Pharmacological Stress Myocardial Perfusion Imaging With the Potent and Selective $A_{2A}$ Adenosine Receptor Agonists ATL193 and ATL146e Administered by Either Intravenous Infusion or Bolus Injection

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Background—Adenosine (Ado) and dipyridamole are alternatives to exercise stress for myocardial perfusion imaging. Though generally safe, side effects frequently occur that cause patient discomfort and sometimes lead to premature termination of the study or require aminophylline administration. Recently, a new class of $A_{2A}$ Ado receptor agonists was synthesized. ATL193 and ATL146e are 2-propynylcyclohexyl-5-$N$-ethylcarboxamido derivatives of Ado. The study goals were to evaluate the potency and selectivity of these new compounds on recombinant canine Ado receptors and to evaluate their hemodynamic properties in dogs to assess their usefulness as vasodilators for myocardial perfusion imaging.

Methods and Results—In assays of recombinant canine Ado receptors, ATL-193 and ATL-146e were highly selective for the $A_{2A}$ over the $A_1$ and $A_3$ receptors and were more potent than MRE-0470 and CGS-21680. In 16 anesthetized dogs, the agonists were administered by infusion (ATL-193; $n=7$ normal) or bolus injection (ATL-146e; $n=9$ critical left anterior descending coronary artery stenosis), and hemodynamic responses were compared with those of Ado. Both agonists produced dose-dependent coronary flow (CF) elevation without provoking the hypotension observed with Ado. After an ATL-146e bolus, the CF increase was sustained for several minutes, providing ample time for injection and myocardial uptake of $^{99m}$Tc-sestamibi, and CF returned to baseline within 20 minutes. The CF increase was completely blocked by the selective $A_{2A}$ antagonist ZM241385 ($3 \mu g \cdot kg^{-1} \cdot min^{-1}$).

Conclusions—ATL-193 and ATL-146e are highly potent and selective Ado $A_{2A}$ receptor agonists with excellent potential for use as vasodilators for myocardial perfusion imaging. An important advantage of ATL-146e is the ability to administer it by bolus injection. (*Circulation. 2001;104:1181-1187.)*

Key Words: adenosine v vasodilation v imaging

Vasodilator stress with adenosine or dipyridamole is an alternative to exercise stress with myocardial perfusion imaging for the detection of coronary artery disease. Although the safety of adenosine and dipyridamole has been well established, undesirable side effects including chest pain, headache, dyspnea, and atrioventricular conduction abnormalities do occur in a majority of patients. In addition, both adenosine and dipyridamole produce severe bronchoconstriction when given to asthmatics. Because of its ultrashort half-life, adenosine must be administered by a constant IV infusion.

Whereas adenosine-induced coronary vasodilatation is mediated primarily by stimulation of the $A_{2A}$ receptor subtype on vascular smooth muscle, the side effects described above are believed to be caused by stimulation of 1 or more of the other 3 adenosine receptor subtypes, $A_1$, $A_{2B}$, and $A_3$. The discovery of highly selective and relatively short-acting adenosine receptor $A_{2A}$ agonists has opened the possibility of a strategy for eliciting the desired coronary vasodilatation without producing side effects. Preliminary experimental studies have been promising. Recently, a new class of selective adenosine $A_{2A}$ receptor agonists has been synthesized at the University of Virginia. Unlike adenosine, which has a half-life of seconds in human blood, the new compounds are esters that are more potent than CGS-21680 at human $A_{2A}$ receptors and have half-lives of several minutes in human blood. This half-life is considered to be optimal for bolus injection with...
pharmacological stress imaging. Accordingly, the major goals of the present study were to (1) compare the potency and selectivity of these new agonists, ATL-193 and ATL-146e, with the previously introduced MRE-0470 and CGS-21680 as agonists of recombinant canine adenosine receptors, which would validate use of the dog model of human hemodynamic responses; (2) compare the hemodynamic and coronary vasodilatory properties of ATL-193 and ATL-146e with those of adenosine to determine whether these new compounds are suitable vasodilators to use for pharmacological stress perfusion imaging; and (3) determine whether the duration of coronary flow (CF) elevation after a bolus injection of ATL-146e is sufficiently long to permit the injection of a radiolabeled tracer (99mTc-sestamibi) for the detection of coronary stenosis by imaging.

Methods

In Vitro Assessment of the Selectivity of ATL-193 and ATL-146e in Recombinant Canine Adenosine Receptors

Our radioligand binding assay methodology has been described previously.13-14 In brief, the A₁, A₂A, and A₃ subtypes of recombinant canine adenosine receptors were stably expressed in human embryonic kidney cells (HEK-293). The inhibition constant (Kᵢ) of test compounds was measured in competition for radioligand binding: [3H]8-cyclopentyl-1,3-dipropylxanthine for A₁ receptors; 125I-ZM241385 for A₂A receptors; and 125I-M membranes for A₃ receptors. The A₂A receptor was not examined because the canine receptor has not been cloned, but results from other species indicate that these new 2-agonists bind to A₂A receptors with very low affinity.15 Median inhibitory concentration and Kᵢ values for competing compounds were derived from the concentrations required to inhibit specific radioligand binding by 50%, as described previously.16

In Vivo Assessment of the Hemodynamic and Vasodilator Properties of ATL-193 and ATL-146e

Sixteen fasted, adult mongrel dogs (mean weight, 24.8±0.6 kg; range, 20.9 to 28.2 kg) were anesthetized with sodium pentobarbital (30 mg/kg), tracheally intubated, mechanically ventilated, surgically prepared, and instrumented as previously described.10 All experiments were performed with the approval of the University of Virginia Animal Care and Use Committee and were in compliance with the position of the American Heart Association on the use of research animals.

Experimental Protocols

Infusion Studies

Incremental doses of the adenosine A₂A receptor agonist ATL-193 (0.05, 0.1, 0.2, 0.3, and 0.4 μg·kg⁻¹·min⁻¹) were infused intravenously over 10 minutes (n=7). The infusion was then terminated and hemodynamic parameters were allowed to return to their baseline values. For comparison, the hemodynamic response to adenosine (250 μg·kg⁻¹·min⁻¹×3 minutes) was also evaluated. For both ATL-193 and adenosine infusions, all hemodynamic parameters were recorded before, during, and after termination of the infusion until the CF response had returned to its preinfusion baseline level.

Bolus Studies

Incremental doses of adenosine (60, 250, 500, and 750 μg/kg) or ATL-146e (0.25, 0.5, 1.0, and 1.5 μg/kg) were administered by IV bolus injection and hemodynamic responses recorded (n=9). The order of the injections was randomized. In 2 of 9 dogs, an IV infusion of adenosine (250 μg·kg⁻¹·min⁻¹×3 minutes) was also given.

Next, the snare ligature was adjusted to create a critical left anterior descending coronary artery (LAD) stenosis. A critical stenosis was defined as one in which resting flow was unchanged but flow reserve was abolished. Radiolabeled microspheres were injected at baseline, after stenosis, and at peak flow obtained with a second bolus injection of ATL-146e (1.0 μg/kg). The microsphere technique is standard in our laboratory and has been previously described.16,17 In 8 dogs, 99mTc-sestamibi (8.0 mCi) was coinjected with the microspheres at peak flow.

To assess the adenosine receptor subtype selectivity in vivo, in 2 of 9 dogs a 10-minute IV infusion of a highly selective adenosine A₂A receptor antagonist, ZM-241385,18 (0.3 and 3.0 μg·kg⁻¹·min⁻¹) was started 5 minutes before a third bolus injection of ATL-146e (1.0 μg/kg). At the end of the experiment, each heart was excised and sliced evenly from apex to base into 4 segments. The slices were imaged ex vivo directly on the collimator of a gamma camera, and image defects were quantified as described previously.16

Statistical Analysis

All statistical computations were made with SYSTAT software (SPSS Inc). Hemodynamic responses to each dose of adenosine or A₂A receptor agonist were compared against the respective preceding baseline period by using a 2-tailed Student’s paired t test. All results were expressed as the mean±SEM. Probability values <0.05 were considered significant.

Results

In Vitro Assessment of Selectivity of ATL-193 & ATL-146e in Recombinant Canine Adenosine Receptors

Figure 1 shows the structures and binding affinities of 4 A₂A agonists to recombinant canine adenosine receptor subtypes. See text for details.

Figure 1. Structures and binding affinities of 4 A₂A agonists to recombinant canine adenosine receptor subtypes. See text for details.
TABLE 1. Hemodynamic Parameters: ATL-193 and Adenosine Infusions

<table>
<thead>
<tr>
<th>Stressor Dose Response</th>
<th>HR, bpm</th>
<th>MAP, mm Hg</th>
<th>Max dP/dt, mm Hg/s</th>
<th>CF, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base 1</td>
<td>Dose 1</td>
<td>Base 2</td>
<td>Dose 2</td>
</tr>
<tr>
<td>Adenosine</td>
<td>122.0 ± 8.1</td>
<td>121.4 ± 8.7†</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ATL-193</td>
<td>122.0 ± 6.6</td>
<td>122.7 ± 5.5</td>
<td>121.7 ± 6.6</td>
<td>128.6 ± 6.9‡</td>
</tr>
<tr>
<td>Adenosine</td>
<td>111.3 ± 2.4</td>
<td>88.3 ± 5.0‡†</td>
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<td>...</td>
</tr>
<tr>
<td>ATL-193</td>
<td>112.1 ± 5.2</td>
<td>111.6 ± 4.1</td>
<td>116.9 ± 5.4</td>
<td>104.6 ± 5.9*</td>
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<td>Adenosine</td>
<td>5.1 ± 0.5‡</td>
<td>5.6 ± 0.5</td>
<td>6.1 ± 0.9</td>
<td>5.0 ± 0.7*</td>
</tr>
<tr>
<td>ATL-193</td>
<td>4.6 ± 0.6</td>
<td>4.7 ± 0.6</td>
<td>6.0 ± 1.9</td>
<td>5.0 ± 0.7*</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LAP, left atrial pressure; and dP/dt, peak positive first derivative of left ventricular pressure with respect to time. Adenosine dose 1 was 250 μg · kg⁻¹ · min⁻¹ × 3 minutes (n = 7). ATL-193 doses 1, 2, 3, 4, and 5 were 0.05 (n = 7), 0.1 (n = 7), 0.2 (n = 7), 0.3 (n = 6), and 0.4 (n = 3) μg · kg⁻¹ · min⁻¹ × 10 minutes, respectively. Values are expressed as mean ± SEM.

*P < 0.05 vs previous baseline.
‡P < 0.05, adenosine vs ATL dose 4 (0.3 μg · kg⁻¹ · min⁻¹).
†P < 0.05 vs previous dose.

In Vivo Assessment of the Hemodynamic and Vasodilator Properties of ATL-193 and ATL-146e

IV Infusion Experiments: Hemodynamics

The mean hemodynamic responses to 5 IV doses of ATL-193 are shown in Table 1. For comparison, the response to an adenosine infusion (250 μg · kg⁻¹ · min⁻¹ × 3 minutes) in the same animals is also shown. There was a dose-dependent increase in mean CF with ATL-193 infusions. With an ATL-193 dose of 0.3 μg · kg⁻¹ · min⁻¹ × 10 minutes (dose No. 4), there was a gradual 4-fold increase in CF, which peaked after ~7 to 8 minutes. The peak increase in CF produced by ATL-193 was greater than that by adenosine (114 vs 101 mL/min), with a lesser decrease in mean arterial pressure (MAP; 102 vs 88 mm Hg). ATL-193 also produced small, positive chronotropic and inotropic effects, consistent with the absence of a significant effect of this compound on myocardial A1 receptors. After the ATL-193 infusion was terminated, CF returned to baseline, with mean half-lives of 2.7 ± 0.6, 3.5 ± 0.5, 4.1 ± 0.5, 5.0 ± 1.6, and 10.4 ± 2.9 minutes for the different doses.

Bolus Injection Experiments: Hemodynamics

The mean hemodynamic responses to 4 IV bolus doses of adenosine and ATL-146e are listed in Table 2. Also shown for comparison is the response to an adenosine infusion (250 μg · kg⁻¹ · min⁻¹ × 3 minutes) in the same dogs. As displayed, there was a dose-dependent increase in mean CF, from 35.3 mL/min at baseline to 142.3 mL/min at the 1.5 μg/kg dose of ATL-146e. ATL-146e was significantly more potent than adenosine, because the 1.0 μg/kg dose of ATL-146e produced a larger increase in CF than did the 500 μg/kg dose of adenosine. Although MAP fell significantly at the higher doses of ATL-146e, tested, the drop in MAP was relatively small (13 to 18 mm Hg) and was markedly less than that produced by all of the adenosine doses (~50 mm Hg). In addition, as noted above, the decrease in MAP was transient with ATL-146e, lasting ~1.5 to 6 minutes after injection. Finally, ATL-146e produced small but statistically significant increases in heart rate and dP/dt at several doses, whereas adenosine produced either no change or a decrease in heart rate at the higher doses.

The upper panel of Figure 2 compares the CF response to an IV bolus of ATL-146e (1.0 μg/kg) with a 3-minute adenosine infusion in the same dog (dog No. 13). As shown, ATL-146e produced a greater and more sustained increase in CF than did the adenosine infusion. The lower panel of Figure 2 shows the MAP responses to both the adenosine infusion and the ATL-146e bolus in the same dog. ATL-146e produced a small, transient decrease in MAP, whereas with the adenosine infusion, there was a large, sustained drop in MAP of ~50 mm Hg.

Figure 3 shows the CF response after a 1.4 μg/kg IV bolus injection of ATL-146e. CF was maximal 2.29 minutes after injection. Although peak flow was delayed, 97% of peak flow occurred in <1 minute. After reaching a peak, there was a plateau in the CF response that persisted for several minutes. Afterward, CF returned to baseline within 20 minutes, with a pharmacodynamic half-life of 2.6 minutes.

Regional Myocardial Blood Flow

Table 3 summarizes absolute regional myocardial blood flow in the stenotic LAD and normal left circumflex coronary artery (LCX) zones at baseline, on setting the LAD coronary
TABLE 2. Hemodynamic Parameters: Adenosine Infusion and Bolus Injections and ATL-146e Bolus Injections

<table>
<thead>
<tr>
<th>Stressor Dose Response</th>
<th>Base 1</th>
<th>Dose 1</th>
<th>Base 2</th>
<th>Dose 2</th>
<th>Base 3</th>
<th>Dose 3</th>
<th>Base 4</th>
<th>Dose 4</th>
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<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Adenosine bolus</td>
<td>126.0±1.0</td>
<td>109.0±7.0</td>
<td>121.5±3.1</td>
<td>109.5±8.0</td>
<td>122.6±2.0</td>
<td>98.6±7.0*</td>
<td>120.7±1.4</td>
<td>82.5±4.4†</td>
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<tr>
<td>ATL-146e bolus</td>
<td>117.4±5.1</td>
<td>124.9±4.9*</td>
<td>119.6±4.5</td>
<td>125.5±5.3*</td>
<td>119.7±3.0</td>
<td>127.7±4.3*</td>
<td>117.8±5.5</td>
<td>137.5±7.2†</td>
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<td>MAP, mm Hg</td>
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<tr>
<td>Adenosine infusion</td>
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<td>77.0±2.0*</td>
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<td>Adenosine bolus</td>
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<td>107.5±2.2</td>
<td>58.8±4.5*</td>
<td>107.8±3.7</td>
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<td>114.0±4.7</td>
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<td>ATL-146e bolus</td>
<td>106.1±3.1</td>
<td>101.3±4.0</td>
<td>106.5±2.7</td>
<td>94.3±4.0†</td>
<td>106.1±3.1</td>
<td>90.6±5.4*</td>
<td>113.7±4.8</td>
<td>95.5±3.2*</td>
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<td>LAP, mm Hg</td>
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<td>Adenosine bolus</td>
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<td>6.0±0.0</td>
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<td>dP/dt, mm Hg/s</td>
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<tr>
<td>Adenosine infusion</td>
<td>1904±61</td>
<td>1769±3</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<td>...</td>
<td>...</td>
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<tr>
<td>Adenosine bolus</td>
<td>1983±23</td>
<td>1779±76</td>
<td>1879±181</td>
<td>1942±207</td>
<td>2100±87</td>
<td>1801±138</td>
<td>2047±76</td>
<td>1606±161†</td>
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<tr>
<td>ATL-146e bolus</td>
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<td>2204±110</td>
<td>1961±91</td>
<td>2132±76</td>
<td>1906±64</td>
<td>2155±104*</td>
<td>1975±92</td>
<td>2325±158*</td>
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<tr>
<td>CF, mL/min</td>
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<tr>
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<td>81.0±0.0*</td>
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<td>...</td>
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<tr>
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<td>39.8±5.0</td>
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<td>111.8±10.4*</td>
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<td>118.1±11.2†</td>
<td>36.2±2.3</td>
<td>142.3±15.1*</td>
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</table>

HR indicates heart rate; LAP, left atrial pressure; and dP/dt, peak positive first derivative of left ventricular pressure with respect to time. Adenosine infusion rate was 250 μg·kg⁻¹·min⁻¹ (n=2). Doses 1, 2, 3, and 4 refer to bolus injections of 60 (n=2), 250 (n=4), 500 (n=5), and 750 (n=6) μg/kg for adenosine and 0.25 (n=7), 0.5 (n=8), 1.0 (n=8), and 1.5 (n=6) μg/kg for ATL-146e, respectively. Values are expressed as mean±SEM.

*P<0.05 vs respective baseline.
†P<0.05 vs previous dose.

Legend: Gg/kg for ATL-146e, respectively. Values are expressed as mean±SEM.

Figure 2. CF (upper panel) and MAP (lower panel) responses of 3-minute IV adenosine infusion with responses after bolus of ATL-146e in same dog. Maximal CF response after bolus of ATL-146e was greater and more sustained than with adenosine. Note that adenosine flow response was blunted and declined ~1 minute after beginning of infusion due to marked drop in systemic pressure. Decline in MAP after bolus of ATL-146e was relatively small and transient.

Figure 3. Total duration of CF response after bolus of ATL-146e. Note plateau in elevated CF response that lasted for several minutes after bolus injection. CF returned to baseline within 20 minutes.
1.0 μg/kg dose of ATL-146e, CF returned completely to baseline in 15.7±0.9 minutes, with a pharmacodynamic half-life of 4.4 minutes. No pharmacodynamic half-life data were reported for the adenosine boluses owing to the fact that the adenosine response was rapid relative to the sampling rate; therefore, not enough samples were acquired to accurately model the data.

Ex Vivo Imaging Results
In the 8 dogs with critical LAD stenoses that were injected with 99mTc-sestamibi after a 1.0 μg/kg IV bolus of ATL-146e, large anteroseptal perfusion defects were observed on ex vivo images of the myocardial slices. The average stenotic to normal 99mTc-sestamibi activity ratio from the 8 dogs was 0.67±0.02.

In Vivo Assessment of Adenosine A2A Receptor Selectivity
Based on the in vitro binding assay data (Figure 1), the selectivity was not great enough to rule out possible effects of these compounds on A3 adenosine receptors. Consequently, we used the highly selective A2A antagonist, ZM-241385, to prove that coronary dilation was mediated by the A2A receptor. The bar graph shown in Figure 4 compares the CF response to a 1.0 μg/kg bolus injection of ATL-146e in the absence and presence of 2 doses of ZM-241385. Note that preadministration with ZM-241385 attenuated (0.3 μg·kg⁻¹·min⁻¹ dose) or abolished (3.0 μg·kg⁻¹·min⁻¹ dose) the adenosine A2A receptor–mediated increase in CF.

Discussion
In this report, we have demonstrated that the 2 novel A2A adenosine receptor subtype agonists, ATL-193 and ATL-146e, are more potent coronary artery vasodilators than are MRE-0470 and CGS-21680. For the first time, the potency and selectivity of these A2A agonists were investigated by radioligand binding assays on membranes from human embryonic kidney (HEK-293) cells expressing recombinant canine A1, A2A, and A3 receptors. We found that the potency

<table>
<thead>
<tr>
<th>TABLE 3. Absolute Regional Myocardial Blood Flow: ATL-146e Bolus Injection (1.0 μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stenotic Zone (LAD)</strong></td>
</tr>
<tr>
<td>Endocardium</td>
</tr>
<tr>
<td>Epicardium</td>
</tr>
<tr>
<td>Transmural</td>
</tr>
<tr>
<td>Endo/epi ratio</td>
</tr>
</tbody>
</table>

Endo/epi ratio indicates endocardial-to-epicardial flow ratio. Myocardial flows are expressed in mL·min⁻¹·g⁻¹. Results are expressed as mean±SEM.

*P<0.05, †P<0.01 vs stenosis.

<table>
<thead>
<tr>
<th>TABLE 4. Kinetic Parameters: Adenosine and ATL-146e Bolus Injections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stressor Dose Response</strong></td>
</tr>
<tr>
<td>Adenosine bolus</td>
</tr>
<tr>
<td>ATL-146e bolus</td>
</tr>
<tr>
<td>Adenosine bolus</td>
</tr>
<tr>
<td>ATL-146e bolus</td>
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</table>

All time values are in minutes. Doses 1, 2, 3, and 4 refer to bolus injections of 60 (n=2), 250 (n=4), 500 (n=5), and 750 (n=6) μg/kg for adenosine and 0.25 (n=6), 0.5 (n=9), 1.0 (n=6), and 1.5 (n=6) μg/kg for ATL-146e, respectively. Values are expressed as mean±SEM.

*P<0.05, †P<0.05 vs previous dose.
and selectivity of these compounds for recombinant canine adenosine receptors were similar to their potency and selectivity for recombinant human adenosine receptors.13 These compounds are thought to produce coronary artery dilatation by activating coronary artery A2A receptors.7,21 All of these compounds have limited selectivity for A2A over A1 receptors on the basis of radioligand binding assays. This selectivity may be underestimated in binding assays, owing to the fact that there are 2 affinity states of G protein–coupled receptors for agonists, and recombinant A2A receptors are poorly coupled to G proteins and consequently, bind agonists selectively to the low-affinity receptor conformation.22 This situation may have contributed to the observation that the functional potency of ATL146e as a coronary vasodilator in vivo appeared to be much greater than its potency as determined from in vitro binding assays (see below).

Comparison With Other Adenosine A2A Receptor Agonists

IV Infusion Experiments

Intravenously infused ATL-193 produced dose-dependent increases in coronary blood flow without a significant drop in MAP. The ATL-193 dose range tested (0.05 to 0.4 μg · kg⁻¹ · min⁻¹) was lower than that used previously for MRE-0470 (0.1 to 3.0 μg · kg⁻¹ · min⁻¹) and CGS-21680 (0.5 to 4.0 μg · kg⁻¹ · min⁻¹),10,11 yet the former produced a nearly equivalent increase in CF. Thus, ATL-193 is more poten than both MRE-0470 and CGS-21680, and these findings are in agreement with what would be predicted on the basis of the in vitro radioligand binding assay data.

Bolus Injection Experiments

When ATL-146e was administered as an IV bolus, we found that it produced dose-related coronary vasodilatation similar to that by IV infusions of ATL-193. Additionally, with bolus doses >0.5 μg/kg, ATL-146e produced approximately equal or greater increases in CF as an adenosine infusion (250 μg · kg⁻¹ · min⁻¹ × 3 minutes) but without provoking significant hypotension. The increase in normal-zone regional myocardial blood flow after a 1.0 μg/kg bolus of ATL-146e was greater than the increase in normal-zone flow reported after either MRE-0470 or CGS-21680 infusions.10,11 MAP fell only slightly after an ATL-146e bolus, such that it remained >90 mm Hg. This result is in marked contrast to adenosine bolus injections that caused MAP to fall precipitously by ≈50 mm Hg.

Of great importance also is that CF remained elevated for several minutes after a bolus injection of ATL-146e. This plateau in the peak CF response was probably due to the longer time required for ATL-146e metabolism by plasma esterases compared with the very short time required for adenosine degradation (2 seconds) in human blood.12 The extended hyperemic flow response after an ATL-146e bolus injection allowed adequate time for ⁹⁹mTc-sestamibi uptake, as evidenced by a defect magnitude (0.67) similar to that previously produced with adenosine infusion.19 CF completely returned to baseline 20 minutes after bolus injection of ATL-146e.

In Vivo Assessment of Adenosine Receptor Selectivity

When a bolus of ATL-146e was injected in the presence of the selective adenosine A2A receptor antagonist ZM-241385, the CF response was markedly attenuated or abolished. This finding indicates that the vasodilatation observed with ATL-146e was indeed mediated by the adenosine A2A receptor subtype. This finding also suggests that the effects of A2A receptor stimulation should be reversible with aminophylline, a nonselective adenosine receptor antagonist that is approved for human use.

Implications for Vasodilator Stress Imaging Protocols

Side effects such as flushing, dizziness, headache, hypotension, and arteriovenous block are frequently observed after dipyridamole or adenosine infusion.1–4 Elimination of side effects will allow for greater patient acceptance of vasodilator stress perfusion imaging and reduce the need for vigilant monitoring of systemic blood pressure or arteriovenous conduction during A2A receptor agonist administration. Elimination of bronchospasm as a side effect will permit the vasodilator stress imaging of patients with asthma and eliminate the need for dobutamine stress in such individuals.

Advantages of ATL-193 and ATL-146e Over Other A2A Agonists

In addition to the advantages of ATL-193 and ATL-146e over adenosine and dipyridamole, this new class of compounds offers several distinct advantages over other A2A agonists. ATL-193 and ATL-146e are esters that can be enzymatically degraded by esterases but are stable as solids or in saline solution. In contrast, MRE-0470 is a hydrazine that is chemically unstable and may have a limited shelf life. In addition, the ability to administer ATL-146e by bolus injection rather than by an infusion would simplify clinical imaging protocols by eliminating the need for an IV infusion pump. Finally, unlike MRE-0470, the 5′-ribose hydroxyl is protected on both ATL-193 and ATL-146e. Phosphorylation of this site by adenosine kinase would have the undesirable effect of generating unnatural intracellular nucleotides that would have unknown effects on adenine nucleotide metabolism.

Study Limitations

One limitation of the present study is that we could not assess the selectivity of ATL-193 and ATL-146e for the A2A over the A3 receptor by the radioligand binding assay technique owing to the fact that the recombinant canine A3 receptor
subtype is unavailable at the present time. A second limitation is that the study was conducted in an anesthetized animal model. Sodium pentobarbital anesthesia is known to produce mild tachycardia and may blunt reflex changes in heart rate and/or contractility. We do not believe that the use of anesthesia was a major limitation, because comparisons were made between the adenosine A2A receptor agonists and adenosine in the same anesthetized animal model. Nevertheless, it would be interesting to test these new A2A receptor agonists in a conscious animal model.

Summary and Conclusions
ATL-193 and ATL-146e are the most highly potent and selective adenosine A2A receptor agonists known. Whether administered by IV infusion or by bolus injection to anesthetized dogs, these compounds increased CF in a dose-dependent manner without provoking significant systemic hypotension. After a bolus injection of ATL-146e, the increase in CF was sustained for several minutes without an associated fall in MAP. These compounds may have significant advantages over dipyridamole, adenosine, and other existing A2A agonists for clinical vasodilator stress imaging because of their highly favorable pharmacokinetics.

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