Intravertebral Artery Adenosine Fails to Alter Cerebral Blood Flow in the Dog

DAVID J. BOARINI, M.D.,* NEAL F. KASSELL, M.D.,*
JAMES A. SPROWELL, B.S.,† JULIE OLIN, B.S.S.†

SUMMARY The effect of intra-arterial adenosine on cerebral blood flow was studied in 11 anesthetized dogs. In a first group of 6 dogs, adenosine was infused into a vertebral artery for 40 minutes at a dose of 0.3 to 0.5 mg/kg/min. Blood flow was determined before, during and after the adenosine infusion using the radioactive microsphere technique. In a second group of 5 dogs, adenosine (3 ± 1 mcg/kg/min) was infused in a similar manner after potentiating its effect with intravenous dipyridamole, and measurements before and after the intravenous dipyridamole and during and after the adenosine infusion were performed.

Systemic arterial pressure and blood gases were unchanged throughout the experiment in both groups of dogs. Blood flow to the cerebral hemispheres, cerebellum, brain stem, paraspinous and temporalis muscles remained unchanged during the adenosine infusion in both groups of dogs.

Adenosine has been implicated as an active agent in the vasodilatory component of cerebral autoregulation. A controversy exists as to whether intervascular or only interstitial adenosine is of physiologic importance. These findings suggest that intra-arterial adenosine does not play a significant role in the regulation of cerebral blood flow.

ADENOSINE is a potent cerebral vaso-dilator which has been implicated as the active agent in the cerebrovascular response to hypoxia, hypotension and seizures.1-4 Despite evidence concerning the activity of parenchymal adenosine levels, the physiologic importance of intravascular adenosine and its effect of cerebral blood flow remain controversial. The purpose of this study was to determine if adenosine delivered into the cerebral arterial circulation would change the regional cerebral blood flow in the dog.

Materials and Methods

Eleven mongrel dogs weighing approximately 15 kg each were anesthetized with intravenous morphine (1.5 mg/kg), 70% nitrous oxide, and 30% oxygen. Muscular paralysis was obtained with pancuronium, 0.1 mg/kg intravenously. Morphine and pancuronium doses were supplemented as needed. The animals were intubated endotracheally, hyperventilated with a pump respirator (Harvard Apparatus, Inc., 150 Dover Mill Road, Millis, MA), and the PaCO2 adjusted to 40 mmHg by adding carbon dioxide to the inspired gas mixture. In all dogs the right vertebral artery was exposed at the base of the neck, catheterized with polyethylene catheter and ligated.

End tidal CO2 was monitored continuously by a capnometer (Hewlett-Packard, 47210A. capnometer, Hewlett-Packard Corporation, 16399 West Bernardo Drive, San Diego, CA). Temperature was measured by a Swan-Ganz catheter thermister (Edwards Laboratory, 17221 Red Hill Avenue, Santa Anna, CA), and was maintained at approximately 37°C with a warming blanket. Blood flow was measured using radioactive microsphere technique with 15 ± 5 micron spheres labeled with CE141, GD131, SC46, SR85, NB95, and SN115 (3M Company Diagnostic Products, 3M Center, St. Paul, MN). Microspheres were injected into the left ventricle via a catheter inserted through the left femoral artery and positioned manometrically. Blood reference samples for blood flow measurements were obtained from catheters in the right femoral and brachial arteries and blood withdrawn at the rate of 1.03 cc/min, beginning 30 sec. prior to microsphere injection and continuing for 3/4 minutes after injection. At the completion of each experiment, the animal was sacrificed and the brain removed and divided into regions. In addition to studying regional flow to the brain, specimens from the paraspinous and temporalis muscle and cervical spinal cord were obtained. Verification of the placement of each catheter was obtained by direct intravertebral injection of radioactive spheres or indigo carmine. Additionally, the vertebral artery was dissected in its entirety at the conclusion of experiment and anatomical verification of correct position of the catheter obtained. Radioisotope activity in all specimens was determined by differential spectroscopy using a scintillation spectrometer (Packard Gamma Scintillation Spectrometer. Packard Instrument Company; 2200 Warrenfield Road, Downers Grove, Illinois). Systemic arterial pressure (SAP) was monitored from a catheter placed in the left brachial artery. Central venous pressure (CVP), pulmonary artery pressure (PAP), and pulmonary artery wedge pressure (PWP) were measured from a Swan-Ganz catheter inserted into the pulmonary artery via the right femoral vein and descending vena cava. Cardiac output (CO) was measured from the Swan-Ganz catheter using the thermal dilution technique. Left ventricular end diastolic pressure (LVEDP) was measured via the pigtail catheter in the left ventricle. Heart rate (HR) was derived from the electrocardiogram. All physio-
logic parameters, except cardiac output, were recorded on an 8-channel strip chart recorder (Hewlett-Packard, 7758B8 channel strip chart recorder, Hewlett-Packard Corporation, 16399 West Barnardo Drive, San Diego, CA).

At the time of each blood flow measurement, blood samples were obtained for the determination of arterial blood gases, hematocrit, and serum electrolytes. Peripheral vascular resistance (PVR) was calculated by dividing the mean arterial pressure by the cardiac index (CI). Cardiac index was calculated by dividing the cardiac output by the animals body weight in kilograms.

In six of the dogs, adenosine (0.4 gm/100cc) was infused into the vertebral artery for 40 minutes at a dose of 0.3 to 0.5 mg/kg/min. In five additional dogs the effect of the adenosine infusion was potentiated by giving 0.5 mg/kg of intravenous dipyridamole every 30 minutes beginning approximately 10 minutes before the start of the adenosine infusion. The infusion rate of intra-arterial adenosine in the second group was chosen to limit the reduction of systemic arterial pressure to approximately 10% of the control value. In this second group of dogs the adenosine infusion was 3 ± 1 mcg/kg/min. In all animals control measurements of cardiovascular and blood flow parameters were performed prior to the beginning of the infusion. Repeat measurements were made in duplicate after 40 minutes of adenosine infusion. A second set of control measurements were then made after a 40 minute recovery following the termination of the adenosine infusion. In those dogs receiving dipyridamole, a single set of measurements was also made approximately 10 minutes after the injection of the first dose of dipyridamole. In all cases, the control measurements were averaged and then compared to the infusion measurements. Statistical analysis where needed was performed with the Pooled T-test.

**Results**

In all dogs, anatomical inspection postmortem revealed that the vertebral artery had in fact been correctly cannulated. This was further verified by the direct vertebral artery injection of indigo carmine and microspheres through the infusion catheter. Both the dye and the radioactive spheres were heavily concentrated in the brain stem, cerebellum and occipital lobes. Lesser amounts of radiation reached the temporalis or paraspinous muscles, as well as the other regions of the cerebrum.

The results of the experiments are discussed in two sections. The first is the dogs who received infusion of adenosine alone. The second is those dogs receiving infusion of adenosine potentiated by intravenous dipyridamole.

Table 1 demonstrates that there were no significant changes in blood gases from control to infusion measurements in the first set of dogs. Temperature, hematocrit and serum electrolytes also remained stable, with the exception of a slight increase in potassium over the course of the experiment.

<table>
<thead>
<tr>
<th>TABLE 1 Intravertebral Adenosine: Blood Gases, Biochemistry, Temperature</th>
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<tr>
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<tr>
<td>PaCO₂ (mmHg)</td>
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<td>PaO₂ (mmHg)</td>
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<tr>
<td>pH</td>
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<td>Temperature (°C)</td>
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* p ≤ .05.

There was no important change in any cardiovascular parameter in this group of dogs. Heart rate, mean arterial pressure, and cardiac work all stayed extremely stable. Cardiac index did show a very slight, non-significant increase during the infusion. The only statistically significant change in any cardiovascular parameter was a slight drop in left ventricular end diastolic pressure during adenosine infusion. (table 2)

There was no change in measured blood flow to any region of the brain, spinal cord, or the temporalis and paraspinous muscles. (table 3)

In the second group of dogs, data are presented for control flow, following the first dose of intravenous dipyridamole, and during the adenosine infusion. Table 4 demonstrates that there was no change in the blood gases between any of these measurements. As in group one, there was no change in temperature, hematocrit or electrolytes except for a slight rise in serum potassium over the course of the experiment.

Inspection of the cardiovascular parameters shows that the heart rate and cardiac index were quite stable, while there was a drop of approximately 11% in the mean arterial pressure. This did not reach statistical significance. The peripheral vascular resistance also

<table>
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<th>TABLE 2 Intravertebral Adenosine: Cardiovascular Parameters</th>
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<tr>
<td>Heart rate (beats/min)</td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
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<tr>
<td>Cardiac index (L/min/kg)</td>
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<tr>
<td>Cardiac work (MAP - LVEDP) x SV x 1.33 x 10^-3)</td>
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<tr>
<td>Stroke volume (cc)</td>
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<tr>
<td>Peripheral vascular resistance (MAP - CVP/C.I.)</td>
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<td>Central venous pressure (mmHg)</td>
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<td>Pulmonary artery pressure (mmHg)</td>
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<td>Pulmonary artery wedge pressure (mmHg)</td>
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<td>Left ventricular end diastolic pressure (mmHg)</td>
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* p ≤ .05
dropped 6%, again not reaching statistical significance. The central venous and pulmonary pressures showed no statistical change over the course of the experiment (table 5).

Regional cerebral and muscle blood flows showed no statistically significant change in any region during the adenosine-dipyridamole infusion (table 6).

Discussion

In recent years, attention has been focused upon the adenine nucleosides as possible metabolic regulators of cerebral blood flow. In particular, adenosine has been proposed as a major factor in cerebral autoregulation. It has been demonstrated that the topical application of adenosine causes dilatation of cerebral vessels. Furthermore, Winn, et al, have shown that adenosine concentrations in the brain rapidly increase in response to cerebral hypoxia, hypotension, and artificially induced seizures. It is known that glial foot processes contain the enzymatic machinery necessary for the regulation of extracellular adenosine concentrations. Furthermore, cerebral vascular smooth muscle has been shown to have receptors and mechanisms for the uptake of adenosine. Despite this evidence that adenosine is an active vasodilator of cerebral vessels, it is known that there is a limited passage of adenosine across the blood-brain barrier. Beck et al have demonstrated in an in vitro blood-brain barrier model that adenosine is taken up by cerebral endothelium, but does not cross an intact blood-brain barrier. Previous in vivo studies have produced conflicting results. Forrester, et al, demonstrated an increase in cerebral blood flow and cerebral metabolic rate of oxygen with the use of intra-arterial ATP. These effects were very short lived even during a continuous infusion. The effect of adenosine in that report was considerably less marked than that of ATP. Marcus et al demonstrated that the intracarotid infusion of adenosine produced no change in cerebral blood flow or CMRO\textsubscript{2} in either a dog or cat model. There was, however, an increase in cerebral blood flow to both intra-arterial adenosine and intravenous dipyridamole in a rabbit model. Their hypothesis was that intravascular adenosine does produce vasodilatation and that this differential effect between species might be due to an extra-cerebral steal. This increased blood flow to the skeletal muscles supplied by the carotid artery in the dog and cat may effectively negate any intracerebral vasodilatation.

The present study was performed in order to further test this hypothesis concerning intra-arterial adenosine's effect in the dog. The vertebral artery, rather than the carotid system, was chosen in order to limit extracerebral factors. The ligation of one vertebral artery in the dog produces no significant change in baseline blood flow. We were able to demonstrate both...
with the tracer dye and with the intra-arterial injection of microspheres that the adenosine infusion was delivered to the structures in question. Despite the fact that adenosine was clearly delivered to the posterior fossa structures, there was no change in regional cerebral blood flow.

The second phase of the study was done by potentiating the intra-arterial adenosine with dipyridamole. Dipyridamole is a known potentiator of adenosine working mainly by preventing transport and degradation of adenosine by red blood cells. It has been previously shown that intravenous dipyridamole in the doses, used in this study, has little effect on regional cerebral blood flow. Even with the additional potentiation of intravascular adenosine with dipyridamole, no change in regional cerebral blood flow could be demonstrated.

There is a large body of experimental evidence now available implicating adenosine as an active agent in cerebral vascular autoregulatory vasodilation. The present study supports the contention that while parenchymal release of adenosine may be of physiologic importance, it is unlikely that intravascular adenosine plays a significant role in physiologic control of cerebral blood flow. This lack of cerebrovascular effect by intravascular adenosine also suggests that it will not have any primary adverse effects on cerebral autoregulation if given pharmacologically as a hypotensive agent.18,19

References
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