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EFFECT OF DORZOLAMIDE ON RABBIT OCULAR BLOOD FLOW AND ISOLATED CILIARY ARTERY

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Abstract

Background

Dorzolamide, the carbonic anhydrase inhibitor, has been reported to have effects on ocular blood flow other than decreasing intraocular pressure (IOP). We investigated the effects of dorzolamide on the phenylephrine induced impairment of rabbit optic nerve head (ONH) blood flow and on phenylephrine induced contraction in isolated rabbit ciliary artery in vitro.

Method

In male Dutch rabbits, dorzolamide was topically administered after phenylephrine hydrochloride instillation. ONH blood flow was measured by the laser speckle method, which expresses blood velocity as a quantitative index, the mean blur rate (MBR). Also, we investigated the effects of dorzolamide on phenylephrine-induced contractions in rabbit ciliary artery in vitro using isometric tension recording method.

Results

MBR showed a maximum decrease by 10% compared to that in the baseline value at 120 min after phenylephrine instillation. The phenylephrine-induced decrease was significantly inhibited by dorzolamide at 120 min ($P=0.0124$). The IOP in the dorzolamide-treated eyes was significantly reduced after phenylephrine instillation. Dorzolamide (30 μM) did not have effect on isolated rabbit ciliary artery which was pre-contracted with 10 μM phenylephrine.

Conclusion

Topical dorzolamide inhibited phenylephrine induced decrease of ONH circulation in Dutch rabbits. However, dorzolamide did not have effect on isolated rabbit ciliary artery pre-contracted with phenylephrine. It is suggested that the dorzolamide improve the ONH blood flow not by direct action to the vessels.

key words : dorzolamide, phenylephrine, ocular blood flow, myograph

Introduction

The high intraocular pressure (IOP) is a major risk factor for the cause or progression of glaucoma, and IOP reduction is the only evidence based treatment for glaucoma. However, even after satisfactory IOP reduction, some patients progress visual field loss[1-3]. The other factor beside IOP has been considered a possibility in the pathogenesis of glaucoma progression. Some clinical studies have shown that blood flow in the optic nerve head (ONH) was significantly lower in open angle glaucoma (OAG) patients than ocular hypertension patients or normal volunteers[4,5]. Impaired ONH blood flow may influence progression of glaucoma[6].

In recent years, there has been considerable interest in the effects of topical anti-glaucoma drugs on ocular

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blood flow (OBF). In patients with newly diagnosed primary open-angle glaucoma, the peak systolic flow velocity in the ophthalmic arteries is higher in patients treated with carbonic anhydrase inhibitor (CAI) than in patients treated with other anti-glaucoma drugs[7]. Moreover, intravenously injected CAI in pigs caused dilation of the retinal blood vessels and increased retinal oxygen tension under normal conditions[8]. On the other hand, reduction of IOP by CAIs may increase ocular perfusion pressure. The pharmacological mechanisms of CAIs on OBF remains to be evaluated.

CAIs reduce IOP by reducing aqueous production[10]. Dorzolamide is a topical CAI used clinically for the treatment of glaucoma. There have been several reports of this agents have effect on ONH blood flow[10-12], but the pharmacological mechanism of topical dorzolamide instillation to ONH blood flow is not still unclear.

The purpose of this study is to clarify the pharmacological mechanisms of dorzolamide on OBF. We examined the effects of dorzolamide on the phenylephrine induced impairment of rabbit ONH blood flow in vivo, and at the same time, we also examine the effect of this drugs on phenylephrine induced contraction of isolated rabbit ciliary artery in vitro.

Materials and Methods

Animals

We used Male Dutch and Albino rabbits (n=17, 2.0-3.0 kg) supplied by Biotech Co. Ltd. (Saga, Japan). They were housed under a 12 hour light-dark cycle, and allowed free access to standard food and water. All animal care and experimental procedures were in accordance with the guidelines of the Akita University Animal Study Committee.

Materials

Dorzolamide ophthalmic solution (1% Trusopt®) was purchased by MSD Co. Ltd. (Tokyo, japan). Phenylephrine ophthalmic solution (5% Neo-Synephrine®) was purchased by Kowa Co. Ltd. (Nagoya, japan).

Ocular blood flow measurements

Ocular blood flow is affected by the depth of anesthesia, and it is difficult to measure it accurately under anesthesia[13]. Therefore, we measured ONH blood flow in rabbits in the conscious state. But in the conscious state, ONH blood flow is affected by excitement of rabbits. In the present study, we have devised for preventing the excitation of rabbits. Rabbits were placed in a holding box, the face and body can move freely to some extent. In addition, we did not use ophthalmic anesthesia, eyelid speculum. No noteworthy abnormalities in the eyes or in the general health of the animals were observed when ONH blood flow measurements were made in the conscious state in this study. The pupil of the eye to be used for measurements was dilated with one drop of 0.4% tropicamide. ONH blood flow was measured using LSFG-MRC™ device (Softcare Co. Ltd. Fukutsu, japan). The LSFG-MRC™ consisted of a fundus camera equipped with a diode laser (wavelength : 830 nm) and a CCD image sensor (750 × 360 pixels). The principle and application of this method have been described previously[14].

To evaluate the microcirculation of the optic nerve head, an index of mean blur rate (MBR) was determined in the optic disk by analyzer of LSFG-NAVI™. MBR, a parameter that yields a theoretically exact measurement of retinal microcirculation, is proportional to blood velocity and showed good correlation with blood flow parameters when measured with other instruments that assess the ocular blood flow[15,16]. All rabbits were examined by one experienced investigator.

Experimental Protocol

The experimental works were performed on 10 male pigmented rabbits. To check their stability in measuring ONH blood flow, MBR were recorded 3 times at 10-min intervals. The inclusion criterion for the stability of measurements was a coefficient of variance (CV) of MBR within 0.05. Averages of measurement values were adopted as baseline values. The IOP was measured with rebound tonometer Icare (M.E. Technica Co. Ltd. Tokyo, Japan). After baseline measurements, a 20 μl of phenylephrine hydrochloride was instilled. And 5 minutes after this treatment, a 20 μl of Dorzolamide or saline was instilled additionally. MBR and IOP measurements were made just before the phenylephrine hydrochloride instillation and at 30, 60, 90, 120, 150, 180 min after instillation.

Isometric tension recording

The detail procedure for mounting the ciliary artery in the Myograph System® has been described in our previous reports[17,18]. In the current in vitro experiments, we used 7 rabbits. Ciliary arteries were isolated at four vessels per eye, and 52 vessels were used in the present in vitro study. Male albino rabbits were anesthetized euthanized by means of an intravenous injection of pentobarbital sodium (Somnopentyl® ; Kyoritsu Pharmaceutical Co. Ltd.). The eyes were immediately enucleated, ensuring that the maximal length of optic nerve was removed, and placed in Krebs solution composed of the following (mM) : NaCl 94.8, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7. This was oxygenated by bubbling with 95% O_2 and 5% CO_2. With the aid of a dissecting microscope, the ciliary artery and surrounding connective tissue were carefully isolated from the optic nerve. A vascular segment (150-300 μm in diameter, 1-2 mm in length) cut from the distal portion of the ciliary artery was immediately mounted in a chamber of a double Myograph System® (Danish Myo Technology, Denmark) which contained 10 mL Krebs solution. The temperature was maintained at 37°C in the chamber. The Myograph System® directly determines vessel isometric tension and simultaneously transmits the data to a computer that displays the tension curves on a monitor. Details of this method of isometric tension recording have been published by Mulvany and Halpern[19]. To induce relaxation of vascular smooth muscle, calbachol (1 μm), a cholinergic agonist that acts on receptors in the endothelium, was added 20 min after contraction. This procedure confirmed the susceptibility of each contracted ciliary artery to cholinergic relaxation before we examined the effects of Dorzolamide. After an equilibration period, the contractions evoked by 10 μM phenylephrine were measured at 20-min intervals to establish preparation viability and stability. The ability of dorzolamide to relax the isolated ciliary artery was determined in segments pre-contracted by 10 μM phenylephrine. Once the phenylephrine-induced contraction had been stable for 40 min, dorzolamide was applied every 10 min in a cumulative manner.

Results

Phenylephrine reduced MBR from 60 min after instillation and reached to the maximum reduction 120 min after. Dorzolamide which applied 5 min after phenylephrine inhibited the effect of phenylephrine induced MBR reduction significantly at 120 min (Student t-test) (Fig. 1).

Phenylephrine alone did not change IOP statistically, however, dorzolamide reduced IOP significantly at 90, 120, 150, 180 min (Student t-test) (Fig. 2).

For rabbit ciliary artery pre-contracted with phenylephrine solution, dorzolamide had little effect up to the concentration of 3×10^{-5} M (Fig. 3).

Discussion

Several previous studies have tried to examine the effect of dorzolamide on ocular blood flow. Among them, method by a single instillation of dorzolamide failed to find significant change[20,21]. However, other study indicating that dorzolamide increased choroidal blood flow[22]. In the present study, we examined the effects of single instillation of dorzolamide on the phenylephrine-induced impairment of rabbit optic nerve head blood flow using LSFG and on phenylephrine-induced vascular contraction in vitro. It is known that phenylephrine decreases ocular blood flow. Optic nerve circulation was significantly decreased after single phenylephrine administration in rabbits in vivo[23], and topical adminisuration of phenylephrine produced significant vasoconstriction in retrobulbar arterioles around the optic nerve in rabbits in vitro[24]. In the present study, the phenylephrine-induced MBR decrease was inhibited by dorzolamide instillation. As a reason for that we detected significant change in vivo experiment, it is an important characteristic that we did not use any anesthesia, and eyelid speculum. This method is not affected by the depth of anesthesia, or by corneal disorder due to drying.

Gugleta have demonstrated that an improvement of OBF by dorzolamide results in preservation of visual field in glaucoma patients[25]. So, it may be important to in-

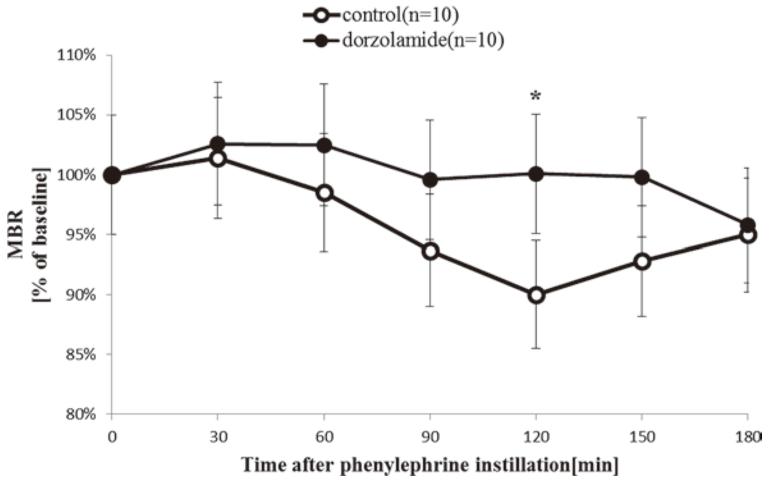

Fig. 1. Effect of dorzolamide on phenylephrine-induced impairment of ONH blood flow in rabbits. Dorzolamide was topically administered at 5 min after the phenylephrine instillation. Difference between dorzolamide and control eyes was significant on 120 min after phenylephrine instillation. Data are expressed as mean ± S. E. M. (n=10). *P< 0.05 vs. time-matched control group (Student t-test).

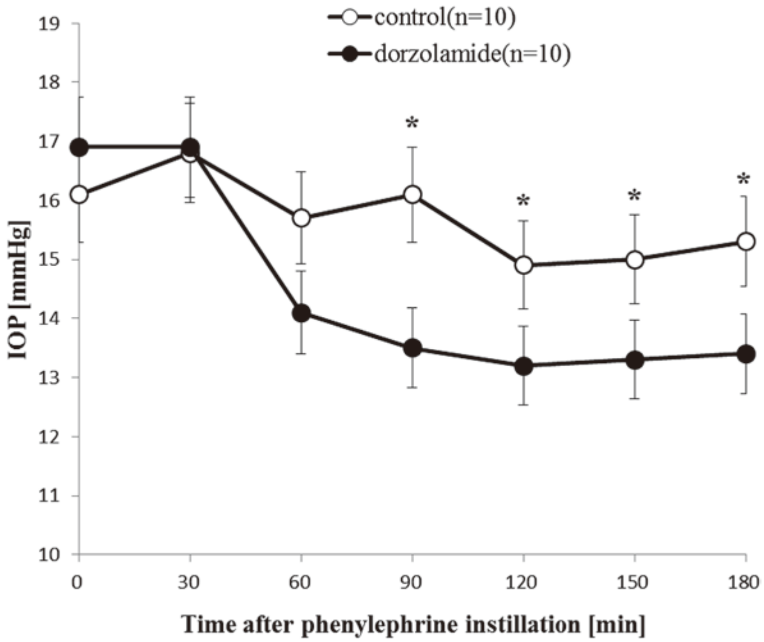

Fig. 2. Effects of dorzolamide on intraocular pressure. Dorzolamide was topically administered at 5 min after the phenylephrine instillation. Difference between dorzolamide and control eyes was significant on 90, 120, 150, 180 min after phenylephrine instillation. Data are expressed as mean ± S. E. M. (n=10). *P< 0.05 vs. time-matched control group (Student t-test).

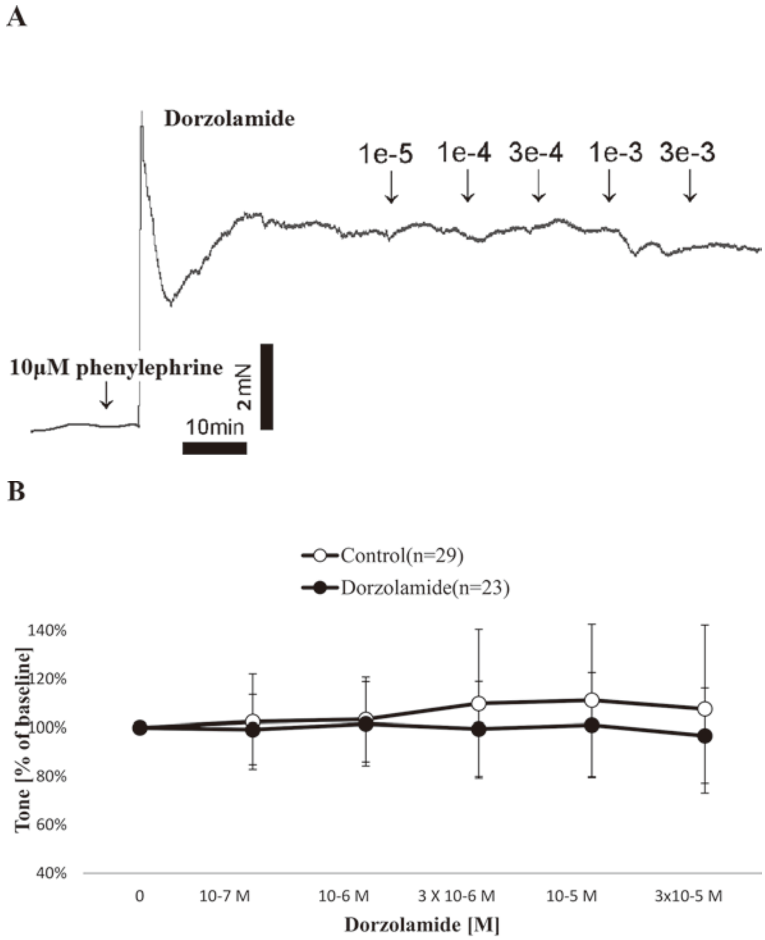

Fig. 3. Effects of dorzolamide on phenylephrine-induced contraction of rabbit ciliary arteries. (A) The figure shows examples of individual experiments as continuous recordings of wall tension. Dorzolamide were added to the recording chamber at the time indicated by the vertical arrows. (B) It showed no significant change between control and dorzolamide.

vestigate the mechanisms of dorzolamide on OBF.

In the present study, topical application of dorzolamide had significant effect on decreasing IOP and increasing OBF in rabbits in vivo. The exact mechanisms of dorzolamide increasing OBF is still not clear. Dorzolamide may have direct effect on the ocular vasculature or indirect effect through IOP reduction. In order to differentiate these two possibility, we studied the effect of dorzolamide on isolated rabbit ciliary arteries. In present study, dorzolamide had no effect on the vascular smooth muscle up to the concentration of 3×10^{-5} M. This concentration seems to be enough to reach the optic nerve after topical application[26]. So, it is concluded that dorzolamide increasing OBF not by primary effect on the ocular vasculature. However, it has been suggested that dorzolamide induced vasorelaxation of intraocular porcine ciliary arteries in vitro which is depends on NO[27]. Moreover, Pickkers et al.[28] have reported that the vasodilator effect of other CAI, acetazolamide, is caused by opening of BK_{Ca} channel on vascular smooth muscle cell. In the present study, we could not find the vasodilating effect of dorzolamide on isolated rabbit ciliary artery. Further study will be needed for definitive conclusion to be made regarding the increasing mechanism of OBF by dorzolamide instillation.

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