Role of Adenosine and Nitric Oxide on the Mechanisms of Action of Dipyridamole

Alfredo Gamboa, MD; Robert Abraham, MD; Andre Diedrich, MD; Cyndya Shibao, MD; Sachin Y. Paranjape, BS; Ginnie Farley; Italo Biaggioni, MD

Background and Purpose—The combination of dipyridamole and aspirin has been shown to be more effective than aspirin alone in the secondary prevention of stroke. Dipyridamole may act by inhibiting adenosine uptake, thus potentiating its actions. Dipyridamole also inhibits cGMP-specific phosphodiesterases (PDE) and, through this mechanism, could potentiate cGMP-mediated actions of nitric oxide.

Methods—To define the mechanism of action of dipyridamole, we studied the local vascular effects of adenosine, acetylcholine (NO-mediated dilation), and nitroprusside (cGMP-mediated dilation) in a double-blind study after treatment with dipyridamole/aspirin (200 mg dipyridamole/25 mg aspirin twice a day) or aspirin control for 7 days in 6 normal volunteers. Vasodilators were administered into the brachial artery in the nondominant arm in random order and forearm blood flow (FBF) was measured by venous occlusion plethysmography.

Results—Adenosine at a dosage of 125 μg/min increased FBF from 4.6±0.9 to 29.4±5.3 (539% increase) with dipyridamole/aspirin and from 3.9±0.8 to 12.5±2 mL/100 mL forearm/min (208% increase) with aspirin alone (P=0.007). In contrast, dipyridamole/aspirin did not alter the response to acetylcholine or to nitroprusside. The magnitude of adenosine-induced vasodilation correlated with plasma dipyridamole concentrations (r²=0.6); no correlation was observed with acetylcholine- or nitroprusside-induced vasodilation. Similar potentiation of adenosine, but not acetylcholine or nitroprusside, was observed in 7 additional subjects when adenosine, acetylcholine, and nitroprusside were given in random order before and 2 hours after a single dose of dipyridamole/aspirin.

Conclusion—The effects of dipyridamole on resistance vessels are preferentially explained by potentiation of adenosine mechanisms rather than potentiation of nitric oxide or other cGMP-mediated actions. (Stroke. 2005;36:2170-2175.)

Key Words: adenosine • aspirin • dipyridamole • endothelium • nitric oxide • regional blood flow

The addition of dipyridamole to aspirin has been found to provide a greater protective effect in the secondary prevention of stroke compared with aspirin alone.1 The molecular mechanism of action of dipyridamole is not completely understood and may be multiple. Dipyridamole inhibits the nucleoside transporter responsible for the cellular uptake of adenosine.2 This transporter explains the extremely short half-life of adenosine, and its blockade by dipyridamole results in greater extracellular levels of adenosine3 and potentiation of its actions.4

Dipyridamole is also a phosphodiesterase (PDE) inhibitor in vitro, particularly of PDE5 and PDE6 involved in cGMP hydrolysis. Dipyridamole, therefore, could act by potentiating the actions of those mediators that work through cGMP, namely nitric oxide (NO; Figure 1).

The study of the mechanisms of actions of dipyridamole is hampered by the extremely short half-life of adenosine and NO, and by the fact that the conditions that modulate their release in vivo are difficult to replicate ex vivo or in vitro. Therefore, we used the isolated forearm vasculature as our experimental model and intrabrachial infusion of acetylcholine to induce NO-dependent vasodilation, nitroprusside to induce cGMP-dependent vasodilation and adenosine, to investigate the molecular mechanisms of action of dipyridamole.

Methods

Subjects
We studied a total of 14 healthy volunteers in 2 separate protocols using a single dose of dipyridamole/aspirin (n=7) in one and repeated doses (n=7) in the other. All subjects were nonsmokers, were not taking medications, and had abstained from methylxanthines for at least 72 hours before taking the designated medication. The Vanderbilt University Institutional Review Board approved the protocol. Volunteers were informed of the characteristics of the study and gave written consent.

Instrumentation
For each study session, subjects were fasted and in the supine position. Heart rate was monitored with a surface electrocardiogram...
Figure 1. Postulated mechanisms of action of dipyridamole. Dipyridamole potentiates endogenous adenosine actions by blocking its cellular uptake, and may potentiate NO by inhibiting cGMP-specific phosphodiesterases (PDE5). The diagram also illustrates the potential interaction between NO and adenosine. NO inhibits protein kinase C (PKC), which activates 5′-nucleotidase (5′-NT), the enzyme responsible for generation of adenosine from 5′-adenosine monophosphate (AMP). ATP indicates adenosine triphosphate; ADP, adenosine diphosphate; cAMP, cyclicAMP; NOS, nitric oxide synthase; cGMP, cyclic GMP.

coupled to a rate computer. A catheter was inserted into the right antecubital vein for safety reasons. An indwelling catheter was placed into the left brachial artery for intraarterial drug administration and was connected through 3-way valves to a pressure transducer. Blood pressure was measured continuously from the brachial artery and through the volume clamp method of the middle finger (Finapres 2300; Ohmeda). Cardiovascular signals were modulated by signal conditioners and digitized using a Windaq system (DA-220; DATAQ Instruments). Forearm blood flow (FBF) was measured using venous occlusion mercury in silastic strain-gauge plethysmography (Hokanson EC4, DE; Hokanson Inc), as previously described.3

Procedures
After instrumentation, subjects were allowed to rest in a quiet room for 30 minutes to ensure that all cardiovascular parameters had returned to resting values. The subjects were warned ahead of time of all procedures to be performed and potential discomforts to avoid anticipatory reactions. After a 10-minute baseline recording, increasing doses of acetylcholine, nitroprusside, or adenosine were administered intrabrachially in random order. Acetylcholine was infused at rates of 1, 3, and 5 μg/min. Nitroprusside was infused at rates of 1, 2, and 3 μg/min. Adenosine was infused at rates of 31.25, 62.5, and 125 μg/min. The infusions were maintained for 5 minutes each. Heart rate, blood pressure, and FBF were measured before and during the last minute of each dose. Sufficient time was allowed between infusions to allow parameters to return toward baseline values.

Study Protocols

Protocol 1: Single-Dose Study
The vasodilatory effects of adenosine, acetylcholine, and nitroprusside were determined in random order, as detailed here, at baseline and 2 hours after a single dose of dipyridamole/aspirin (200 mg dipyridamole and 25 mg aspirin).

Protocol 2: Repeated Dosing Study
Subjects were randomized to receive either 25 mg aspirin or dipyridamole/aspirin (200 mg dipyridamole and 25 mg aspirin) orally twice daily for 7 days in a double-blind, crossover fashion at least 1 month apart. The vasodilatory effects of adenosine, acetylcholine, and nitroprusside were determined in random order, as detailed here, the morning of the seventh day, approximately 3 hours after the last dose of dipyridamole/aspirin or aspirin alone. This was followed by measuring the maximal increase in FBF after a 3-minute total occlusion of the forearm induced by inflating a proximal cuff to 200 mm Hg. Plasma dipyridamole levels were measured at the beginning of each study day.

Drugs and Statistical Analysis
Medications (aspirin and dipyridamole/aspirin) were prepared by Vanderbilt’s Investigational Pharmacy. We used a commercially available dipyridamole/aspirin combination (Aggrenox) containing a slow-release formulation of dipyridamole with greater bioavailability than previous oral preparations.5 Acetylcholine (Miochol-E), nitroprusside (sodium nitroprusside injection), and adenosine (Adenocard) were purchased from our local pharmacy. All statistical analyses were carried out using SPSS version 11. Continuous variables are expressed as mean±standard error of mean. Groups were compared using the general linear model analysis of variance for repeated measures. Statistical significance was accepted at P<0.05.

Results

Protocol 1
At baseline, adenosine increased FBF from 8.7±1.9 to 14.1±2.3, 19.8±4.3, and 28.1±4.6 mL/100 mL/min (for the first, second, and third doses, respectively; Figure 2). Acetylcholine increased FBF from 6.0±0.9 to 16.5±5.5, 28.4±7.1, and 31.7±7.3 for the first, second, and third doses, respectively. Nitroprusside increased FBF from 5.2±0.8 to 11.3±1.7, 16.9±2.8, and 19.2±2.9 for the first, second, and third doses, respectively. Two hours after a single dose of dipyridamole/aspirin, adenosine produced a significantly greater increase in FBF, from 8.6±1.0 to 25.5±3.7, 30.1±3.9, and 35.2±4.2 for the first, second, and third doses, respectively. No significant effect of dipyridamole/aspirin was observed with any of the 2 other vasodilators (Figure 2).

Protocol 2
Of the 7 subjects originally studied, one had undetectable plasma levels of dipyridamole after he had presumably received dipyridamole/aspirin for 7 days. Therefore, the data of this subject was not included in the analysis. The remaining 6 subjects were 32±1 years of age and had a weight of 85±7 kg and a height of 1.76±0.05 m. Basal hemodynamic measurements taken after 7 days of aspirin or dipyridamole/ aspirin are shown in Table 1. Basal FBF was mildly but significantly elevated, and forearm vascular resistance lower, after dipyridamole/aspirin compared with aspirin control (Table 1).

After 7 days of aspirin control, adenosine increased FBF from 3.9±0.8 to 5.5±1.2, 10.4±2.5, and 12.0±2.5 mL/100 mL per minute (for the first, second, and third doses, respectively; Figure 3). Acetylcholine increased FBF from 3.3±0.6 to 3.2±0.6, 11.1±2.7, and 13.5±3.4 for the first, second, and third doses, respectively. Nitroprusside increased FBF from 3.3±0.6 to 6.1±2.3, 17.1±3.3, and 18.8±3.7 for the first, second, and third doses, respectively.

After 7 days of dipyridamole/aspirin, adenosine increased FBF from 4.6±0.9 to 7.6±2.5, 24.3±5.3, and 36.4±6.5 mL/100mL per minute. Acetylcholine increased FBF from
Nitroprusside increased FBF from 3.7 ± 0.5 to 11.1 ± 4.8, 20.2 ± 4.0, and 25.1 ± 3.7 for the first, second, and third doses, respectively. Only the increase in FBF produced by adenosine was significantly greater in the dipyridamole/aspirin day compared with the aspirin day (Figure 3). There was a trend toward a greater increase in acetylcholine-induced vasodilation during the dipyridamole/aspirin day, but it did not reach statistical significance.

Plasma dipyridamole levels ranged from 1.1 to 5.5 μg/mL after 7 days of treatment, with an average of 2.99 ± 0.8 μg/mL. Resting forearm blood flow ranged from 1.4 to 7.5 mL/100 mL per minute and correlated significantly with plasma concentrations of dipyridamole ($r^2 = 0.87$, $P = 0.007$). The magnitude of vasodilation induced by 125 μg/min adenosine correlated with plasma dipyridamole concentrations ($r^2 = 0.6$), but the relationship did not reach statistical significance ($P = 0.07$; Figure 4). No correlation was observed between dipyridamole plasma levels and acetylcholine- or nitroprusside-induced vasodilation.

Forearm blood flow increased from 4.5 ± 0.9 to 12.2 ± 1.9 mL/100 mL per minute in response to a 3-minute ischemia on the aspirin control day and from 6.1 ± 1.5 to 17.2 ± 3.3 mL/100 mL per minute on the dipyridamole/aspirin day. Although there was a trend toward greater reactive hyperemia during dipyridamole/aspirin, this did not reach statistical significance.

**Discussion**

Dipyridamole, given as a slow-release preparation in combination with aspirin, was found in a large randomized clinical trial (ESPS 2) to be more effective than aspirin alone in the secondary prevention of stroke. A metaanalysis of comparable trials concluded that the dipyridamole/aspirin combination was effective in secondary prevention of vascular events but expressed concern that this was the result of the inordinate contribution of a single clinical trial, ESPS 2. A subsequent metaanalysis confirmed the effectiveness of dipyridamole/aspirin in preventing recurrence of ischemic stroke and other vascular events, even when results from ESPS 2 were not included in the analysis. A post hoc analysis of the data from ESPS 2 showed that the efficacy of the dipyridamole/aspirin combination was greater in patients with the highest risk of stroke and those who had other cardiovascular diseases.

Thus, available information supports the efficacy of dipyridamole/aspirin combination in stroke prevention, and an ongoing clinical trial will compare the efficacy and safety of dipyridamole/aspirin and clopidogrel in stroke prevention.
It is not known, however, if this is the predominant mechanism of action of dipyridamole. In particular, dipyridamole inhibits phosphodiesterases that hydrolyze cyclic-GMP. Because this is the main signaling pathway for nitric oxide, it is possible that dipyridamole also works by enhancing the effects of nitric oxide, another potent vasodilator and inhibitor of platelet aggregation. In this study, we used the isolated forearm vascular bed to explore these possibilities and found that dipyridamole preferentially potentiates the vasodilatory effects of adenosine while having relatively less effect on the vasodilation induced by nitric oxide pathways.

There are substantial data from animal studies indicating that dipyridamole potentiates adenosine actions by blocking the nucleoside transporter. It is not certain, however, that results derived from animal studies are applicable to humans because there are significant animal and genetic differences in the adenosine nucleoside transporter. Nonetheless, there is evidence that the nucleoside transporter is particularly efficacious in humans, accounting for the extremely short half-life of adenosine in blood of less than 1 second. Furthermore, we have previously shown that dipyridamole produces approximately a 4-fold potentiation of the cardiovascular effects of systemically administered adenosine. Additionally, we have shown that adenosine, infused into the brachial artery at doses that produce near maximal forearm vasodilation, fails to increase interstitial levels of adenosine unless the vascular barrier provided by the nucleoside transporter is blocked by dipyridamole. Finally, the cardiovascular effects of dipyridamole are inhibited by the adenosine receptor antagonists theophylline and caffeine in humans. Thus, there is evidence that dipyridamole modulates adenosine actions in vivo in humans.

It should be noted that previous studies examining the effect of dipyridamole on adenosine’s actions used an intravenous preparation of dipyridamole at doses that result in rapid increases in plasma dipyridamole levels. This mode of administration is used as a cardiac stress test to induce perfusion defects, based on the concept that stenotic vessels dilate less than normal ones. Therefore, to the use of dipyridamole in patients requiring treatment for stroke prevention who often have coronary artery disease. In this regard, it is reassuring that myocardial ischemia has not been reported when the current slow-release formulation of dipyridamole is used, and this preparation apparently can be safely given to patients with coronary artery disease. We found that the plasma levels of dipyridamole produced by oral dipyridamole/aspirin are also suffi-
cient to potentiate the vascular effects of adenosine, either when given acutely or after repeated dosing.

Dipyridamole is considered a selective inhibitor of PDE5, an isoenzyme that hydrolyzes cGMP. Recent studies, however, indicate that PDE isoenzymes that hydrolyze cAMP are also sensitive to dipyridamole.10,17 It is not clear if these in vitro findings are relevant to the in vivo actions of dipyridamole, but they raise the possibility that dipyridamole potentiates the effects of adenosine not only by inhibiting its uptake, but also by enhancing its cAMP-mediated actions. It is also possible that dipyridamole potentiates other agents that have cAMP as their signaling pathway such as prostacyclin.

Dipyridamole has been shown to enhance the effects of inhaled NO in the pig pulmonary circulation,18 presumably by inhibition of PDE in vivo. The predominant PDE isoenzymes present in human smooth muscle and endothelial cells are PDE1 and PDE5, and dipyridamole inhibits the latter.19,20 It was reasonable to propose, therefore, that the vasodilatory effects of dipyridamole are mediated by PDE inhibition, but our results suggest that its effects in the forearm vasculature are preferentially mediated by potentiation of endogenous adenosine. Our studies are limited to examination of resistance vessels, and they may or may not apply to the microcirculation. In platelets, the predominant isoenzymes are PDE2, PDE3, and PDE5, and in vivo dipyridamole selectively inhibits PDE5 at therapeutic concentrations, but not other PDE isoforms.21,22 We cannot rule out the possibility that dipyridamole may modulate NO pathways in platelets more efficiently than in the vasculature. Dipyridamole was shown recently to inhibit the synthesis of inflammatory gene products produced by human platelet–monocyte aggregates in vitro. The mechanisms proposed to explain this effect include potentiation of the effects of adenosine, NO/cGMP, and others.23

Potential interactions between adenosine and NO mechanisms are worth discussing. Adenosine, which enhances intraplatelet cAMP levels and inhibits platelet aggregation, has been suggested to cause also an increase in platelet cGMP concentrations through a mechanism that involves NO synthesis.24 Conversely, some studies have shown that acute inhibition of NO synthesis produces a significant increase in adenosine levels. Kostic and Schrader first reported that inhibition of NO synthesis was associated with increased adenosine formation at rest and during reactive hyperemia in buffer-perfused isolated guinea pig hearts.25 Woolfson et al found a 5-fold increase in adenosine concentrations in the coronary perfusate of isolated rabbit hearts perfused with the nitric oxide synthase inhibitor Nω-nitro-ω-arginine methyl ester (1-NAME)26 and suggested that this increase could explain the paradox effect of 1-NAME to limit infarct size in this model.27 Recent in vivo studies in dogs found a 4.2- to 5.6-fold increase in coronary venous blood during intracoronary 1-NAME perfusion28 and enhanced adenosine release in animals treated with 1-NAME during increased myocardial oxygen consumption induced by cardiac pacing.29 The mechanism by which NO inhibits adenosine formation is not completely known, but may involve modulation of protein kinase C (PKC; Figure 1). It is known that PKC increases ecto-5′-NT activity, which produces adenosine from AMP in the myocardium.30 Conversely, both NO and NO donors inhibit PKC and, through this mechanism, decrease ecto-5′-NT activity.31 The increase in adenosine levels induced by ischemia in isolated coronary vessels can be blunted by an ecto-5′-NT inhibitor, and PKC inhibitors blunt both the increase in adenosine and ecto-5′-NT activation.32 Furthermore, Minamino et al concluded, in an in vivo study in dogs, that 1-NAME increases both adenosine production (4.2- to 5.6-fold) and ecto-5′-NT activity through the activation of PKC via a cGMP-independent mechanism.28

Although our studies were limited to the isolated forearm vasculature, we used this model to investigate molecular mechanisms of action of dipyridamole that are likely relevant to other vascular beds. We cannot be certain, however, if this assumption also applies to the cerebral circulation. In rabbits, intracarotid infusion of adenosine or dipyridamole increases cerebral blood flow.33 Studies in humans have been limited to intravenous administration of these drugs. Results from these studies are confounded by the prominent increase in ventilation, as a result of arterial chemoreceptors activation34 and in sympathetic activity35 produced by adenosine. After correcting for adenosine-induced hyperventilation, Birk et al found that systemic administration of adenosine did not increase cerebral blood flow,36 but the effect of dipyridamole on cerebral blood flow autoregulation has not been defined.

In conclusion, we found that the effects of dipyridamole on forearm resistance vessels are preferentially explained by potentiation of adenosine mechanisms rather than potentiation of NO or other cGMP-mediated actions. It remains to be determined if this conclusion applies to other vascular beds, in particular the cerebral circulation, and to the effects of dipyridamole on platelets.

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