis (32.5 ± 8.1% reduction from baseline compared with a 68.7 ± 10.4% reduction in patients without atherosclerosis, P = 0.04 between groups; Figure 2). Similarly, ADP-induced platelet aggregation during ACh infusion was significantly inhibited only in patients without atherosclerosis, in whom it was 48.2 ± 10% lower than at baseline (P = 0.008). In contrast, the 16.7 ± 19% reduction in patients with atherosclerosis did not reach statistical significance.

The lesser increase in flow velocity with ACh administration in patients with versus patients without atherosclerosis trended toward but did not achieve significance (97 ± 22% versus 148 ± 29%, respectively; P = 0.17).

Univariate and multivariate stepwise regression analyses were performed to investigate whether any demographic characteristics, risk factors, presence of atherosclerosis, or vascular flow responses were predictive of the magnitude of inhibition of collagen-induced platelet aggregation with ACh. Age (r = 0.39, P = 0.03), hypertension (r = 0.37, P = 0.05), diabetes (r = 0.52, P = 0.004), presence of atherosclerosis (r = 0.38, P = 0.05), and total number of risk factors (r = 0.57, P = 0.0014) were all univariate predictors of the effect of ACh.
on platelet aggregation. Multivariate analysis demonstrated that the magnitude of the inhibitory effect of ACh on collagen-induced platelet aggregation was independently predicted by the total number of risk factors ($r=0.57, P=0.004$).

Relation Between Vascular and Antiplatelet Responses to ACh
A significant correlation was present between the percent change in flow velocity with ACh and the percent decrease in collagen-induced platelet aggregation with ACh ($r=-0.50, P=0.03$). Similarly, there was a significant correlation between femoral venous saturation with ACh (a measure of the magnitude of increase in blood flow) and percent decrease in collagen-induced platelet aggregation with ACh ($r=-0.52, P=0.004$), suggesting that patients with greater vasodilation with ACh also had greater inhibition of platelet aggregation and vice versa.

Effect of Sodium Nitroprusside
Intra-arterial sodium nitroprusside did not alter systemic arterial blood pressure but did increase femoral blood flow velocity by 125±17% ($P=0.005$), from a mean 7.7 to 17.5 cm/s. Femoral venous saturation increased simultaneously from 68.8±2.5% to 77.9±2.3% ($P<0.0001$).

Despite a 50.9±17.3% reduction in collagen-induced platelet aggregation with ACh in these patients, sodium nitroprusside infusion did not alter platelet aggregation; in response to collagen, platelet aggregation in femoral venous blood changed from 288±32 to 302±33 Ω·s ($P=0.3$). No platelet inhibition was evident in patients with or without atherosclerosis, and both groups had similar vasodilation with sodium nitroprusside.

Effect of L-Arginine
With intra-arterial infusion of L-arginine, there was no significant alteration in femoral arterial flow velocity (−3±11% change; $P=0.3$) in the 9 patients with atherosclerosis. Combined administration of L-arginine with ACh (150 μg/min) did not increase the vasodilator response compared with ACh alone; flow velocity with ACh was 12.6±3.2 m/s before and 11.3±2 m/s after L-arginine ($P=0.3$). Similarly, venous oxygen saturation during ACh remained unchanged before compared with after L-arginine (72±3.3% versus 79±3.7%, respectively; $P=0.13$).

ACh infusion produced 29±11% inhibition of platelet aggregation in response to collagen ($P=0.02$) (Figure 3). Compared with baseline, L-arginine produced a 36.9±13.3% reduction in collagen-induced platelet aggregation ($P=0.012$). Combined administration of ACh with L-arginine did not further change platelet aggregation (Figure 3) compared with L-arginine alone or with control ACh infusion ($P=0.11$). There was no significant change in arterial platelet aggregation in response to collagen during L-arginine infusion, indicating that its effects were localized to the femoral circulation.

Effect of L-NMMA
Intra-arterial femoral infusion of 16 μmol/min L-NMMA resulted in a 19.6±4.5%, decrease in blood flow velocity ($P<0.001$); however, there was no significant alteration in whole blood platelet aggregation in femoral venous blood, either with collagen (from 270±40 to 274±6 Ω·s; $P=0.9$) or with ADP (from 193±21 to 226±28 Ω·s; $P=0.24$). There was no significant alteration in platelet aggregation in patients with or those without atherosclerosis.

In Vitro Studies
To investigate whether the observed in vivo effects on platelets of ACh, L-arginine, and L-NMMA were due to an action of these drugs on vascular endothelial NO production or to a direct intraluminal effect on platelets, we performed in vitro studies in which platelet aggregation was measured after incubation of whole blood with increasing concentrations of these agents. Neither ACh nor L-NMMA produced any significant alteration in whole blood platelet aggregation at concentrations at or above those estimated to be achieved in vivo, suggesting that basal release of NO does not contribute to platelet passivation. Finally, intra-arterial L-arginine caused no change in resting or ACh-stimulated blood flow but did inhibit agonist-stimulated platelet aggregation to approximately the levels observed with ACh. L-Arginine resulted in similar inhibition of platelet aggregation in vitro.

Discussion
Our study demonstrates that stimulation of endothelium-derived relaxing factor release into the lumen by ACh causes inhibition of platelet aggregation. Patients with atherosclerosis who had a depressed vasodilator response to ACh also had reduced inhibition of platelet aggregation, indicating that endothelial dysfunction not only results in attenuated abluminal, agonist-mediated NO activity but also causes reduced luminal bioavailability of NO. Inhibition of tonic basal endothelial release of NO resulted in the expected reduction in blood flow but did not increase platelet aggregation ex vivo, suggesting that basal release of NO does not contribute to platelet passivation. Finally, intra-arterial L-arginine caused no change in resting or ACh-stimulated blood flow but did inhibit agonist-stimulated platelet aggregation to approximately the levels observed with ACh. L-Arginine resulted in similar inhibition of platelet aggregation in vitro.
nating that its effects were exerted directly on luminal platelets and not via the vascular endothelium.

Inhibition of Platelet Aggregation With ACh

The vascular endothelium releases relaxing factors that not only modulate vascular smooth muscle tone but also influence platelet function and cell proliferation.3–14,30 NO inhibits platelet adhesion and, to a lesser extent, aggregation by increasing levels of cytosolic soluble cGMP. This leads to phosphorylation of cGMP-dependent phosphoproteins that attenuate agonist-mediated increases in intracellular calcium.31 Human platelets contain both constitutive and inducible forms of NO synthase,32–34 and it appears that platelet responsiveness is regulated by both endothelium-derived and platelet-produced NO.35,36 Thus, a single passage of platelets through the guinea pig coronary circulation results in a cGMP-dependent decrease in platelet aggregation,10 and exogenous donors of NO inhibit platelet activity by directly increasing intraplatelet cGMP levels.4,37–40

In addition to stimulating endothelial NO, ACh may also release endothelium-derived hyperpolarizing factor, which does not possess platelet inhibitory properties.41 Prostacyclin, a powerful, endothelium-derived, antiplatelet aggregatory agent, is not released from human vasculature in response to ACh.42,43 Therefore, it is likely that the observed effects of ACh in the present study are a result of promotion of NO release by ACh in vivo, as has been corroborated by previous animal studies.30–32

We44 have previously shown that adducts of NO inhibit platelet aggregation in vitro and in vivo and that the effect of NO donors is attenuated in whole blood compared with platelet-rich plasma preparation. The fact that endothelium-derived NO is rapidly inactivated in whole blood by hemoglobin and other oxidants has raised doubts regarding the in vivo role of luminaly released NO.15–17 These concerns were strengthened by a reported lack of platelet inhibitory effects of ACh in human forearm circulation.18 However, a study in human coronary circulation has demonstrated inhibition of platelet aggregation with substance P33 and, together with the present results, helps demonstrate the platelet inhibitory effects of NO stimulation in vivo.

Basal NO and Platelet Aggregation

Inhibition of basal NO production with L-NMMA did not alter agonist-stimulated ex vivo platelet aggregation but produced the expected reduction in flow, indicating that the lack of effect on platelets was not due to inadequate blockade of NO synthase. It is possible that under resting conditions, platelet passivation is not dependent on basal NO release into the lumen and that NO-mediated antiplatelet aggregatory effects become evident only during conditions that cause stimulation of NO production, such as during situations when blood flow and shear stress increase.8,61,62 Moreover, it is likely that only a minority of platelets (those close to the vessel wall and not those in the center of the lumen) will be exposed to tonically released NO under resting conditions. Thus, L-NMMA is likely to activate a minority of platelets along the vessel wall during their passage across the femoral circulation, and measurement of platelet aggregation in all platelets may obscure the activation of a few. Nevertheless, the present study demonstrates that stimulation of NO with ACh inhibits aggregation in sufficient numbers of platelets in vivo to result in a net inhibition of aggregation, suggesting that larger quantities of luminal NO released during ACh infusion are available to more platelets or are able to more profoundly inactivate platelets. To further clarify these issues, it will be necessary to measure platelet cGMP content during L-NMMA and to study platelet aggregation during maneuvers that activate platelets before and after L-NMMA. Animal studies examining the effect of NO inhibition on platelet aggregation, performed in platelet-rich plasma or in washed platelets, have demonstrated an increase in platelet aggregation.35,36 In a dog model of cyclic flow variation, systemic L-NMMA also produced enhancement of platelet aggregation.36

The present study examined local blockade of NO in human peripheral circulation and failed to demonstrate an effect of NO inhibition on platelets. These differences may be due to differences in species, lack of counterregulatory influences because of local delivery in the present study, and the fact that the present studies were performed in whole blood without preparation of platelet-rich plasma, which may in itself cause changes in platelet function. In vitro L-NMMA at concentrations achieved in vivo failed to demonstrate an effect on whole blood platelet aggregation, suggesting that tonic activity of platelet NO synthase was also not responsible for basal platelet activation.

Comparison of ACh With Sodium Nitroprusside

Intra-arterial infusion of sodium nitroprusside, a donor of NO, did not alter platelet aggregation despite an increase in femoral blood flow to levels observed with ACh.46,47 With
few exceptions, it has been observed previously that nitrovasodilators at therapeutic concentrations do not inhibit agonist-induced platelet aggregation.\textsuperscript{38-54} We\textsuperscript{29} have previously demonstrated that sodium nitroprusside in therapeutic concentrations does not inhibit whole blood platelet aggregation in vitro; inhibition of aggregation was only observed at concentrations 10- to 100-fold higher.

Sodium nitroprusside releases NO after intracellular metabolism, and it appears that this conversion occurs more efficiently in the vascular smooth muscle cells, leading to relaxation, but occurs at higher concentrations in platelets, so that at concentrations that lead to vasodilation in vivo, there are no apparent antiaggregatory effects. Nevertheless, in previous studies,\textsuperscript{27,29} we demonstrated that despite a lack of baseline effect on platelet aggregation, sodium nitroprusside inhibited increased platelet aggregation during cardiac pacing in patients with coronary artery disease and that intravenous nitroglycerin inhibited platelet aggregation in patients with unstable angina, indicating that the antiplatelet effects of nitrovasodilators may become evident only when platelets are activated.

**Endothelial Dysfunction and Platelet Activation**

A depressed dilator response to ACh is an indicator of reduced basal and stimulated activity of NO and is associated with attenuated vasodilation during physiological stresses such as cardiac pacing, mental stress, exercise, and hyperemia.\textsuperscript{19-21,55-59} Stimulation of NO bioavailability with increasing shear not only causes vasodilation that serves to decrease shear forces but also compensates for the direct proaggregatory effects of increased shear stress on platelets.\textsuperscript{55,59,60} Thus, in the normal circulation, endothelium-mediated platelet inhibition appears to be of critical importance at branch points where shear forces are dramatically increased. In this regard, we\textsuperscript{28} have previously demonstrated platelet activation during conditions of increased coronary blood flow in patients with significant narrowing of epicardial arteries. In contrast, patients without stenoses had no activation of platelets during passage through the unobstructed coronary circulation.

The results of the present study indicate that the impaired platelet-inhibitory properties of the vessel wall in atherosclerosis may be responsible, at least in part, for the previous observations. Thus, patients with atherosclerosis have attenuated NO-mediated inhibition of platelet aggregation when coronary blood flow and thus shear stress increase, and this defect results in platelet activation. Similar activation of platelets during conditions of increased shear stress in the cerebral and other vascular beds may account for the increased incidence of thrombotic events in patients with atherosclerosis and provides an important pathway for development of future antiplatelet therapeutic strategies.

**\textsuperscript{L-Arginine}**

NO is formed after oxidation of the terminal guanidino nitrogen of \textsuperscript{L-arginine}, the substrate for all isoforms of NO synthase.\textsuperscript{3} Because intracellular concentrations of \textsuperscript{L-arginine} approach 1 mmol/L and the $K_a$ for \textsuperscript{L-arginine} ranges between 1 and 3 $\mu$mol/L, it is believed that the availability of \textsuperscript{L-arginine} is not rate limiting for NO synthesis.\textsuperscript{61-64} Incubation of platelets with \textsuperscript{L-arginine} at concentrations estimated to be produced in vivo in the present study resulted in inhibition of whole blood platelet aggregation to the same extent as during intra-arterial infusion of \textsuperscript{L-arginine}, suggesting that the effect of \textsuperscript{L-arginine} is exerted directly on platelets in the lumen and not via stimulation of the endothelial NO pathway. This direct platelet inhibitory effect of \textsuperscript{L-arginine} appears to be secondary to increased platelet NO activity,\textsuperscript{14,65} and human studies that used oral supplementation of \textsuperscript{L-arginine}, which increases plasma \textsuperscript{L-arginine} levels by up to 2-fold, have also shown inhibition of platelet aggregation in normal individuals and patients with hypercholesterolemia.\textsuperscript{66-68}

**Study Limitations**

Our study was not designed to determine whether baseline platelet aggregation was increased in patients with atherosclerosis compared with those without, although previous studies\textsuperscript{67-70} have demonstrated increased baseline ex vivo platelet aggregation in patients with atherosclerosis and those with hypercholesterolemia. Our observations that stimulation of endothelial NO activity produces less inhibition of platelet aggregation in patients with atherosclerosis, coupled with previous observations of increased platelet aggregation in this group, suggest that decreased NO activity during stress, a powerful stimulus for endothelial NO production, may contribute to this observed difference in patients with atherosclerosis.

One limitation of platelet aggregation studies performed ex vivo is that they may not accurately reflect what occurs at the arterial wall surface because the first step in development of a thrombus, adhesion of a platelet monolayer, is not replicated by test tube aggregation studies.

Because patients participating in our study had atherosclerosis and were exposed to a variety of risk factors for atherosclerosis, it is not possible to determine whether the observed attenuation in platelet inhibitory effects with ACh was attributable to 1 or more risk factors for atherosclerosis. The observed correlation between attenuation of vasodilation and reduction in platelet inhibition with ACh suggests that it is the magnitude of endothelial dysfunction that determines the degree of reduction in platelet inhibition, and previous studies\textsuperscript{66} have shown that the number of risk factors correlate with the degree of endothelial dysfunction observed with ACh.

**Conclusions**

We have demonstrated that stimulation of NO release from the vascular endothelium promotes inhibition of whole blood platelet aggregation in human peripheral circulation and that this platelet inhibitory effect is attenuated in patients with atherosclerosis. \textsuperscript{L-Arginine} inhibits platelet aggregation in patients with atherosclerosis mainly by its direct effect on platelets. This reduced antiplatelet aggregatory property of the vasculature may predispose patients with atherosclerosis to thrombotic vascular events and, because of the known contribution of platelet-derived products to atherosclerosis, to more rapid progression of atherosclerosis.\textsuperscript{78,79} \textsuperscript{L-Arginine} supplementation and other therapies designed to improve endothelial NO activity should be tested for their long-term antithrombotic potential in atherosclerosis.
Acknowledgments

The authors would like to thank Dr Xu Fu for his technical assistance for some of the in vitro platelet studies and William Schenke for technical assistance with preparation of the manuscript.

References


Atherosclerosis and Platelet Activation in Humans


Atherosclerosis and Platelet Activation in Humans


Effect of Atherosclerosis on Endothelium-Dependent Inhibition of Platelet Activation in Humans

Jean G. Diodati, Nader Dakak, David M. Gilligan and Arshed A. Quyyumi

_Circulation_. 1998;98:17-24
doi: 10.1161/01.CIR.98.1.17

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/98/1/17

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/