EXTENDED REPORT

Adenosine A_{2A} receptor mediated protective effect of 2-(6-cyano-1-hexyn-1-yl)adenosine on retinal ischaemia/reperfusion damage in rats

T Konno, A Sato, T Uchibori, A Nagai, K Kogi, N Nakahata

Br J Ophthalmol 2006;90:900–905. doi: 10.1136/bjo.2006.091496

See end of article for authors' affiliations

Correspondence to:
Takashi Konno, Drug
Research Section II,
Fukushima Research
Laboratories, Toa Eiyo Ltd,
1 Tanaka, Yuno, Iizaka,
Fukushima 960-0280,
Japan; konno.takashi@
toaeyo.co.jp

Accepted for publication
4 March 2006

Aims: To determine the effect of 2-(6-cyano-1-hexyn-1-yl)adenosine (2-CN-Ado), an adenosine A_{2A} receptor agonist, on retinal ischaemia/reperfusion damage in rats.

Methods: Retinal ischaemia/reperfusion damage was induced by elevating the intraocular pressure of one eye to 130 mm Hg for 60 minutes and returning it to normal. 7 days later, retinal ischaemia/reperfusion damage was histologically quantified by measuring the thickness of retinal layers. Intraocular pressure was measured by pressure transducer.

Results: Retinal ischaemia/reperfusion caused cell loss in the ganglion cell layer and thinning of the inner plexiform and nuclear layer. Both ocular topical and intravenous administration of 2-CN-Ado caused a reduction of retinal ischaemia/reperfusion damage. A selective A_{2A} receptor antagonist, 1,3,7-trimethyl-8-(3-chlorostyryl) xanthine (CSC), but not a selective A₁ receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), or a selective A_{2B} receptor antagonist, alloxazine, reduced the protective effect of 2-CN-Ado. While ocular topical administration of 2-CN-Ado caused a sustained reduction of intraocular pressure, intravenous administration of 2-CN-Ado showed a transient ocular hypotensive effect.

Conclusions: These results suggest that 2-CN-Ado attenuates retinal ischaemia/reperfusion damage, and at least some of this protective effect of 2-CN-Ado might be mediated via activation of the adenosine A_{2A} receptor.

Retinal ischaemia/reperfusion caused the degeneration of retinal ganglion cells or inner retinal neurons.^{1,2} It is believed that ischaemia induced retinal injury is a possible cause of retinal diseases such as glaucoma.^{3–5}

It is known that adenosine has an important role in eye function.^{6–8} For instance, adenosine is thought to participate in retinal ischaemia,⁹ since retinal ischaemia/reperfusion results in elevation of adenosine and its metabolites in the retina-choroid in rats.^{10–11} In addition, it has been shown that adenosine receptor agonists or antagonists influenced the retinal function and structure after ischaemia/reperfusion in rats.^{9–12–13} Moreover, adenosine has been reported to have a role in neuroprotection through adenosine A₁ or A_{2A} receptors in the retina.^{12–16} Therefore, it is possible that the detailed analyses of adenosine action in the retina may develop adenosine based therapies in retinal ischaemic disorders such as glaucoma.

Adenosine derivatives with substituents at the 2-position are assumed to be relatively selective to adenosine A₂ receptors.¹⁷ Previously, we reported that some 2-alkynyladenosine derivatives had potent and relatively selective binding activity to adenosine A_{2A} receptor,¹⁸ and some 2-alkynyladenosine derivatives decreased intraocular pressure via adenosine A_{2A} receptor.¹⁸ Moreover, these compounds decreased intraocular pressure in an ocular hypertensive model induced by water loading or α -chymotrypsin in rabbits, indicating that 2-alkynyladenosine derivatives might be useful drugs for the treatment of eye diseases such as glaucoma.¹⁹

However, it is unknown whether 2-alkynyladenosine derivatives protect retinal degeneration induced by ischaemia/reperfusion. In the present study, we examined the effect of 2-(6-cyano-1-hexyn-1-yl)adenosine (2-CN-Ado) on retinal ischaemia/reperfusion damage and intraocular pressure in rats.

MATERIALS AND METHODS

Animals

All animal experiments were reviewed and approved by the experimental animal committee of the drug research department, Toa Eiyo (Fukushima and Oomiya, Japan). Male Sprague-Dawley rats weighing 175–400 g were used in the study (Japan SLC Co, Ltd, Hamamatsu, Japan). Rats were housed in stainless steel cages under a 12 hour light/dark cycle in temperature controlled rooms, and were allowed free access to food and tap water for a minimum of 1 week before the experiments.

Materials

2-(6-Cyano-1-hexyn-1-yl)adenosine (2-CN-Ado), a 2-alkynyladenosine derivative, was synthesised by Yamasa Corporation (Chiba, Japan) and Toa Eiyo (Tokyo, Japan), and its chemical structure is shown in figure 1.¹⁸ An adenosine A_{2A} receptor agonist, 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS-21680), an adenosine A₁ receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an adenosine A_{2A} receptor antagonist, 1,3,7-trimethyl-8-(3-chlorostyryl) xanthine (CSC), an adenosine A_{2B} receptor antagonist, alloxazine, and a N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, were purchased from Sigma (St Louis, MO, USA). Intraocular irrigating solution (Opeguard-MA) and atropine 1% ophthalmic solution were purchased from Senju Pharmaceutical (Osaka, Japan), and gentamicin ophthalmic

Abbreviations: CGS-21680, 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine; 2-CN-Ado, 2-(6-cyano-1-hexyn-1-yl)adenosine; CSC, 1,3,7-trimethyl-8-(3-chlorostyryl) xanthine; DMSO, dimethyl sulfoxide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; ip, intraperitoneal; IPL, inner plexiform layer; IRL, inner retinal layer; iv, intravenous; NMDA, N-methyl-D-aspartate; ONH, optic nerve head

solution (Gentacin ophthalmic solution) was purchased from Schering-Plough (Osaka, Japan). 2-CN-Ado was dissolved in 2% boric acid buffer solution (2% boric acid and 2% sodium borate, pH 7–7.5) containing 0.5% polysorbate 80, or dissolved in saline containing 1% dimethyl sulfoxide (DMSO). MK-801 was dissolved in 0.9% saline solution. DPCPX, CSC, or alloxazine was dissolved in a vehicle composed of >50% DMSO and <50% ethanol by volume of 200 µl/kg (intraperitoneal, ip). As a control, the same volume of the vehicle was used as the corresponding drug.

Pressure induced retinal ischaemia in rats

Pressure induced retinal ischaemia was assessed in rats as described previously.²⁰ Briefly, rats were anaesthetised with sodium pentobarbital (50 mg/kg, ip). Anaesthesia was maintained throughout the experiment by supplemental doses of the barbiturate if necessary. The anterior chamber of the right eye was cannulated with a 27 gauge needle attached to an infusion line from a bottle of Opeguard-MA. Retinal ischaemia was induced by elevating the intraocular pressure to 130 mm Hg for 60 minutes (by lifting the bottle of Opeguard-MA). After 60 minutes of ischaemia, the needle was withdrawn from the anterior chamber, and the retina was allowed to reperfuse. The left eye was kept intact. The rectal temperature was kept at approximately 37°C using a heat blanket and heat pad throughout the experiment and until the animal recovered from the anaesthesia. One drop of atropine 1% ophthalmic solution and Gentacin ophthalmic solution were applied topically to the experimental eye before and after cannulation of the anterior chamber.

Systemic administrations of 2-CN-Ado, CGS-21680, and MK-801

2-CN-Ado (100 µg/kg, intravenous, iv) or MK-801 (1 mg/kg, iv) was injected 5 minutes before retinal ischaemia. In addition, CGS-21680 (3 mg/kg, ip) was injected 30 minutes before retinal ischaemia. To assess the involvement of adenosine receptors on the effects, we used DPCPX (3 mg/kg, ip), CSC (3 mg/kg, ip), or alloxazine (10 mg/kg, ip). Each adenosine receptor antagonist was injected 30 minutes before retinal ischaemia.

Topical administrations of 2-CN-Ado in rats

2-CN-Ado (three times daily, around 7 am, 1 pm, and 7 pm) was instilled 1 day before ischaemia, and instillation was continued until 1 day after reperfusion. 2-CN-Ado (0.3% and 1%, 30 µl) was instilled into one eye, and the vehicle was instilled into the other eye. As a control, the vehicle was

instilled into both eyes of rats. Moreover, to examine the involvement of adenosine A_{2A} receptor, CSC (0.3 mg/kg, ip) was injected when 2-CN-Ado (1%) was instilled.

Histological procedures

The right (experimental) and left (control) globes were enucleated 7 days after the ischaemia/reperfusion insult. They were fixed in 1% glutaraldehyde and 4% formalin in phosphate buffer overnight, then in 10% formalin neutral buffer solution overnight at 4°C before sectioning. The globes were sectioned in the vertical meridian, and processed to paraffin embedded sections. Sections through the optic disc of the eye were cut 3 µm thick and stained with haematoxylin and eosin. The histological assessment used a light microscope (BX50, Olympus Optical Co Ltd, Tokyo, Japan). The retinal layers in each section for approximately 1–2 mm either side of the centre of the optic nerve head (ONH) were recorded onto disk as image data using a 3 CCD camera (XC-003, Sony Co Ltd, Tokyo, Japan) connected to light microscope at 20× magnification. The images were analysed using an imaging processor (Image Processor for Analytical Pathology-Windows, Sumika Technoservice, Osaka, Japan). The thickness of two different retinal layers was measured to evaluate the retinal ischaemia/reperfusion damage; (i) the inner retinal layer (IRL), which extends from the inner limiting membrane to the boundary of the outer plexiform layer and the outer nuclear layer, and (ii) the inner plexiform layer (IPL). The values used for retinal thickness were averaged from three measurements in each eye. The retinal layers (IRL and IPL) were expressed as a percentage of the values of the contralateral non-ischaemic eye.

Measurement of intraocular pressure in rats

Rats were anaesthetised with sodium pentobarbital (50 mg/kg, ip). Anaesthesia was maintained throughout the experiment by supplemental doses of the barbiturate if necessary. The left femoral vein was cannulated for intravenous drug administration. A hypodermic needle (30 gauge) (M-S Surgical Mfg, Tokyo, Japan) was inserted into the anterior chamber through the cornea and connected to a pressure transducer (1829, NEC san-ei Instruments, Tokyo, Japan) to monitor the intraocular pressure. One drop of atropine 1% ophthalmic solution was applied topically to the experimental eye before cannulation of the anterior chamber. After the intraocular pressure became stable, drugs were administered via ocular topical or intravenous routes. The intraocular pressure was measured immediately before administration, and 30, 60, 90, and 120 minutes after administration. Vehicle or 2-CN-Ado (100 µg/kg) was injected into the left femoral vein. In the instillation study, vehicle or 2-CN-Ado (1%) was instilled as a volume of 30 µl.

Data analysis

Statistical analysis was performed using the SPSS statistical package (SPSS Japan, Tokyo, Japan). Data were analysed using a paired or unpaired Student's *t* test, or a one way analysis of variance followed by Dunnett's test. For all evaluations, *p* values less than 0.05 were considered statistically significant.

RESULTS

Effects of 2-CN-Ado, CGS-21680, and MK-801 on retinal ischaemia/reperfusion damage in rats

Rats were subjected to pressure induced retinal ischaemia for 60 minutes under anaesthesia, and then the reperfusion of the blood vessels of the eye. On the seventh day after ischaemia/reperfusion, the loss of cells in the ganglion cell layer and the thinning of inner plexiform or inner nuclear layer were observed (fig 2B), compared with normal eye

Figure 1 Chemical structure of 2-(6-cyano-1-hexyn-1-yl)adenosine (2-CN-Ado).

Figure 2 Ischaemia/reperfusion induced retinal changes in rats. Photograph taken on the seventh day after ischaemia/reperfusion, as described in Materials and methods. (A) Normal eye (the contralateral eye). (B) Ischaemia/reperfusion eye in a vehicle treated animal. (C) Ischaemia/reperfusion eye in a 2-CN-Ado (100 μ g/kg, iv) treated animal. (D) Ischaemia/reperfusion eye in a CGS-21680 (3 mg/kg, ip) treated animal. (E) Ischaemia/reperfusion eye in MK-801 (1 mg/kg, iv) treated animal. 2-CN-Ado or MK-801 was injected 5 minutes before retinal ischaemia. CGS-21680 was injected 30 minutes before retinal ischaemia. IRL; inner retinal layer, which extends from the inner limiting membrane to the boundary of the outer plexiform layer and the outer nuclear layer. IPL; inner plexiform layer. Scale bar = 25 μ m.

(fig 2A). In contrast, the administration of 2-CN-Ado (100 μ g/kg, iv) 5 minutes before ischaemia resulted in partial attenuation of the pathological changes (fig 2C). In addition, the administration of CGS-21680, an adenosine A_{2A} receptor agonist, and MK-801, an NMDA receptor antagonist, also reduced retinal ischaemia/reperfusion damage (figs 2D and 2E).

The thickness of IPL or IRL was determined from the photographs, and the quantitative results are shown in figure 3 to evaluate the potency of the drug. IPL and IRL in the ischaemia/reperfusion eye were thinner than those in the normal eye (the contralateral eye) on the seventh day after ischaemia/reperfusion, consistent with the previous reports by ligation of the optic nerve^{13,21} and by raising the intraocular pressure.²⁰ In addition, these degenerative changes were more marked in the IPL than in the IRL, indicating that the IPL might be more sensitive to ischaemia/reperfusion insult than the IRL. 2-CN-Ado significantly reduced retinal ischaemia/reperfusion damage when compared with the control (fig 3). CGS-21680 and MK-801 also significantly reduced retinal ischaemia/reperfusion damage (fig 3).

Figure 3 Effects of 2-CN-Ado, CGS-21680, and MK-801 on retinal ischaemia/reperfusion damage in rats. IPL or IRL was expressed as the thickness ratio of retinal layers of the ischaemia/reperfusion eye to that of the contralateral (control) eye (%). (Top) IPL, (bottom) IRL. Vehicle (iv, n=12), 2-CN-Ado (100 μ g/kg, iv, n=13) or MK-801 (1 mg/kg, iv, n=9) was injected 5 minutes before ischaemia. CGS-21680 (3 mg/kg, ip, n=11) was injected 30 minutes before ischaemia. Data are expressed as the means (SE). * $p < 0.05$, statistically significant compared with the vehicle (Dunnett's test).

Figure 4 Effects of adenosine receptor antagonists on CGS-21680 induced protection of retinal ischaemia/reperfusion damage in rats. IPL or IRL was expressed as the thickness ratio of retinal layers of the ischaemia/reperfusion eye to that of the contralateral (control) eye (%). (Top) IPL, (bottom) IRL. Vehicle (ip, n=20), CGS-21680 (3 mg/kg, ip, n=10) was injected 30 minutes before ischaemia. DPCPX (3 mg/kg, ip, n=10), CSC (3 mg/kg, ip, n=10), or alloxazine (10 mg/kg, ip, n=8) was injected 30 minutes before ischaemia. Data are expressed as the means (SE). * $p < 0.05$, statistically significant compared with the drug alone (Dunnett's test).

Effects of adenosine receptor antagonists on 2-CN-Ado induced protection of retinal ischaemia/reperfusion damage in rats

Since 2-CN-Ado causes the attenuation of retinal ischaemia/reperfusion damage as shown in figures 2 and 3, we examined the subtype of adenosine receptors involved in the protective effect. The protective effect of CGS-21680 was significantly inhibited by CSC, but not by DPCPX or alloxazine (fig 4). Similarly, the protective effect of 2-CN-Ado was significantly inhibited by CSC, but not by alloxazine. In contrast, the protective effect of 2-CN-Ado on the change in the IPL was somewhat attenuated by DPCPX without statistical significance (fig 5).

Effect of topical administration of 2-CN-Ado on retinal ischaemia/reperfusion damage in rats

To evaluate the possibility of 2-CN-Ado as an ophthalmic drug for the treatment of glaucoma, we attempted to determine whether topical administration of 2-CN-Ado

Figure 5 Effects of adenosine receptor antagonists on 2-CN-Ado induced protection of retinal ischaemia/reperfusion damage in rats. IPL or IRL was expressed as the thickness ratio of retinal layers of the ischaemia/reperfusion eye to that of the contralateral (control) eye (%). (Top) IPL, (bottom) IRL. Vehicle (iv, $n=20$) or 2-CN-Ado (100 $\mu\text{g}/\text{kg}$, iv, $n=21$) was injected 5 minutes before ischaemia. DPCPX (3 mg/kg, ip, $n=9$), CSC (3 mg/kg, ip, $n=8$), or alloxazine (10 mg/kg, ip, $n=9$) was injected 30 minutes before ischaemia. In the single administration group, DPCPX (3 mg/kg, ip, $n=9$), CSC (3 mg/kg, ip, $n=10$), or alloxazine (10 mg/kg, ip, $n=7$) was injected 30 minutes before ischaemia. Data are expressed as the means (SE). * $p<0.05$, statistically significant compared with the drug alone (Dunnett's test).

(0.3% and 1%) affected retinal ischaemia/reperfusion damage (fig 6). The repeated instillation of 2-CN-Ado at a dose of 1% resulted in a significant reduction in retinal ischaemia/reperfusion damage (fig 6). Moreover, the protective effect of 2-CN-Ado was significantly attenuated by the co-administration of CSC (fig 7).

Effect of 2-CN-Ado on intraocular pressure in normal rats

The intraocular pressure was determined when 2-CN-Ado was administered as an intravenous injection or topical application (fig 8). The basal intraocular pressure ranged from 12.2 (0.4) mm Hg to 13.7 (0.6) mm Hg before drug treatment. Intraocular pressure tends to decrease initially after iv administration of 2-CN-Ado, and returned towards control values at 120 minutes, but the ocular hypotension was not obviously changed. In contrast, topical administration of 2-CN-Ado caused a sustained reduction of intraocular pressure (fig 8).

DISCUSSION

In the present study, we showed that 2-CN-Ado attenuated retinal ischaemia/reperfusion damage. In addition, the protective effect of 2-CN-Ado was inhibited by CSC, but not by alloxazine or DPCPX. Moreover, the CGS-21680 induced protective effect was also inhibited by CSC, but not by DPCPX or alloxazine. These results suggest that 2-CN-Ado attenuates retinal ischaemia/reperfusion damage through the adenosine A_{2A} receptor, consistent with the report that neuroprotection in the retina occurs through an adenosine A_{2A} receptor

Figure 6 Effect of topical administration of 2-CN-Ado on retinal ischaemia/reperfusion damage in rats. IPL or IRL was expressed as the thickness ratio of retinal layers of the ischaemia/reperfusion eye to that of the contralateral (control) eye (%). (Top) IPL, (bottom) IRL. Vehicle (30 μl , $n=8$), 2-CN-Ado (0.3%, 30 μl , $n=6$), or 2-CN-Ado (1%, 30 μl , $n=8$) was instilled into one eye, and the vehicle was instilled into the other eye. 2-CN-Ado was instilled three times daily (around 7 am, 1 pm, and 7 pm) from 1 day before ischaemia, and instillation was continued until 1 day after reperfusion. Data are expressed as the means (SE) ($n=6-8$). * $p<0.05$, statistically significant compared with the vehicle (Dunnett's test).

dependent mechanism.¹⁴⁻¹⁶ In addition, we showed that the topical administration of 2-CN-Ado also exhibited the histological protection against retinal ischaemia/reperfusion damage. And the protective effect of 2-CN-Ado was also attenuated by the co-administration of CSC. These results indicate that the topical administration of 2-CN-Ado might give protection against retinal ischaemia/reperfusion damage mediated via an adenosine A_{2A} receptor.

The opposite observation has been reported, that CSC protected against retinal ischaemia/reperfusion damage in rats.^{9,13} Thus, we cannot totally exclude the possibility that the attenuation of adenosine A_{2A} receptor after ischaemia/reperfusion improves. However, Li and Roth¹⁴ showed that CGS-21680 could precondition the retina against ischaemic injury. Taken together, we speculate that the modest activation of the adenosine A_{2A} receptor before the ischaemic insult and the subsequent attenuation of this receptor during ischaemia contributed to the protection against retinal ischaemia/reperfusion damage.

The protective effect of 2-CN-Ado on IPL was somewhat attenuated by DPCPX without statistical significance. It has been shown that stimulation of the adenosine A_1 receptor results in protection against retinal ischaemia/reperfusion damage.^{9,12,13} Therefore, the partial involvement of the adenosine A_1 receptor may not be ruled out in the protective effect of 2-CN-Ado.

It has been shown that the carbonic anhydrase inhibitor dorzolamide might protect against retinal degeneration in a rat experimental glaucoma model through ocular hypotension.²² Our study showed that 2-CN-Ado produced ocular hypotension, indicating that 2-CN-Ado might reduce retinal ischaemia/reperfusion damage partly through ocular

Figure 7 Effect of an adenosine A_{2A} receptor antagonist on the protection of retinal ischaemia/reperfusion damage induced by topical administration of 2-CN-Ado in rats. IPL or IRL was expressed as the thickness ratio of retinal layers of the ischaemia/reperfusion eye to that of the contralateral (control) eye (%). (Top) IPL, (bottom) IRL. Instillation of vehicle (30 μ l, n = 12), 2-CN-Ado (1%, 30 μ l, n = 8) alone or combined with CSC (0.3 mg/kg, ip, n = 12) was maintained three times daily (around 7 am, 1 pm, and 7 pm) from 1 day before ischaemia, and administration was continued until 1 day after reperfusion. Data are expressed as the means (SE). * p < 0.05, statistically significant compared with the drug alone (unpaired Student's t test).

hypotension. We reported previously that 2-CN-Ado reduced intraocular pressure via the adenosine A_{2A} receptor, and 2-CN-Ado induced ocular hypotension is the result of an increased outflow facility in rabbits.^{18, 19} Therefore, we speculate that 2-CN-Ado produced ocular hypotension via a similar mechanism in rats.

It has been shown that ischaemic retinal degeneration consists of a number of mechanisms involving retinal artery occlusion, acute inflammatory responses, platelet aggregation, and increased glutamatergic stimulation,^{5, 23, 24} indicating that pharmacological therapy may be a novel approach to treat ischaemic retinal injury.

Adenosine is known as a modulator of ocular blood flow.^{6, 8, 25–27} Recently, Hein *et al*²⁸ reported that adenosine evokes retinal arteriolar dilatation via the activation of adenosine A_{2A} receptor, and the subsequent production of NO and opening of K_{ATP} channels. Our preliminary experiments showed that an intravitreal injection of 2-CN-Ado resulted in increased ocular blood flow in the rabbit eye via adenosine A_{2A} receptor (Konno *et al*, unpublished observation). Therefore, it is possible that 2-CN-Ado may produce a protective effect in part through an increased ocular blood flow via the activation of adenosine A_{2A} receptor.

It is well known that activation of the adenosine A_{2A} receptor is involved in antiplatelet and anti-inflammatory effects.^{29, 30} In addition, 2-alkynyladenosine derivatives showed the reduction of rabbit platelet aggregation³¹ and the inhibition of leucocyte activation in rat liver.³² Moreover, our recent preliminary experiments showed that 2-CN-Ado inhibited the aggregation of rabbit platelets induced by ADP (Konno *et al*, unpublished observation). Therefore, it is possible that antiplatelet and/or anti-inflammatory effects may be involved in the protective effect of 2-CN-Ado.

Figure 8 Effect of 2-CN-Ado on intraocular pressure in normal rats. Δ Intraocular pressure was expressed as the difference in mm Hg from the intraocular pressure before drug treatment. (Top) The effect of intravenous administration of 2-CN-Ado (100 μ g/kg, from femoral vein). Vehicle (open symbols, n = 5) and 2-CN-Ado (solid symbols, n = 5). (Bottom) Effect of topical administration of 2-CN-Ado (1% 2-CN-Ado as a volume of 30 μ l). Vehicle (open symbols, n = 5) and 2-CN-Ado (solid symbols, n = 6). Data are expressed as the means (SE). * p < 0.05, statistically significant compared with the vehicle (unpaired Student's t test).

Moreover, there is a report that stimulation of the adenosine A_{2A} receptor is responsible for the suppression of excitatory amino acid induced toxicity,¹⁵ indicating that stimulating the adenosine A_{2A} receptor attenuates retinal ischaemia/reperfusion damage through the antagonism of excitatory amino acid. Taken together, it is possible that 2-CN-Ado might protect the retina by mechanisms other than simply lowering intraocular pressure, although further study is required to identify the mechanism of the protective effect of 2-CN-Ado.

In conclusion, the present study demonstrated that 2-CN-Ado attenuated retinal ischaemia/reperfusion damage, and at least some of this protective effect of 2-CN-Ado might be mediated via activation of the adenosine A_{2A} receptor.

ACKNOWLEDGEMENTS

The authors would like to thank Ms Yuu Kanazawa, Mr Shin-ya Ohnuma, Mr Kazuhiro Uemoto, Dr Fumiya Yoneyama, and Dr Yoichi Manome for their excellent technical assistance. We are also grateful to Yamasa Corporation for supplying the adenosine derivatives.

Authors' affiliations

T Konno, A Sato, T Uchibori, A Nagai, K Kogi, Drug Research Section II, Fukushima Research Laboratories, Toa Eiyo Ltd, Fukushima, Japan

N Nakahata, Department of Cellular Signaling and 21st Century COE program "CRESCENDO", Graduate School of Pharmaceutical Sciences, Tohoku University, Miyagi, Japan

Competing interests: none.

REFERENCES

- 1 Weber M, Bonaventure N, Sahel JA. Protective role of excitatory amino acid antagonists in experimental retinal ischemia. *Graefes Arch Clin Exp Ophthalmol* 1995;233:360–5.
- 2 Lam TT, Siew E, Chu R, *et al*. Ameliorative effect of MK-801 on retinal ischemia. *J Ocul Pharmacol Ther* 1997;13:129–37.
- 3 Flythe TJ. A rationalization of treatment of central retinal artery occlusion. *Trans Ophthalm Soc UK* 1974;94:468–79.

- 4 **Kaushik S**, Pandav SS, Ram J. Neuroprotection in glaucoma. *J Postgrad Med* 2003;**49**:90–5.
- 5 **Osborne NN**, Melena J, Chidlow G, *et al.* A hypothesis to explain ganglion cell death caused by vascular insults at the optic nerve head: possible implication for the treatment of glaucoma. *Br J Ophthalmol* 2001;**85**:1252–9.
- 6 **Braunagel SC**, Xiao JG, Chiou GC. The potential role of adenosine in regulating blood flow in the eye. *J Ocul Pharmacol* 1988;**4**:61–73.
- 7 **Blazynski C**, Perez MT. Adenosine in vertebrate retina: localization, receptor characterization, and function. *Cell Mol Neurobiol* 1991;**11**:463–84.
- 8 **Polska E**, Ehrlich P, Luksch A, *et al.* Effects of adenosine on intraocular pressure, optic nerve head blood flow, and choroidal blood flow in healthy humans. *Invest Ophthalmol Vis Sci* 2003;**44**:3110–14.
- 9 **Ghiardi GJ**, Giddy JM, Roth S. The purine nucleoside adenosine in retinal ischemia-reperfusion injury. *Vis Res* 1999;**39**:2519–35.
- 10 **Roth S**, Park SS, Sikorski CW, *et al.* Concentrations of adenosine and its metabolites in the rat retina/choroid during reperfusion after ischemia. *Curr Eye Res* 1997;**16**:875–85.
- 11 **Roth S**, Rosenbaum PS, Osinski J, *et al.* Ischemia induces significant changes in purine nucleoside concentration in the retina-choroid in rats. *Exp Eye Res* 1997;**65**:771–9.
- 12 **Larsen AK**, Osborne NN. Involvement of adenosine in retinal ischemia. Studies on the rat. *Invest Ophthalmol Vis Sci* 1996;**37**:2603–11.
- 13 **Li B**, Rosenbaum PS, Jennings NM, *et al.* Differing roles of adenosine receptor subtypes in retinal ischemia-reperfusion injury in the rat. *Exp Eye Res* 1999;**68**:9–17.
- 14 **Li B**, Roth S. Retinal ischemic preconditioning in the rat: requirement for adenosine and repetitive induction. *Invest Ophthalmol Vis Sci* 1999;**40**:1200–16.
- 15 **Ferreira JM**, Paes-de-Carvalho R. Long-term activation of adenosine A_{2A} receptors blocks glutamate excitotoxicity in cultures of avian retinal neurons. *Brain Res* 2001;**900**:169–76.
- 16 **Rego AC**, Agostinho P, Melo J, *et al.* Adenosine A_{2A} receptors regulate the extracellular accumulation of excitatory amino acids upon metabolic dysfunction in chick cultured retinal cells. *Exp Eye Res* 2000;**70**:577–87.
- 17 **Matsuda A**, Shinozaki M, Yamaguchi T, *et al.* Nucleosides and nucleotides. 103. 2-Alkynyladenosines: a novel class of selective adenosine A₂ receptor agonists with potent antihypertensive effects. *J Med Chem* 1992;**35**:241–52.
- 18 **Konno T**, Murakami A, Uchibori T, *et al.* Involvement of adenosine A_{2A} receptor in intraocular pressure decrease induced by 2-(1-Octyn-1-yl)adenosine or 2-(6-cyano-1-hexyn-1-yl)adenosine. *J Pharmacol Sci* 2005;**97**:501–9.
- 19 **Konno T**, Ohnuma S, Uemoto K, *et al.* Effects of 2-alkynyladenosine derivatives on intraocular pressure in rabbits. *Eur J Pharmacol* 2004;**486**:307–16.
- 20 **Toriu N**, Akaie A, Yasuyoshi H, *et al.* Lomerizine, a Ca²⁺ channel blocker, reduces glutamate-induced neurotoxicity and ischemia/reperfusion damage in rat retina. *Exp Eye Res* 2000;**70**:475–84.
- 21 **Stefansson E**, Wilson CA, Schoen T, *et al.* Experimental ischemia induces cell mitosis in the adult rat retina. *Invest Ophthalmol Vis Sci* 1988;**29**:1050–5.
- 22 **Seki M**, Tanaka T, Matsuda H, *et al.* Topically administered timolol and dorzolamide reduce intraocular pressure and protect retinal ganglion cells in a rat experimental glaucoma model. *Br J Ophthalmol* 2005;**89**:504–7.
- 23 **Osborne NN**, Casson RJ, Wood JP, *et al.* Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog Retin Eye Res* 2004;**23**:91–147.
- 24 **Pop E**. Trends in neuroprotection. *Arch Soc Esp Ophthalmol* 2002;**77**:295–7.
- 25 **Campochiaro PA**, Sen HA. Adenosine and its agonists cause retinal vasodilation and hemorrhages. Implications for ischemic retinopathies. *Arch Ophthalmol* 1989;**107**:412–16.
- 26 **Giddy JM**, Park TS. Microcirculatory responses to adenosine in the newborn pig retina. *Pediatr Res* 1993;**33**:620–7.
- 27 **Matsugi T**, Chen Q, Anderson DR. Adenosine-induced relaxation of cultured bovine retinal pericytes. *Invest Ophthalmol Vis Sci* 1997;**38**:2695–701.
- 28 **Hein TW**, Yuan Z, Rosa RH Jr, *et al.* Requisite roles of A_{2A} receptors, nitric oxide, and KATP channels in retinal arteriolar dilation in response to adenosine. *Invest Ophthalmol Vis Sci* 2005;**46**:2113–9.
- 29 **Paul S**, Feoktistov I, Hollister AS, *et al.* Adenosine inhibits the rise in intracellular calcium and platelet aggregation produced by thrombin: evidence that both effects are coupled to adenylate cyclase. *Mol Pharmacol* 1990;**37**:870–5.
- 30 **Sullivan GW**. Adenosine A_{2A} receptor agonists as anti-inflammatory agents. *Curr Opin Investig Drugs* 2003;**4**:1313–19.
- 31 **Kogi K**, Uchibori T, Aihara K, *et al.* Pharmacological profile of the 2-alkynyladenosine derivative 2-octynyladenosine (YT-146) in the cardiovascular system. *Jpn J Pharmacol* 1991;**57**:153–65.
- 32 **Harada N**, Okajima K, Murakami K, *et al.* Adenosine and selective A_{2A} receptor agonists reduce ischemia/reperfusion injury of rat liver mainly by inhibiting leukocyte activation. *J Pharmacol Exp Ther* 2000;**294**:1034–42.