Translational Therapeutics of Dipyridamole
Hyung-Hwan Kim, James K. Liao

Abstract—Dipyridamole (DP) is a phosphodiesterase inhibitor that increases the intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP) by preventing their conversion to AMP and GMP, respectively. By increasing cAMP and cGMP levels in platelets, DP reversibly inhibits platelet aggregation and platelet-mediated thrombotic disease. In addition, DP may potentiate some of the vascular protective effects of endothelium-derived nitric oxide (NO), which increases cGMP by stimulating soluble guanylyl cyclase. Endothelium-derived NO is an important regulator of vascular tone, blood flow, and tissue perfusion. Indeed, endothelial NO synthase-deficient (eNOS−/−) mice exhibit elevated systemic blood pressure and have larger myocardial and cerebral infarct size after ischemic injury. Other NO/cGMP-dependent effects that may be potentiated by DP include inhibition of vascular smooth muscle proliferation and prevention of endothelial-leukocyte interaction. In addition, DP increases local concentrations of adenosine and prostacyclin, which could affect vascular tone and inflammation. Finally, DP has antioxidant properties, which could stabilize platelet and vascular membranes as well as prevent the oxidation of low-density lipoprotein. These platelet and nonplatelet actions of DP may contribute to some of its therapeutic benefits in vascular disease. (Arterioscler Thromb Vasc Biol. 2008;28:s39-s42)

Key Words: platelets • endothelium • vascular • dipyridamole • oxidation • inflammation • perfusion

This article is part of a multi-part CME-certified activity titled Translational Therapeutics at the Platelet Vascular Interface. In order to achieve all of the activity’s learning objectives, please read all of the components of the activity listed in the Table of Contents and follow the “Instructions for Participation and Obtaining CME Credit” outlined prior to the Introduction.

Antiplatelet therapy such as aspirin (ASA) has been the cornerstone for the treatment of cardiovascular disease, particularly ischemic strokes. However, the relatively small magnitude of benefits derived from aspirin monotherapy, ie, 14% to 20% relative risk (RR) reduction compared with placebo, has spurred the search for more effective antiplatelet agents or regimens.1–3 Surprisingly, the Management of placebo, has spurred the search for more effective antiplatelet agents or regimens.1–3 Surprisingly, the Management of

Adenosine and Platelet Inhibition
DP was initially found to increase extracellular levels of adenosine by inhibiting adenosine uptake by red blood cells, thereby leading to inhibition of platelet aggregation (Figure 1).8 Adenosine is released from vascular wall cells and platelets into the extracellular space as a breakdown product of adenosine triphosphate (ATP). Released adenosine nucleotides are rapidly converted to adenosine by nucleases. In circulating blood, free adenosine is rapidly removed from plasma by a specific adenosine carrier into red blood cells. At clinically relevant doses, DP inhibits adenosine uptake by red blood cells by >90% and increases plasma adenosine levels by 60%.9,10 Adenosine, acting through adenosine receptors, stimulates adenylyl cyclase in platelets and increases intracellular levels of cyclic adenosine monophosphate (cAMP), which is a potent inhibitor of platelet activation.1 It should be noted that DP can also increase intracellular levels of cAMP in platelets by preventing the breakdown of cAMP via inhibition of phosphodiesterase (PDE).12,13 Indeed, DP has been shown to inhibit platelet aggregation in whole blood in

by about 20% compared with ASA alone, without incurring excess bleeding.6,7 Surprisingly, the risk of bleeding was less with DP plus ASA compared with ASA alone. These findings suggest that DP may exert vascular protective effects beyond platelet inhibition.
vitro and potentiate the antiaggregatory effect of adenosine in vitro.\textsuperscript{14,15}

### Vasodilation and Perfusion

By inhibiting cyclic guanine monophosphate (cGMP) PDE, DP enhances cGMP-dependent downstream vasodilatory effects in smooth muscle (Figure 1).\textsuperscript{16} DP can also stimulate prostacyclin (PGI\textsubscript{2}) production by increasing intracellular levels of cAMP.\textsuperscript{17} PGI\textsubscript{2} is not only a potent inhibitor of platelet aggregation, but also a vasodilator. PGI\textsubscript{2} is generated by a cyclooxygenase-dependent pathway in a variety of cells, including endothelial cells.\textsuperscript{18} Finally, DP can potentiate vasodilation by increasing local adenosine levels.\textsuperscript{8} Thus, DP can exert direct and indirect vasodilatory effects on vascular smooth muscle.

Because of its vasodilatory properties, DP is often used in conjunction with electrocardiographic or imaging studies to detect underlying coronary ischemia.\textsuperscript{19,20} The basis of these studies is to augment the difference in myocardial perfusion, ie, coronary steal, via non-rate-limiting atherosclerotic coronary arteries compared with that of fixed rate-limiting lesions. The DP myocardial imaging studies are performed with IV infusion of DP, which results in 4 to 5 times higher acute blood levels of DP than what can be achieved with oral dose. A smaller increase in myocardial perfusion is observed with sustained-release oral DP, showing improved hyperemic myocardial blood flow and left ventricular systolic function in patients with ischemic cardiomyopathy.\textsuperscript{21}

### Antioxidative Effects

The molecular structure of DP allows it to accept electrons, thus functioning as a free radical scavenger and antioxidant. Using lipid oxidation assays based on the generation of peroxyl radicals by azo compounds, DP was found to scavenge both hydrophilic and hydrophobic radicals.\textsuperscript{22} Compared with ascorbic acid, \textalpha;\text{-tocopherol, and probucol, DP was more efficient in inhibiting chemically or cellularly induced low-density lipoprotein (LDL) oxidation as monitored by diene formation, evolution of hydroperoxides and thiobarbituric acid reactive substances, apoprotein modification, and by the fluorescence of cis-parinaric acid.\textsuperscript{23}

The antioxidative effects of DP could also occur at the cellular level. At clinically relevant concentrations, DP protects erythrocyte membranes from oxidation and spares the antioxidant power of erythrocytes.\textsuperscript{24} Furthermore, DP suppresses oxygen free radical formation in platelets and endothelial cells and improves cellular redox status.\textsuperscript{25} These antioxidative effects of DP may extend the half-life and increase the bioavailability of endothelium-derived nitric oxide (NO), which is vascular protective.

### Antiinflammatory Effects

Besides indirect antiinflammatory effects of DP via adenosine and PGI\textsubscript{2}, DP may also exert direct antiinflammatory effects through inhibition of platelet-monocyte interaction. For example, activated platelets adhere to and stimulate monocytes, causing monocytes to secrete monocyte chemo tactic protein-1 (MCP-1) and matrix metalloproteinase-9 (MMP-9).\textsuperscript{26} Treatment of activated platelets with DP, but not ASA, prevented monocyte secretion of MCP-1, MCP-9, and tissue factor.\textsuperscript{26,27} DP has also been shown to inhibit the adhesion of neutrophils to the vascular endothelium in ischemic stroke patients via a specific downregulation of Mac-1.\textsuperscript{28} Furthermore, DP inhibits lymphocyte recruitment, activation, and secretion of proinflammatory mediators.\textsuperscript{29,30} Thus, the potential antiinflammatory effects of DP may contribute to some of its clinical benefits.

### Potentiation of NO-Mediated Pathways

By increasing intracellular levels of cGMP, DP could augment many of the downstream signaling pathways of NO (Figure 2). Loss of endothelial-derived NO activity leading to reduction in intracellular cGMP levels contributes to impaired vascular responses,\textsuperscript{31} enhanced platelet aggregation,\textsuperscript{32} and vascular smooth muscle proliferation.\textsuperscript{33} Inhibition of endothelial NO production by the endothelial NO synthase...
Table. Vascular Effects of DP

<table>
<thead>
<tr>
<th>Endothelial cell</th>
<th>↑ cGMP and potentiate downstream actions of endothelium-derived NO</th>
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<tbody>
<tr>
<td></td>
<td>↑ PGlu production</td>
</tr>
<tr>
<td></td>
<td>↓ Thrombus formation</td>
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<td></td>
<td>↓ Oxidative stress</td>
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<td></td>
<td>↓ Inflammation</td>
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<td></td>
<td>↑ Angiogenesis</td>
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<tr>
<td>Smooth muscle cell</td>
<td>↓ Migration and proliferation</td>
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<tr>
<td></td>
<td>↓ Reactive oxygen species</td>
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<tr>
<td></td>
<td>↑ Vasorelaxation</td>
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<tr>
<td>Platelet</td>
<td>↓ Platelet reactivity via increase in local adenosine levels</td>
</tr>
<tr>
<td></td>
<td>↓ Platelet aggregation via increase in intracellular cAMP and cGMP</td>
</tr>
<tr>
<td></td>
<td>↑ Stabilization of platelet membranes</td>
</tr>
<tr>
<td>Monocyte/macrophage</td>
<td>↓ Platelet-monocyte interaction</td>
</tr>
<tr>
<td></td>
<td>↓ MMP-9 expression and secretion</td>
</tr>
<tr>
<td></td>
<td>↓ MCP-1 secretion</td>
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<tr>
<td></td>
<td>↓ Tissue factor expression and activity</td>
</tr>
<tr>
<td></td>
<td>↓ Interleukin-8 secretion</td>
</tr>
<tr>
<td>Vascular inflammation</td>
<td>↓ High-sensitivity C-reactive protein level</td>
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<tr>
<td></td>
<td>↓ Leukocyte-endothelial cell adhesion</td>
</tr>
<tr>
<td></td>
<td>↓ CD40/CD40L expression</td>
</tr>
<tr>
<td>Other effects</td>
<td>↑ LDL oxidation</td>
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<tr>
<td></td>
<td>↑ Plasma adenosine levels</td>
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</table>

(eNOS) inhibitor, Nω-monomethyl-l-arginine (l-NMA), causes vasoconstriction and vascular inflammation by promoting endothelial-leukocyte adhesion. Indeed, lower vascular cGMP levels in mutant mice lacking eNOS are associated with systemic and pulmonary hypertension, greater propensity for intimal smooth muscle proliferation in response to vascular cuff injury, and larger stroke sizes in response to cerebral ischemia. Thus, by inhibiting cGMP PDE, DP may potentiate the downstream effects of NO. Indeed, DP has been shown to potentiate NO/cGMP vasodilatory and platelet antiaggregatory effects, enhance ischemia-induced angiogenesis, increase myocardial perfusion in heart failure and stable coronary artery disease, and ameliorate the severity of ischemic strokes via NO- and adenosine-mediated effects.

Translational Benefits of DP in Secondary Stroke Protection in Antiplatelet Clinical Trials

The mechanism by which DP, especially the extended-release formulation, could reduce the risks for secondary strokes without incurring excess bleeding may be attributable to some of its effects beyond platelet inhibition on the vascular wall (Table). As previously mentioned, DP could augment many of the downstream signaling pathways of NO (Figure 2). Indeed, lower vascular cGMP levels in mutant mice lacking eNOS are associated with larger stroke sizes in response to cerebral ischemia. These findings suggest that DP may protect against stroke, in part, through a platelet-independent mechanism. Similar mechanisms may occur with other cardiovascular agents such as statins (Stroke Prevention by Aggressive Reduction in Cholesterol Levels [SPARCL] trial), angiotensin-converting enzyme inhibitors (Heart Outcomes Prevention Evaluation [HOPE] trial), and angiotensin II receptor blockers (Losartan Intervention for Endpoint Reduction [LIFE] Study), which protect the vascular wall and confer stroke protection without any direct effects on platelet aggregation. Indeed, the combination of DP plus lower doses of statins synergizes to protect against ischemia-reperfusion injury and ischemic stroke (Hyung-Huan Kim and James K. Liao, 2008).

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