Blockade of Nucleoside Transport Is Required for Delivery of Intraarterial Adenosine Into the Interstitium
Relevance to Therapeutic Preconditioning in Humans
Alfredo Gamboa, MD; Andrew C. Ertl, PhD; Fernando Costa, MD; Ginnie Farley; M. Lisa Manier; David L. Hachey, PhD; André Diedrich, MD; Italo Biaggioni, MD

Background—Adenosine, a known mediator of preconditioning, has been infused into the coronary circulation to induce therapeutic preconditioning, eg, in preparation for angioplasty. However, results have been disappointing. We tested the hypothesis that endothelial nucleoside transporter acts as a barrier impeding the delivery of intravascular adenosine into the underlying myocardium and that this can be overcome with dipyridamole, a nucleoside transporter blocker.

Methods and Results—We infused saline or adenosine (0.125 and 0.5 mg/min) into the brachial artery while monitoring forearm blood flow (FBF) and interstitial adenosine levels with microdialysis probes implanted in the flexor digitorum superficiales of the forearm in 7 healthy volunteers during intravenous administration of saline or dipyridamole (loading dose, 0.142 mg/kg per min for 5 minutes followed by 0.004 mg/kg per min). Adenosine produced near maximal forearm vasodilation, increasing FBF from 4.0 ± 0.7 to 10.4 ± 1.9 and 13.1 ± 1.6 mL/100 mL per min for the low and high doses, respectively, but did not increase muscle dialysate adenosine concentration (from 88 ± 21 to 65 ± 23 and 85 ± 26 nmol/L).

Intravenous dipyridamole enhanced resting muscle dialysate adenosine (from 77 ± 25 to 147 ± 50 nmol/L), adenosine-induced increase in FBF (from 4.1 ± 0.8 to 12.6 ± 3 and 15.1 ± 3 mL/100 mL per min for the low and high dose, respectively), and the delivery of adenosine into the interstitium (to 290 ± 80 and 299 ± 143 nmol/L for the low and high dose, respectively, \( P = 0.04 \)).

Conclusions—Intravascular adenosine is likely ineffective in inducing myocardial preconditioning because of poor interstitial delivery. This can be overcome by blocking the nucleoside transporter with dipyridamole. (Circulation, 2003;108:2631-2635.)

Key Words: adenosine ■ ischemia ■ endothelium ■ blood flow

Adenosine is an immediate product in the breakdown of ATP and accumulates in the interstitium of metabolically active tissues when oxygen demands exceed supply, eg, during ischemia. Adenosine is considered a retaliatory autacoid, whose main function is to protect tissues against ischemia-related injury. Many of its actions are indeed consistent with such a role. Adenosine contributes to reactive hyperemia and produces maximal coronary vasodilation, inhibits norepinephrine and renin release, and inhibits neutrophil activation and platelet aggregation.

Adenosine is also considered an important mediator of ischemic preconditioning, a phenomenon by which an initial brief period of ischemia protects the tissue from the damage produced by a subsequent more intense ischemic episode. It is believed that during transient coronary occlusion, adenosine and other mediators are released and initiate a cascade of intracellular events involving protein kinase C activation that are responsible for the protective effect to subsequent ischemia. There has been interest, therefore, in replicating ischemic preconditioning by infusing adenosine and using this pharmacological preconditioning as a therapeutic approach for cardiac protection. A situation where this approach may be of practical relevance is angioplasty, where ischemia may be induced transiently during the procedure. Indeed, adenosine has been infused into the coronary circulation to protect the myocardium from subsequent ischemia during balloon inflation. However, results have been disappointing and the reason for negative outcomes has not been clear.

In interpreting results from these clinical studies, it is important to consider the clinical pharmacology of adenosine. The half-life of adenosine in human blood is estimated to be less than 10 seconds because of cellular uptake of adenosine through a high-affinity nucleoside transporter. Dipyridamole is an inhibitor of this nucleoside transporter and is proposed to act by blocking adenosine uptake, thus increasing...
plasma and interstitial levels of adenosine and potentiating adenosine actions in humans. High-affinity nucleoside transporters are expressed in endothelial and vascular smooth muscle cells and may provide a barrier to intravascular adenosine reaching myocardial cells. Indeed, animal studies suggest that most of the adenosine infused into the arterial circulation might be trapped by the endothelium. The effectiveness of this barrier will obviously depend on the levels of expression of the nucleoside transporter, which differs between species. We are not aware of previous studies addressing this issue in humans.

Therefore, we tested the hypothesis that the nucleoside transporter acts as a barrier in humans, impeding the delivery of intravascular adenosine into the underlying tissue, and that this can be overcome by pretreatment with dipyridamole. We used the forearm vasculature as a model to test this hypothesis. If this hypothesis is true, then blocking endothelial uptake of adenosine can be used as an approach to improve the delivery of adenosine into the underlying tissue for the purpose of inducing preconditioning.

Methods

Subjects

We studied a total of 7 healthy volunteers, age 21 to 42 years (mean, 29±2 years). Subjects were nonsmokers, were not taking medications, and had abstained from methylxanthines for at least 72 hours before the study. 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 surface ECG coupled to a rate computer. A catheter was inserted into the right antecubital vein for drug administration. An indwelling catheter was placed into the left brachial artery for intraarterial drug administration and 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

Forearm blood flow (FBF) was measured using venous occlusion mercury-in-silastic strain-gauge plethysmography (Hokanson EC4, DE; Hokanson Inc). Four FBF curves per minute were recorded. The forearm was elevated to at least ~10 cm above the level of the right atrium to ensure that the forearm veins were drained at the beginning of each flow measurement. The wrist cuff was inflated to 200 mm Hg 30 seconds before each series of forearm flow measurements to exclude blood flow through the hand and ensure that measurements only reflected the forearm vascular bed. The last 3 measurements of each series were averaged for the determination of the FBF for each period.

Transcutaneous Interstitial Muscle Microdialysis

A microdialysis probe, CMA/20 (CMA), was introduced into the flexor digitorum superficialis muscle of the left forearm, as previously described in detail. The probe had a dialysis tubing size of 10×0.5 mm (20 000 molecular mass cutoff) and was perfused continuously with saline at a rate of 2 µL/min (perfusate) with a microinjection pump (CMA/102 Microdialysis Pump). The effluent (dialysate) was recovered with a fraction collector.

Analytical Methods

Because of the relatively low concentrations and small volumes recovered with microdialysis, we developed a sensitive and specific ion-pair liquid chromatographic electrospray (ESI) MS/MS assay. Briefly, microdialysates were diluted 10-fold with water containing 10 nmol/L [UIC10,023-15N5] adenosine as an internal standard. HPLC separations were performed using a 5-µm C18 column using pentadecylfluoroacetanoic acid as an ion-pairing reagent. Mass spectrometric analysis was performed on a Finnigan MAT TSQ-7000 triple-quadrupole mass spectrometer using positive-ion ESI. Selected reaction monitoring was used to measure adenosine (m/z from 268 to 136) and the internal standard (from 282 to 145). The calibration range was 23 to 1150 femtomoles on column (10 to 500 nmol/L adenosine in original microdialysate). The chromatographic peak at the lower limit of quantitation (10 nmol/L; 25 femtomoles on column) gave a signal-to-noise ratio of 25. The intern run coefficient of variability was 8.1%.

Experimental Protocol

The intramuscular microdialysis probe was inserted as described above, and after a 1-hour equilibration period, 2 consecutive 15-minute dialysate samples were collected to determine baseline adenosine levels. We then infused saline or adenosine intravenously at 2 different doses, 0.125 and 0.5 mg/min (low and high dose, respectively), into the brachial artery while collecting dialysate to estimate interstitial adenosine levels during simultaneous intravenous administration of saline or dipyridamole (loading dose, 0.142 mg/kg per min for 4 minutes followed by 0.004 mg/kg per min). All 7 subjects received sequentially intravenous saline and then dipyridamole. The dialysate collection period was shifted by 1 minute in relation to the infusion period to account for the lag time produced by the length of the collecting tubing. FBF was measured before each drug infusion and during the last minute of each dose.

In vitro calibration was performed in all microdialysis probes to estimate the fraction of adenosine recovered across the microdialysis membrane. Probes were removed from the muscle at the end of the study, placed in a solution containing 2.5 µmol/L adenosine, and continuously perfused with saline at 2 µL/min. The dialysates were collected over 30 minutes in 2 15-minute fractions. Two 30-µL samples were also collected directly from the 2.5-µmol/L adenosine solution. These 2 sets of samples were processed, and the percentage recovery was calculated by dividing the dialysate concentration by the adenosine concentration measured from the 2.5-µmol/L adenosine solution.

Drugs and Statistical Analysis

Adenosine (Adenocard) and dipyridamole were purchased from our local pharmacy. All statistical analyses were carried out using SPSS v.11. Continuous variables are expressed as mean±SEM. Groups were compared by means of the General Linear Model ANOVA for repeated measures. Statistical significance was accepted at P<0.05.

Results

We studied 7 subjects 40±4 years of age with the following clinical characteristics: weight, 78±7.2 kg; height, 1.7±0.03 m; systolic blood pressure, 111±4 mm Hg; diastolic blood pressure, 63±2 mm Hg; and heart rate, 62±4 bpm.

Intrabrachial adenosine had no effect on systemic hemodynamics (Table). Adenosine increased FBF from 4.1±0.7 to 10.4±1.9 and 13.1±1.6 mL/100 mL per min for the low and high dose, respectively, Figure 1) but did not increase muscle dialysate adenosine concentration significantly (from 88±21 to 65±23 and 73±23 nmol/L, Figure 2).

Intravenous dipyridamole increased basal heart rate from 60±3 to 80±5 bpm but had no effect on blood pressure (from 88±8 after saline recovery to 88±7 mm Hg, Table). Dipyridamole increased resting muscle dialysate adenosine (from...
Systemic Cardiovascular Values During Intraarterial Infusion of Adenosine With Concomitant Intravenous Infusion of Saline or Dipyridamole

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ado 1</th>
<th>Ado 2</th>
<th>Recovery</th>
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</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td></td>
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<tr>
<td>Saline</td>
<td>58±3</td>
<td>58±8</td>
<td>60±3</td>
<td>60±3</td>
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<tr>
<td>Dipyridamole</td>
<td>80±5*</td>
<td>79±7*</td>
<td>83±8*</td>
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<tr>
<td>Mean arterial blood</td>
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<td>pressure, mm Hg</td>
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<tr>
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<td>76±4</td>
<td>68±7</td>
<td>82±8</td>
<td>88±9</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>88±7</td>
<td>82±4</td>
<td>93±5</td>
<td>94±7</td>
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Dipyridamole indicates 0.142 mg/kg per min for 4 minutes followed by 0.004 mg/kg per min; Ado 1, adenosine 0.125 mg/min; and Ado 2, adenosine 0.5 mg/min.

*Significant changes (P<0.05) with respect to corresponding saline values. No significant changes were produced by adenosine with respect to baseline values.

77±24 after saline recovery to 147±50 nmol/L, potentiated the increase in FBF induced by adenosine (from 4.1±0.8 to 12.6±3 and 15.1±3 mL/100 mL per min for the low and high dose, respectively; P<0.05, Figure 1), and enhanced the delivery of adenosine into the interstitium (to 290±80 and 299±143 nmol/L for the low and high dose; P=0.04, Figure 2). In vitro recovery of adenosine through the microdialysis probe was 24±1.6%.

Discussion

We found that intrabrachial adenosine, infused at doses that produced near maximal forearm vasodilation, failed to increase the interstitial level of adenosine. These results demonstrate that intraarterial administration of adenosine is not an effective delivery system if the goal is to reach the underlying tissue (eg, the myocardium) to induce preconditioning. The nucleoside transporter is largely to blame, because we were able to overcome this limitation with dipyridamole, a known nucleoside transport blocker.

We propose that the endothelial or vascular smooth muscle layer acts as an effective barrier to adenosine, impeding its diffusion from the intravascular compartment to the interstitium. Alternative explanations that will limit the effectiveness of adenosine should be considered. Nucleoside uptake is present in red blood cells, and it is possible that, given their number, these cells act as a sink for adenosine. Also, adenosine can be degraded to inosine by adenosine deaminase and phosphorylated to AMP by adenosine kinase. These processes are unlike explanations of our results, because adenosine produced a profound local vasodilator effect, even in the absence of dipyridamole.

This phenomenon explains why previous studies using intracoronary or intravenous adenosine to induce myocardial preconditioning often yield contradictory results. Patients receiving intracoronary adenosine tolerated significantly longer balloon inflation times, but there is controversy as to whether preconditioning was induced or not. Intracoronary adenosine did not improve significantly the left ventricular dysfunction normally observed during angioplasty; by comparison, increasing endogenous adenosine by intracoronary infusion of dipyridamole completely prevented angioplasty-induced ventricular dysfunction. There is also controversy about the effectiveness of intravascular adenosine to induce myocardial protection during cardiac surgery. Surprisingly, the possibility of an endothelial/vascular barrier impeding the delivery of adenosine into the myocardium has not been previously considered as an explanation for the negative results of these clinical studies.

Our findings do not negate the potential value of giving adenosine systemically or into the coronary circulation for cardiac protection. Adenosine has many other functions that may protect the heart against ischemia or reperfusion injury, independent of its ability to penetrate the myocardium. Adenosine, acting within the intravascular compartment, may inhibit platelet aggregation, inhibit neutrophil adhesion and activation, induce endothelial preconditioning, and have other beneficial actions. Our conclusions, therefore, are limited to the inability of intravascular adenosine to induce myocardial preconditioning.

Potential limitations in study design should be considered. The assumption is made that the forearm vasculature is an adequate model for the coronary vascular bed. The validity of this assumption is supported by the study of Auchampach and Gross, who found that intracoronary adenosine only induced preconditioning if dogs were pretreated with dipyridamole, suggesting that both vascular beds behave in a similar manner.
We studied only healthy subjects, and it is also possible that the endothelial barrier is impaired in atherosclerotic vessels or during hypoxia, allowing for greater delivery of adenosine in diseased subjects. This seems unlikely, given the negative results from clinical studies. For example, Heidland et al. found that angioplasty provoked a virtually identical degree of deterioration of ventricular function when subjects were pretreated with adenosine or saline, but these abnormalities were prevented when dipyridamole was administered. Thus, neither diseased vessels nor hypoxia allows for a more effective delivery of intraarterial adenosine into the interstitium.

Pharmacological therapeutic preconditioning could be induced with intravascular adenosine if dipyridamole is used. Intracoronary administration would have the advantage of avoiding the systemic effects of dipyridamole. At the time we designed these studies, there was limited human experience with intraarterial administration of dipyridamole. Recent studies have safely used this route of administration. Intracoronary dipyridamole seems to induce preconditioning even without adenosine, but the addition of adenosine is likely to provide greater protection. This cardioprotective effect seems paradoxical, because high-dose dipyridamole is used as a stress test to unmask areas of myocardial ischemia. This is probably related to its ability to vasodilate normal vessels to a greater extent than diseased ones, producing an apparent “steal” phenomenon. High-dose intravenous dipyridamole may, nonetheless, induce actual ischemia in diseased vascular beds, and it is not known if this could be avoided by intracoronary administration of dipyridamole or by the use of lower doses. For example, systemic administration of low-dose dipyridamole reportedly improves exercise capacity in patients with coronary artery disease and may induce preconditioning. Ischemic preconditioning is mediated by activation of A1 and perhaps A2 receptors. Stable analogs of these receptor subtypes could be developed for this indication. A short-acting A1 agonist may be preferable, because sinus node and AV node slowing are mediated by this receptor subtype and would be undesirable side effects for this indication.

We have previously shown that intrabrachial bolus injections of adenosine elicit a systemic sympathetic activation because of stimulation of forearm afferent fibers. This phenomenon is elicited by relatively high doses of adenosine given as bolus injections, and it is possible that the high local concentrations reached are able to overcome the endothelial barrier. Similarly, the ability of adenosine to induce AV prolongation is seen only if given as bolus injections but not during continuous infusion.

Our results evoke questions about the importance of the endothelium to adenosine-induced vasodilation, an area that remains controversial. Adenosine receptors are expressed in endothelial cells and could induce endothelium-mediated vasodilation. Animal studies are in disagreement about the ability of adenosine to induce NO release; studies in favor and against have been reported, and this may depend on the vascular bed and species studied. In the human forearm, adenosine-induced vasodilation was attenuated in the presence of the NOS inhibitor L-NMMA in one study but not in another. Adenosine-induced coronary vasodilation is believed to be independent of NO release in humans, and intracoronary adenosine is routinely used as an indicator of endothelium-independent coronary vasodilation. It is possible, however, that adenosine induces the release of other vasodilating endothelial factors, such as EDHF. Our studies were not designed to contribute to this controversy. Actions at the level of arterioles are likely to be responsible for most of adenosine-induced vasodilation. Even if we did not observe an overall increase in interstitial adenosine, it is possible that intraarterial adenosine is able to reach the arteriolar vascular smooth muscle.

In conclusion, our findings indicate that intravascular adenosine is likely ineffective in inducing myocardial preconditioning because of poor interstitial delivery. This limitation can be overcome by blocking the nucleoside transporter with dipyridamole.

Acknowledgments

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References

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