RELATIONSHIP BETWEEN CAFFEINE-INDUCED OCULAR HYPERTENSION AND ULTRASTRUCTURE CHANGES OF NON-PIGMENTED CILIARY EPITHELIAL CELLS IN RATS

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ABSTRACT — The purpose of this study was to morphologically assess a possible mechanism for caffeine-induced ocular hypertension. Taking into consideration the relationship between the secretion of aqueous humor and the ultrastructure of the ciliary body, the time course of the morphological features in the ciliary epithelium when caffeine was administered intravenously to male Wistar rats was investigated by electron-microscopy. These morphological findings were also compared with the changes in the intraocular pressure (IOP).

A significant increase in IOP was noted 15 min and 1 hr after a single dosing of caffeine alone. This change disappeared in all animals within 2 hr after dosing. The IOP in the animals receiving caffeine and the β-blocker befunolol, which lowers the IOP by inhibiting aqueous humor secretion, decreased significantly from 15 min after dosing, and this change persisted 2 hr after dosing. In electron-microscopy 15 min and/or 1 hr after dosing with caffeine, a slight dilatation in the lateral intercellular spaces near the basement membrane of the non-pigmented ciliary epithelium was observed and the interdigitations between the non-pigmented epithelial cells were intact. Reversal of these changes was observed 2 hr after dosing. On the other hand, the lateral intercellular spaces between the non-pigmented epithelial cells were markedly dilated and the interdigitations were disorganized following dosing with caffeine alone and in combination with befunolol.

These results described here indicate that the intravenous administration of caffeine causes ocular hypertension and also changes in the non-pigmented ciliary epithelium, suggesting an enhancement of aqueous humor transportation. This paradigm in the rat is considered to be useful to further assess caffeine-induced ocular hypertension and for use as an animal model in glaucoma research associated with an aqueous humor secretion.

KEY WORDS : Caffeine, Intraocular pressure, Non-pigmented ciliary epithelium, Rat

INTRODUCTION

It has been reported that an intravenous injection of the most widely used methylxanthine derivatives caffeine, theobromine and aminophylline as elevated doses in rabbits often leads to ocular hypertension (Yamashita et al., 1983). However, these methylxanthines are known to increase the cyclic-AMP (cAMP) level by inhibiting phosphodiesterase in many tissues, and when, administered topically to the eye, decreasing the intraocular pressure (IOP) (Harison, 1994). It
was also demonstrated that intracameral injection of high concentrations of cAMP caused a marked decrease in rabbit IOP (Neufeld et al., 1972). Thus, the effects of methylxanthines on IOP are conflicting, and cannot be explained only by an elevation of cAMP level in the aqueous humor.

It is highly desirable for experimental glaucoma research to move from expensive non-human primate models to smaller models. Recently, it was reported that the rat eye was a suitable model for experimental studies on ocular diseases that were characterized by chamber angle anomalies or congenital glaucoma because of the striking morphological similarities with the analogous region of the human eye (Nucci et al., 1992). Rodents are also a preferable species, as the experimental conditions for rodents are much more easily controlled and they are more easily handled and less expensive as models.

To the best of our knowledge, data for rats on the relationship between IOP and the morphological findings in the ciliary body after an intravenous administration of caffeine have not been reported. The aim of this work was to electron-microscopically investigate the time course morphological features of the non-pigmented ciliary epithelium when caffeine was administered intravenously to male Wistar rats, and to compare these morphological findings with the changes in the IOP.

MATERIALS AND METHODS

Animal
A total of 48 male Wistar rats (CLEA Japan Inc., Japan) weighing 353 to 412 g were used. At the start of treatment, the animals were 21 weeks old; based on the limitations of the rat body weight for IOP measurements using a Perkin's applanation tonometer, these animals were selected for the present study. Each animal was housed individually in a stainless steel cage in a room having a constant 12 hr light-dark cycle (light on from 8:00 to 20:00). The room temperature was maintained at 23±3°C with a relative humidity of 55±15%. The animals were allowed a laboratory diet (CE-2, CLEA Japan Inc., Japan) and tap water. Maintenance and experimental conditions conformed to the Guide for the Care and Use of Laboratory Animals of Takeda Chemical Industries, Ltd.

Drugs and administrations
Caffeine sodium benzoate, a commercial product [Annaka 10%(w/v) for injection, Fuso Pharmaceutical Co., Ltd.], was diluted in physiological saline at a concentration of 1%(w/v). The pH of the solutions was 6.7 to 7.2. Befunolol hydrochloride, a commercial product [Bentos 1%(w/v) for instillation, Kaken Pharmaceutical Co., Ltd.], was used to reduce the ocular hypertensive effect by caffeine.

First, on the basis of body weight, sixteen male rats for caffeine injection alone were allocated randomly to 4 groups, each comprised of 4 animals for the time course study (pre-drug, 15 min, 1 and 2 hr after dosing of caffeine). The dosage level of caffeine to investigate the relationship between the ocular hypertension and the morphological changes of ciliary body was selected as 100 mg/kg, based on the results of a preliminary IOP study of caffeine in Wistar rats in which an intravenous injection of 100 mg/kg caffeine caused ocular hypertension. The dosage volume for this study was 10 ml/kg. A single intravenous injection of caffeine was conducted generally in the morning. The dosing solution was administered intravenously in the tail vein at about 2 ml/min. Next, the other 16 male rats for the combination with befunolol were allocated to 4 groups, each comprised of 4 animals for the time course study (pre-drug, 15 min, 1 and 2 hr after dosing of caffeine). 100 mg/kg of caffeine immediately after each instillation of 100 μl of 1%(w/v) befunolol for both eyes was injected in the tail vein. Control animals (4 rats/each point) received physiological saline intravenously in the same manner.

Intraocular pressure (IOP) measurements
The IOP for each right eye was measured with a Perkin's applanation tonometer (Clement Clarke Ltd., London). The technique and calibration of the tonometer for use on Wistar rats were described previously (Kurata et al., 1996). Breifly, animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.m.) and droperidol hydrochloride (1 mg/kg, i.m.). The rats were then positioned in ventral recumbency after the instillation of a local anesthetic for ophthalmic examination, oxybuprocaaine hydrochloride (0.01 μl/drop). The rat cornea was then stained with a
fluorescein indicator (FLUORES Test Paper, Showa Pharmaceutical Ltd.). The IOP was then measured before dosing commenced (predosing values) and 15 min, 1 and 2 hr after a single injection of caffeine, or with caffeine in combination with befunolol or physiological saline. Three readings using the tonometer were recorded. The corneal surface was moistened properly with physiological saline during the measurement. Body temperature measured before and after each IOP measurement was 37.2±0.3°C and was well maintained during the course of experiments.

**Morphologic study**

Following IOP measurements for each time course, 2 rats/time course point were deeply anesthetized with ether and perfused via the ventricle with a phosphate-buffered solution of 2.5% glutaraldehyde-2% formaldehyde. Both eyes were removed from the skull, and were gently infused with a phosphate-buffered solution of 4% glutaraldehyde (pH 7.4) using a glass pipette. The right eyes were immersed in 1% osmium and placed on a rotator for 1 hr. After processing using conventional histologic techniques, the right eyes were embedded in Epon-Araldite. Following polymerization of the Epon-Araldite, 3 sections (approximately 20 μm apart and 6 μm thick) were cut with a glass knife on an ultramicrotome. Sections were mounted on glass slides and were stained with toluidine blue. After the ciliary body was identified light-microscopically, 4 to 5 sections for transmission electron microscopy were stained with uranyl acetate and lead citrate and were examined by electron microscope (JEM-1200EX, JEOL Ltd., Japan).

**Statistical analysis**

The data of IOP were averaged for each group in each measurement period. Dunnett's test was performed to compare the control with the treated groups. Also, differences between pre-

![Graph showing changes of intraocular pressure in rats given caffeine alone and in combination with β-blocker befunolol.](image)

**Fig. 1.** Changes of intraocular pressure in rats given caffeine alone and in combination with β-blocker befunolol. ○, control (n=4); ●, caffeine (100 mg/kg, i.v., n=4); ▲, befunolol [100 μl of 1%(w/v) solution, each instillation for both eyes] and caffeine (100 mg/kg, i.v.) (n=4). Each value is presented as the mean with standard deviation. IOP significantly increased 15 min to 1 hr after dosing of caffeine alone. IOP in the animals receiving caffeine and befunolol decreased throughout the postdosing period. *,** Significantly different from the control group at p<0.05 and p<0.01, respectively. #,** Significantly different from the predosing value at p<0.05 and p<0.01, respectively.
dosing and postdosing values in each group were evaluated using a paired t test. All the statistical tests were conducted at the 5% and 1% probability levels (two-tailed).

RESULTS

**IOP measurements**

In the control rats, the mean values for the IOPs were 17.8 to 19.0 mm Hg (n=4). Each value was within the confidence interval of 95% and 99% as we reported previously. The values for each animal were reproducible during the experiment period, and there were no statistical differences between the predosing value and the values obtained at 15 min, 1 and 2 hr after dosing with saline (Fig. 1).

A significant increase in IOP was noted 15 min and 1 hr after a single dose of 100 mg/kg caffeine alone. This increase disappeared for all animals within 2 hr after dosing. The IOP in the animals receiving caffeine and befunolol decreased significantly from 15 min after caffeine dosing and this change persisted for 2 hr after dosing (Fig. 1).

**Morphologic study**

Alterations of the non-pigmented ciliary epithelium were not present in the rats 1 hr after dosing with physiological saline (Photo 1). Photo 2 shows the changes 15 min after dosing with 100 mg/kg caffeine. Slight dilatation in the lateral intercellular spaces near the basement membrane of the non-pigmented ciliary epithelium was observed but the interdigitations between the non-pigmented epithelial cells were intact. The severity of these changes increased markedly 1 hr after dosing (Photo 3). These changes were not noted 2 hr after dosing (Photo 4). Photo 5 represents the alterations 1 hr after dosing with caffeine and with caffeine in combination with befunolol. The intercellular spaces between non-pigmented epithelial cells were markedly dilated and the interdigitations were disorganized. These changes persisted for 2 hr after dosing.

![Photo 1](image_url). Non-pigmented ciliary epithelium in male Wistar rat receiving physiological saline. No morphological changes in the epithelium were seen. ×15000. Bar = 1 μm.
Photo 2. Non-pigmented ciliary epithelium 15 min after dosing of 100 mg/kg caffeine. Note slight irregularity in the basal surface of the epithelial cells (asterisk). ×15000. Bar = 1 μm.

Photo 3. Non-pigmented ciliary epithelium 1 hr after dosing of 100 mg/kg caffeine. Note slight dilatation in the lateral intercellular spaces of the epithelial cells (asterisks). ×15000. Bar = 1 μm.
Photo 4. Non-pigmented ciliary epithelium 2 hr after dosing of 100 mg/kg caffeine. No abnormalities in the epithelial cells were noted. ×15000. Bar = 1 μm.

Photo 5. Alterations 1 hr after dosing of caffeine and β-blocker bifuralol. Note a marked dilatation in the intercellular spaces between the epithelial cells and the disorganized interdigitations (asterisk). ×6000. Bar = 2 μm.
DISCUSSION

The results presented here indicate that a single intravenous injection of caffeine causes an ocular hypertension in rats and the change in intraocular pressure (IOP) is most evident 15 min to 1 hr after dosing. The results also show that the instillation of a β-blocker, buprenoril, inhibits the ocular hypertensive effect of caffeine throughout the postdosing period. The modification of IOP seen in this study using male Wistar rat is in agreement with the previous observations of the ocular hypertensive effect of caffeine in rabbits (Yamashita et al., 1983).

It has been reported that the slight dilatation in the lateral intercellular spaces indicates an increase in the transport of Na⁺ and H₂O in the epithelial cells (Berridge et al., 1972). Therefore, following the intravenous injection of caffeine, the slight dilatation in the intercellular spaces near the basement membrane seen in the present study was considered to be due to an increased transport of water in the epithelial cells. Furthermore, the intact interdigitations between the non-pigmented epithelial cells suggest that this may be an important site for the secretion of the aqueous humor (Tormey, 1966). The intact interdigitations in the present study may be associated with the secretion of aqueous humor. Therefore, the marked dilatation in the intercellular spaces and the disorganized interdigitation observed in the combination group were considered to suggest an inhibition of the secretion of the aqueous humor.

In the present study, the morphological features seen were indicative of an enhancement of the transportation of the aqueous humor when caffeine was administered intravenously to the rats. Thus, caffeine-induced ocular hypertension may be involved in the increased secretion of the aqueous humor.

Nevertheless, it was reported that instillation of the pivaloxyloxymethyl ester of griseolic acid, a potent inhibitor of cAMP-phosphodiesterase, induces in the rabbit a dose-dependent decrease in IOP (Yamazaki et al., 1990). More recently, it has been demonstrated that the kinetics of the ocular hypotensive activity of 8-bromo-cAMP depend on its concentration in the aqueous humor (Busch et al., 1992). With respect to the phosphodiesterase inhibitors, including caffeine, the mechanisms underlying the ocular hypertension after an intravenous injection and/or the ocular hypotension after an instillation are not yet resolved.

The wide variety of pharmacological and toxicological effects of caffeine seems to reflect molecular interactions between caffeine and several different receptors or processes within the cells. These include adenosine receptors of various subtypes, phosphodiesterase, intracellular fluxes of calcium, and undefined regulators of the cell cycle (Barone and Grice, 1990). Also, it was suggested that caffeine, theophylline and isobuty1-1-methylxanthine, and probably other cAMP-phosphodiesterase inhibitors, have an inhibitory effect on cGMP-phosphodiesterase (Helfman and Kuo, 1982). This enzyme is found in the ciliary body, and its inhibition leads to an increase in cGMP levels; cGMP is another second messenger involved in the regulation of IOP (Nathanson, 1987). Thus, the effects of the methylxanthines on IOP cannot be explained solely by an elevation of cAMP level in the aqueous humor. Furthermore, an additional action of the methylxanthine derivatives on IOP could be mediated through effects on the blood pressure.

These results described here indicate that the ocular hypertension induced by an intravenous administration of caffeine to rats is associated with morphological changes characterized by dilatation of the lateral cellular spaces near the basement membrane of the non-pigmented ciliary epithelium with intact interdigitations among the cells, suggesting enhanced aqueous humor transportation. This paradigm in the rat is considered to be useful to further assess caffeine-induced ocular hypertension and for use as an animal model in glaucoma research associated with an aqueous humor secretion.

REFERENCES


