Effects of Adenosine on Ocular Blood Flow

Maria Portellos, Charles E. Riva, Stephen D. Cranstoun, Benno L. Petrig, and Alexander J. Brucker

Purpose. To determine the effect of intravascular adenosine on blood flow in the ocular fundus and to examine indirectly whether the blood–brain barrier to adenosine, which exists in the cerebrovasculature of the cat, is present in the eye of this animal.

Methods. The noninvasive techniques of laser Doppler flowmetry and velocimetry along with fundus photography were used to measure the change in optic nerve head and choroidal and retinal blood flow during intravenous infusions of 0.18 and 0.6 mg/kg per minute of adenosine.

Results. Infusions of adenosine induced significant increases in choroidal blood flow (60% with 0.6 mg/kg per minute) but not in optic nerve head or retinal blood flows.

Conclusions. The lack of effect of intravenously infused adenosine on the optic nerve and retinal circulations is most likely caused by the tight junctions in the vessels of these vascular beds, which prevent adenosine from reaching its receptors. Perivascular adenosine in the choroid most likely accounts for the increase in blood flow in this tissue. Invest Ophthalmol Vis Sci. 1995;36:1904–1909.

Adenosine, a breakdown product of cellular adenosine triphosphate, is known to be a potent vasodilator. It has been demonstrated that this nucleoside is involved in the metabolic regulation of blood flow to the heart, skeletal muscles, and brain. A recent study by Gidday et al suggested that adenosine also may play a mediating role in the autoregulation of retinal blood flow during hypoxia and hypotension. Other studies have shown that intravitreal injections of adenosine cause an increase in retinal vessel diameter, as well as in retinal and choroidal blood flows.

Intravascular administration is less invasive than intravitreal injection and, therefore, would be more suitable in an acute clinical situation. Thus, the purpose of this study was to determine the effect of intravenous adenosine on blood flow in various tissues of the ocular fundus and to examine whether a blood–retinal barrier exists that is similar to the known blood–brain barrier of this nucleoside.


MATERIALS AND METHODS

Animal Preparation

Twenty seven cats weighing, each 2.1 to 3.5 kg, were prepared as described in detail elsewhere. All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and to the Presbyterian Medical Center of Philadelphia Guidelines on Animal Research for the Use of Animals in Ophthalmic and Vision Research. Each cat was premedicated with atropine (0.04 mg/kg, subcutaneously) and anesthetized with intramuscular ketamine hydrochloride (22 mg/kg) and acepromazine maleate (2 mg/kg). Catheters were placed in a femoral artery and vein, and a tracheostomy was performed. A loading dose of pancuronium bromide (0.2 mg/kg) was given intravenously, and the animal was ventilated with 21% O2 and 79% N2 using a variable volume respirator. Mean arterial blood pressure (MABP), tidal CO2, and heart rate were monitored continuously. Arterial pH, Pco2, and Po2 were monitored intermittently using a blood gas analyzer, and adjustments of the inspired gas mixture, tidal volume, and respiration rate were made to keep pH ~ 7.4, Pco2 ~ 31 mm Hg, Po2 ≥ 90 mm Hg, and mean blood pressure between 85 and 110 mm Hg. Rectal temperature was maintained at ~38°C. Enflurane
(1.7% to 2.5%) was administered, and pancuronium bromide (0.10 mg/kg per hour) was infused continuously. The pupils were dilated with 1% tropicamide and 10% phenylephrine, and the cat was placed prone on a table with the head secured in a stereotactic headholder. A ring was sutured to the eye with three stitches at the limbus and held in a fixed position to prevent eye motion. A zero diopter contact lens was placed on the cornea protected with Healon.

**Relative Choroidal Blood Flow**

In eight cats, a laser Doppler fundus camera that allows delivery of a laser beam to any site of the posterior pole was placed in front of the eye. An area of the fundus (30°) was illuminated by a tungsten source for observation. As described in detail elsewhere, measurements were made under conditions that would ensure that the flow measured represented choroidal blood flow in the choriocapillaris. These conditions included that the probing laser diode beam (λ = 789 or 812 nm) was focused at an intervascular site in the tapetal region of the fundus; the Doppler shift power spectrum of the light scattered by the red blood cells had the shape and frequency range typical of a microvascular bed; and the recorded flow did not decrease by more than 5% when the cat was given 100% O₂ to breathe for 4 minutes. If a greater decrease in flow was observed, the laser beam was moved to another intervascular site and the 100% O₂ test was performed again. Laser light scattered by the red blood cells was collected in the image plane of the fundus camera by an optical fiber (diameter at the fundus, ~150 μm) and detected by an avalanche photodiode. The collected light was guided by the fiber to a photodetector, and the resultant photocurrent was fed into the electronic system of a TSI Laser Flo (BPM403A; Vasamedics, St. Paul, MN) or a Periflux PF3 (Perimed, Stockholm, Sweden) laser Doppler flowmeter. When the laser beam is focused on a tissue, these instruments provide a measurement of relative flux, F, of red blood cells within the illuminated volume of tissue. From recordings under normal conditions and after the administration of adenosine, we calculated the corresponding changes in blood flow.

**Relative Optic Nerve Head Blood Flow**

In 21 cats, the laser beam was focused at the center of the disk at a site away from visible blood vessels when the fundus was observed through the fundus camera. In 7 and 17 cats, respectively, 0.18 or 0.6 mg/kg per minute adenosine were infused. Laser Doppler flowmetry was applied as described in detail elsewhere to determine the change in optic nerve blood flow.

**Relative Retinal Blood Flow**

Laser Doppler velocimetry was applied in 12 cats to determine relative centerline velocity, \( V_{\text{max}} \), of red blood cells in retinal vessels, as described in detail. The laser beam was focused on a retinal arteriole close to the disk. All the light scattered by red blood cells into the input pupil of the fundus camera was collected by a single optical fiber to detect the maximum amount of scattered light, thereby maximizing the signal-to-noise ratio of the Doppler measurements. Consequently, with this detection scheme, only relative \( V_{\text{max}} \) was determined. \( V_{\text{max}} \) was obtained using a computer-assisted method. In the first four cats, a loss of the high-frequency components of the Doppler spectrum was observed during the infusion of 0.6 mg/kg per minute adenosine, along with a 20% to 40% drop in blood velocity. This loss was attributed to the observed motion of the eye fundus resulting from the drop in MABP, causing the beam to move away from the center of the vessel by approximately \( 1/T \) to 1-vessel diameter. Therefore, in the next eight cats, the laser beam was repositioned at the center of the vessel approximately every 30 seconds during each infusion.

In five cats, red-free fundus photographs were taken with a Topcon TRC-RE fundus camera at 1-minute intervals during an infusion rate of 0.6 mg/kg per minute. Vessel diameter was measured from photographic negatives projected on the screen of a Macbeth prooflite, model V-135 projector using an electronic caliper. Change in retinal blood flow was then calculated using the relationship \( Q = \frac{k \cdot D^2 \cdot V_{\text{max}}}{1/T} \), where D is the diameter of the vessel, \( V_{\text{max}} \) is the centerline velocity, and k is a constant of proportionality assumed to be unaffected by the infusion of adenosine.

**Adenosine Infusions**

Adenosine (Medco Research, Los Angeles, CA) was supplied in a concentration of 3 mg/ml. It was diluted with lactated Ringers solution and infused through a femoral vein using a Razal syringe pump. Infusion rates from 0.06 to 1.2 mg/kg per minute were tested initially. Rates < 0.4 mg/kg per minute caused no change in MABP, whereas rates > 0.6 mg/kg per minute consistently decreased systemic blood pressure by 10% to 30%. Therefore, experiments were performed at infusion rates of either 0.18 mg/kg per minute or 0.6 mg/kg per minute. Durations of infusion ranged from 5 to 8 minutes. Controls were run using lactated Ringers solution.

**Statistical Analysis**

Percentage of changes in the measured quantities were expressed as mean ± 95% confidence limits. Paired Student’s t-test and nonparametric statistics
RESULTS

Figure 1 displays representative time courses of choroidal blood flow and MABP during an adenosine infusion of 0.6 mg/kg per minute. Adenosine produced a gradual drop in MABP that occurred within 2 minutes of onset to reach a relatively constant level. The group average decrease in MABP, measured between 2 and 4 minutes after the start of the infusion, amounted to 12.4% ± 4% (n = 24) when adenosine was infused at a rate of 0.6 mg/kg per minute. At a rate of 0.18 mg/kg per minute, the change in MABP was not significant. Heart rate remained unchanged at both doses.

Figure 2 shows changes in choroidal blood flow at infusion rates of 0.18 and 0.6 mg/kg per minute. These changes are expressed as the percent change between choroidal blood flow at baseline, i.e., average flow over 1 minute before infusion of adenosine, and during the 6th minute of the infusion, i.e., 2 minutes after the average peak effect on choroidal blood flow, which was found to be at 4 minutes. There were significant increases in choroidal blood flow at both infusion rates. Choroidal blood flow increased by 26% ± 22% (n = 6) at infusion rates of 0.18 mg/kg per minute and by 64% ± 16% (n = 6) at infusion rates of 0.6 mg/kg per minute. After infusions of 0.6 mg/kg per minute, choroidal blood flow in all six cats remained elevated for more than 5 minutes. In 3 of 6 cats, more than 15 minutes elapsed before normalization of choroidal blood flow. At infusion rates of 0.18 mg/kg per minute, choroidal blood flow returned to baseline within 1 minute in 4 of 6 cats in which there had been an initial increase in choroidal blood flow. In one cat, choroidal blood flow did not return to baseline for more than 15 minutes. Adenosine at 0.6 mg/kg per minute had been infused 3 hours earlier.

Optic nerve blood flow increased by 27% ± 25% (n = 7, P < 0.05 with Student’s t-test; P > 0.05 with nonparametric test) and 16.5% ± 18% (n = 17, P > 0.05 with both parametric and nonparametric tests) at rates of 0.18 and 0.6 mg/kg per minute, respectively, as depicted in Figure 3. In all cats, optic nerve blood flow returned to baseline 7 minutes after stopping the infusion at the dose of 0.18 mg/kg per minute and within 10 minutes of the infusion of 0.6 mg/kg per minute.

DISCUSSION

This study demonstrates that intravenous infusions of adenosine induce significant increases in choroidal blood flow and marginal increases in optic nerve head blood flow. In the choroid, blood flow increased by an average of 64% with an infusion dose of 0.6 mg/kg despite a significant decrease in perfusion pressure. Because choroidal capillary walls have fenestrations that are permeable to substances with molecular weights as large as 160,000,13 adenosine, which has a molecular weight of 267.25, must leak into the extravascular compartments and exert its vasodilatory effect.
Adenosine and Ocular Blood Flow

FIGURE 2. Average percentage change in choroidal blood flow between the value at baseline and that between 5th and 6th minute of each adenosine infusion. With infusion rates of 0.18 and 0.6 mg/kg per minute, the average changes in choroidal blood flows were 26% ± 22% (n = 6) (left) and 64 ± 16% (n = 6) (right), respectively.

on the choroidal vasculature. After infusions of 0.6 mg/kg per minute, choroidal blood flow remained elevated for a prolonged period of time, suggesting the presence of adenosine in the extravascular fluid. Adenosine has a half-life of <10 seconds in the plasma.14 However, based on observations in the brain, adenosine may be degraded less rapidly in the cerebrospinal fluid, which does not contain enzymes that metabolize this nucleoside or its degradative products, inosine and hypoxanthine.15 A similar effect could occur in the extravascular choroidal fluid and be responsible for the prolonged increase in choroidal blood flow.

In examining a potential dose response ratio of choroidal blood flow to infused amount of adenosine, we observed that this flow increased by an average factor of 2.3, with a range from 1 to 20 ([64 - 16]/[26 + 22]) and ([64 + 16]/[26 - 22]) when the concentration of adenosine was increased by a factor of 3.3. Because of the great variability of the increase with the smaller dose of adenosine, the difference between the increase in choroidal blood flow and adenosine concentration is not significant.

The increase in optic nerve blood flow at the low dose of adenosine was marginally significant with the Student's t-test but was not significant with nonpara-

FIGURE 3. Average percentage change in optic nerve head blood flow between the value at baseline and that between the 5th and 6th minute of adenosine infusions. With infusions rates of 0.18 and 0.6 mg/kg per minute, the average blood flow changes were 27% ± 25% (n = 7) (left) and 16.5% ± 18% (n = 17) (right), respectively.
metric tests. It was not significant at the higher dose of adenosine with both parametric and nonparametric tests and also was not dose dependent. There were two outliers with the high dose. These could have resulted from undetected motion of the disk relative to the site of the laser beam and aperture of the light-scattering detecting fiber. For example, the drop in blood pressure when the larger dose of adenosine was infused could have resulted in displacement of the disk (estimated to be approximately 50 µm from the motion of the laser beam when this beam was focussed on a large arteriole) and a subsequent apparent increase in blood flow. This is so because the blood flow measured at the center of the disk is generally lower than in the periphery.

Previous studies have shown that the optic nerve capillaries are endowed with the property of a blood–brain barrier, as evidenced by electron microscopic findings that have shown that these capillaries do not have fenestrae and that their endothelial cells have tight junctions. Furthermore, Grayson and Laties showed that there was no direct leakage of fluorescein out of these capillaries. This blood–brain barrier also may have prevented adenosine from reaching the smooth muscle. On the other hand, the studies of these authors and that of Ben–Sira and Riva suggest that some adenosine, like fluorescein, could reach the nerve by passive diffusion from the choroidal extravascular space. The slowness of such a process, compared to the short lifetime of adenosine in the plasma and the lack of a dose response of the flow to the drug, makes this interpretation unlikely.

Blood flow in the retina, as in the optic nerve, did not change significantly after the infusion of adenosine because no change in vessel diameter nor in $V_{max}$ could be demonstrated. The limited sensitivity of our retinal blood-flow measurements, however, does not allow us to assert that there was no change below 15%. Our results differ clearly from those obtained when adenosine was injected intravitreally and large increases in retinal vessel diameter and blood flow on the order of at least 40% were demonstrated. In the heart, the mechanism for vasodilation is primarily through $A_1$ and $A_2$ receptors found in endothelial and vascular smooth muscle cells. Activation of these receptors increases guanylate cyclase activity, and the subsequent increased cyclic guanosine 3,5'-monophosphate levels relax vascular smooth muscle. Assuming that a similar mechanism exists in the retinal circulation, $A_1$ and $A_2$ receptors in retinal vessels, which are probably involved in vasodilation, may not be exposed to intravascular adenosine but are activated by perivascular adenosine, as happens when adenosine is injected into the vitreous. This is supported by studies on the action of adenosine in the cerebral vasculature, demonstrating that adenosine dilates cerebral vessels when applied topically but not when given intravascularly. Thus, it seems that the vasculature of the retina, like that of the brain, has a tight barrier that prevents adenosine-induced vasodilation and alteration in blood flow.

In conclusion, intravenously infused adenosine has different actions on different vascular beds of the cat fundus. Although it clearly increases choroidal blood flow, it probably has no effect on optic nerve blood flow and no demonstrable effect on retinal blood flow, making this nucleoside unlikely to be of any therapeutic use in acute retinal ischemic syndromes of the eye, such as central retinal artery occlusion.
Adenosine and Ocular Blood Flow

Key Words
adenosine, blood—retinal barrier, choroidal blood flow, laser Doppler flowmetry, optic nerve head blood flow, retinal blood flow

References