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Effects of Tetramethylpyrazine on Prostaglandin E_2- and Prostaglandin E_2 Receptor Agonist-induced Disruption of Blood-Aqueous Barrier in Pigmented Rabbits

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Purpose: To evaluate the effect of tetramethylpyrazine on the elevation of aqueous flare and intraocular pressure (IOP) induced by prostaglandin (PG) E_2 and PGE_2 receptor (EP) agonists.

Methods: PGE_2 or EP agonists (11-deoxy PGE_1, EP_2 agonist; 17-phenyl trinor PGE_2, EP_1 and EP_3 agonist; or sulprostone, EP_1 and EP_3 agonist), 25 μg/mL, were transcorneally administered to pigmented rabbits. Animals were pretreated with tetramethylpyrazine intravenously (10 or 30 mg/kg) or topically (0.1% solution). Aqueous flare was measured using a laser flare-cell meter, and the intensity was expressed as the area under the curve (AUC). Intraocular pressure was measured using a noncontact tonometer.

Results: After administration of PGE_2, aqueous flare and IOP increased and then gradually decreased. The AUC of eyes pretreated with tetramethylpyrazine, 10 or 30 mg/kg, intravenously, or topical 0.1% solution, was significantly smaller than that of the controls. The mean Δ IOP of eyes pretreated with tetramethylpyrazine, 30 mg/kg intravenously, was significantly lower than that of the controls. After administration of 11-deoxy PGE_1, aqueous flare increased and then gradually decreased. 17-phenyl trinor PGE_2 and sulprostone did not disrupt the blood-aqueous barrier. The AUC of eyes pretreated with tetramethylpyrazine, 10 or 30 mg/kg, intravenously, before 11-deoxy PGE_1 application was significantly smaller than that of the controls.

Conclusion: The results indicated that tetramethylpyrazine inhibited PGE_2- or 11-deoxy PGE_1-induced elevation of aqueous flare and IOP. **Jpn J Ophthalmol 2001;45:227–232**
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Key Words: Aqueous flare elevation, EP agonists, pigmented rabbits, prostaglandin E_2, tetramethylpyrazine.

Introduction

Sino-Korean-Japanese traditional herbal (Kampo) medicine has been safely and widely used for more than 3,000 years. The extract of Ligusticum wallichii Franch (Chung chong in Chinese, and Senkyu in Japanese) has been clinically used for treatment of angina pectoris and cerebral stroke.[1–3] Tetramethylpyrazine (Figure 1),an active ingredient of Ligusticum wallichii Franch, reportedly has multiple pharmacological modes of actions.[4–8]

In particular, this drug increases retinal and choroidal blood flow but does not affect systemic blood pressure or heart rate in rabbits.[9] Previous investigators have shown that PGE_2 induces disruption of the blood-aqueous barrier and a transient rise of intraocular pressure (IOP).[10,11] PGE_2 binds with high affin-

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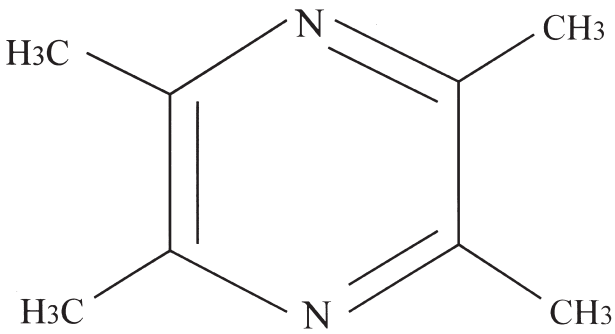

Figure 1. Chemical structure of tetramethylpyrazine.

ity to the PGE_2 receptor (EP).[12] In the iris-ciliary body of rabbits, three subtypes of EP (EP_1, EP_2, and EP_3) have been identified.[13] To our knowledge, the effect of tetramethylpyrazine on the disruption of the blood-aqueous barrier has not been reported. In the present study, we evaluated the effect of tetramethylpyrazine on the disruption of the blood-aqueous barrier and the acute rise of IOP induced by transcorneally applied PGE_2, and examined the effect of EP agonists on the blood-aqueous barrier in pigmented rabbits. We also studied the effects of 11-deoxy PGE_1 (EP_2 agonist), 17-phenyl trinor PGE_2 (EP_1 and EP_3 agonist), and sulprostone (EP_1 and EP_3 agonist).[14] In addition, we evaluated the effect of tetramethylpyrazine on aqueous flare elevation induced by 11-deoxy PGE_1 in pigmented rabbits.

Materials and Methods

Animals

Fifty-seven Japanese mongrel pigmented male rabbits weighing 2.5 to 3.0 kg were used. The animals were housed and treated according to the Association for Research in Vision and Ophthalmology (USA) Resolution on Use of Animals in Research.

Chemicals

Tetramethylpyrazine (molecular weight: 136.2) was obtained from Wako Pure Chemical (Osaka). For intravenous injection, tetramethylpyrazine was dissolved in 100% ethanol and diluted to 5% ethanol with 0.9% NaCl just before use. For topical instillation, the agent was diluted to 5% ethanol plus 0.4% polysorbate 80. PGE_2, 17-phenyl trinor PGE_2, sulprostone, and 11-deoxy PGE_1, all purchased from Funakoshi Company (Tokyo), were dissolved in 100% ethanol, and stored at −70°C. PGE_2 and the EP agonist solution were diluted (5% ethanol in 0.9% NaCl) just before use.

Pretreatment of Animals

The animals were divided into nine groups. In the first group, rabbits received 5 mL of the vehicle (5% ethanol in 0.9% saline, intravenous) 30 minutes before PGE_2 application. In the second group, rabbits received intravenous injection of tetramethylpyrazine (10 mg/kg) 30 minutes before PGE_2 application. In the third group, rabbits received intravenous injection of tetramethylpyrazine (30 mg/kg) 30 minutes before PGE_2 application. In the fourth group, rabbits received topical instillation of 0.1% tetramethylpyrazine solution 120, 105, 90, 75, 60, 45, 30, and 15 minutes before PGE_2 application. In the fifth, sixth and seventh groups, rabbits received 5 mL of the vehicle (5% ethanol in 0.9% saline; intravenous) 30 minutes before 17-phenyl trinor PGE_2, sulprostone, or 11-deoxy PGE_1 application. In the eighth group, rabbits received intravenous injection of tetramethylpyrazine (10 mg/kg) 30 minutes before 11-deoxy PGE_1 application. In the ninth group, rabbits received intravenous injection of tetramethylpyrazine (30 mg/kg) 30 minutes before 11-deoxy PGE_1 application.

PGE_2 and EP Agonist Administration

Transcorneal diffusion of PGE_2, 17-phenyl trinor PGE_2, sulprostone, or 11-deoxy PGE_1 was performed as described by Kaji et al.[15] Briefly, a glass cylinder (11 mm in diameter) was placed on the rabbit cornea. Then 600 μL of PGE_2, 17-phenyl trinor PGE_2, sulprostone, or 11-deoxy PGE_1, 25 μg/mL, was put into the cylinder. After 4 minutes, the solution was pipetted out and the cylinder was removed. The corneal surface and conjunctival sac were rinsed with 20 mL of 0.9% NaCl solution.

Aqueous Flare Measurement

Aqueous flare (photon counts/milliseconds) was measured in the animals using a flare-cell meter (FC-1000; Kowa, Tokyo), according to the method described by Sawa et al.[16] The laser flare-cell meter measured intracameral protein. Five measurements were performed at each time point and the mean value was calculated. Aqueous flare elevation was expressed as the area under the curve (AUC) in arbitrary units.

IOP Measurement

Intraocular pressure was measured using a noncontact tonometer (Konan, Tokyo). Two measurements were performed at each point to obtain the

mean value. Intraocular pressure was measured just before and 10, 20, 30, and 60 minutes after PGE_2 application. Maximal change in IOP from baseline was expressed as Δ IOP.

Statistical Analysis

The results were expressed as mean value ± standard deviation. Statistical analysis was performed using the Dunn multiple comparisons procedure. $P < .05$ was considered statistically significant.

Results

Changes in aqueous flare following transcorneal diffusion of PGE_2 to the cornea in the first group are shown in Figure 2. After transcorneal diffusion of PGE_2 to the cornea with the use of a glass cylinder, aqueous flare increased up to 90 minutes, gradually decreased, and returned to baseline levels at 8 hours.

The AUCs of the PGE_2-induced aqueous flare elevation in the first through fourth groups are shown in Figure 3. The mean AUC of the PGE_2-induced aqueous flare elevation after pretreatment with the vehicle in the first group was 1,809 ± 101 arbitrary units (n = 9 eyes). The mean AUCs of rabbits pretreated with tetramethylpyrazine, 10 mg/kg intravenous (the second group), 30 mg/kg intravenous (the third group), and topical 0.1% solution (fourth group) were 1,417 ± 179, 1,016 ± 95, and 1,285 ± 59 arbitrary units, respectively (n = 5 eyes in each group). These values were significantly lower than those in the controls (the first group) ($P < .01$, or $P < .001$).

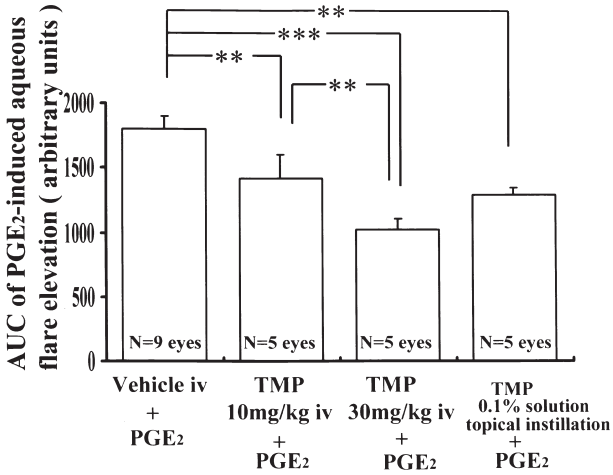

Figure 3. Area under curve (AUC) of PGE_2-induced aqueous flare elevation after various pretreatments. Rabbits were pretreated by iv injection of tetramethylpyrazine (TMP; 10 mg/kg, or 30 mg/kg) or topical instillation of TMP (0.1%). Animals pretreated by iv injection of vehicle served as controls. PGE_2 (25 μg/mL) was applied to cornea. Means ± standard deviations are plotted. Statistical analysis was performed using Dunn multiple comparisons procedure. $^{**}P < .01$; $^{***}P < .001$

The acute increase in IOP following topical application of PGE_2 to the cornea is shown in Figure 4. After transcorneal diffusion of PGE_2 to the cornea with the use of a glass cylinder, the IOP increased,

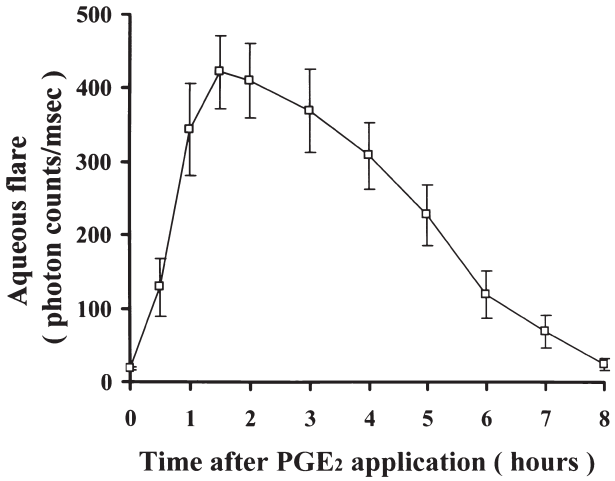

Figure 2. Changes in aqueous flare after transcorneal diffusion of PGE_2 in rabbits pretreated with vehicle. PGE_2 (25 μg/mL) solution was applied to cornea using glass cylinder. Means ± standard deviations are plotted (n = 9 eyes).

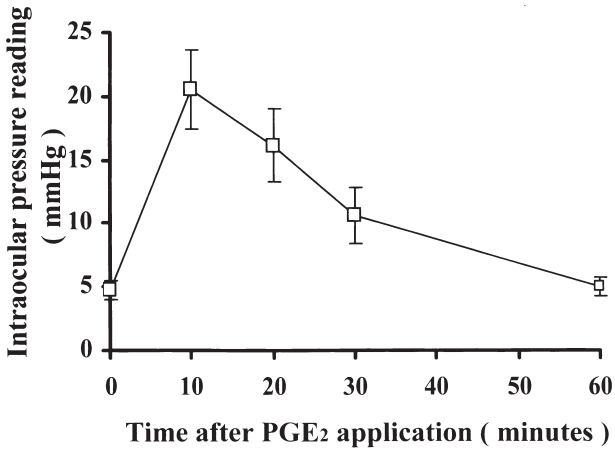

Figure 4. Changes in intraocular pressure elevation after transcorneal diffusion of PGE_2 in rabbits pretreated with vehicle. PGE_2 solution (25 μg/mL) was applied to cornea using glass cylinder. Means ± standard deviations are plotted (n = 9 eyes).

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reached a maximum at 10 minutes, and decreased thereafter.

The mean Δ IOP of PGE_2-induced IOP elevation in the pretreated groups is shown in Figure 5. The mean Δ IOP of the eyes of rabbits pretreated with vehicle in the first group was 15.3 ± 2.9 mm Hg. The mean Δ IOP of the eyes of rabbits pretreated with tetramethylpyrazine 10 mg/kg intravenous (the second group), 30 mg/kg intravenous (the third group), and topical 0.1% solution (the fourth group) were 13.5 ± 2.4, 10.8 ± 1.2, and 11.0 ± 2.9 mm Hg, respectively (n = 5 eyes in each group). The mean Δ IOP of rabbits treated with tetramethylpyrazine, 30 mg/kg intravenous (the second group), was significantly lower than the control value (the first group) ($P < .05$).

Changes in aqueous flare following transcorneal diffusion of 11-deoxy PGE_1 (the seventh group), 17-phenyl trinor PGE_2 (the fifth group) and sulprostone (the sixth group) to the cornea are shown in Figure 6. After transcorneal diffusion of 11-deoxy PGE_1 to the cornea with the use of a glass cylinder, the aqueous flare increased up to 90 minutes, gradually decreased, and returned to the baseline levels at 12 hours. 17-phenyl trinor PGE_2 and sulprostone did not elevate aqueous flare.

The AUCs of the 11-deoxy PGE_1-induced aqueous flare elevation after various pretreatments are shown in Figure 7. The mean AUC of 11-deoxy

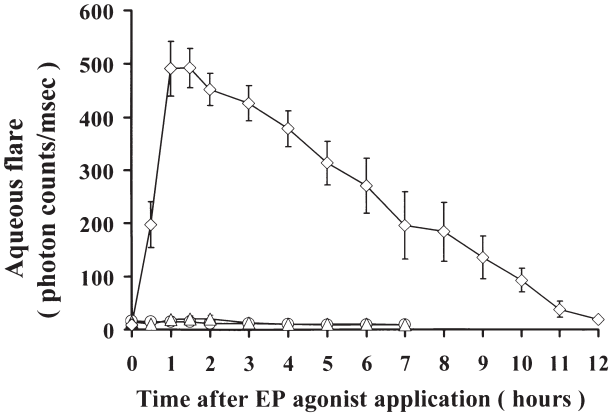

Figure 6. Changes in aqueous flare after transcorneal diffusion of EP agonists in rabbits pretreated with vehicle. 11-deoxy PGE_1 (◇) (n = 9 eyes), 17-phenyl trinor PGE_2 (○) (n = 6 eyes), or sulprostone (△) (n = 6 eyes), 25 μg/mL, respectively, was applied to cornea using glass cylinder. Means ± standard deviations are plotted.

PGE_1-induced aqueous flare elevation in rabbits pretreated with the vehicle (5% ethanol in 0.9% saline) was 2,173 ± 141 arbitrary units (the seventh group) (n = 9 eyes). The mean AUCs of the eyes of rabbits pretreated with tetramethylpyrazine, 10 mg/kg intravenous (the eighth group), and 30 mg/kg intravenous (the ninth group), were 1,802 ± 116 and 1,480 ± 44 arbitrary units, respectively. These values

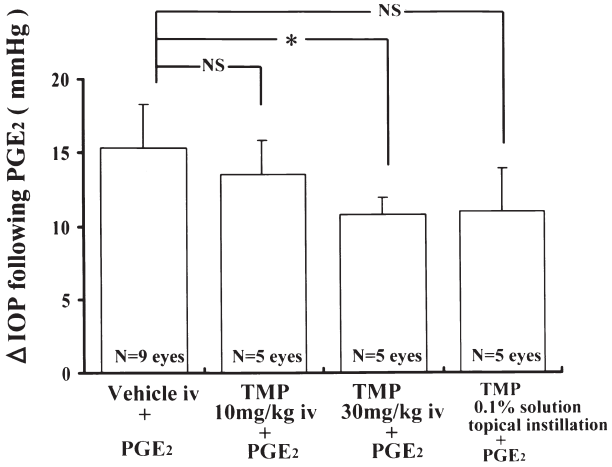

Figure 5. Maximal changes in intraocular pressure (IOP) following PGE_2 and various pretreatments. Rabbits were pretreated by iv injection of tetramethylpyrazine (TMP; 10 mg/kg, or 30 mg/kg) or topical instillation of TMP (0.1%). PGE_2, 25 μg/mL, was applied to cornea. Maximal change in IOP from baseline is expressed as Δ IOP. Means ± standard deviations are plotted. Statistical analysis was performed using Dunn multiple comparisons procedure. *$P <$ .05; NS: not significant.

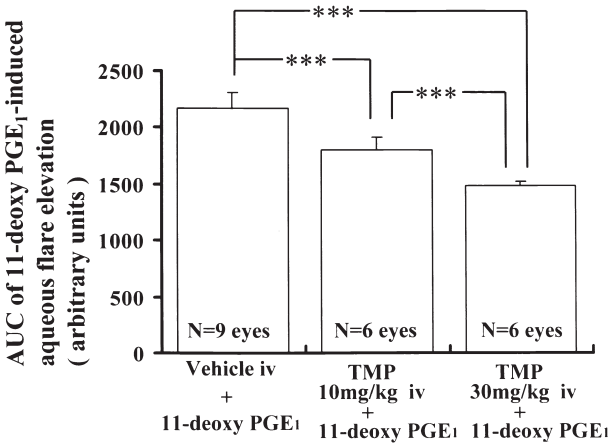

Figure 7. Area under curve (AUC) of 11-deoxy PGE_1-induced aqueous flare elevation after various pretreatments. Rabbits were pretreated by intravenous injection of tetramethylpyrazine (TMP; 10 mg/kg, or 30 mg/kg). Animals pretreated by intravenous injection of vehicle served as controls. 11-deoxy PGE_1 (25 μg/mL) was applied to cornea. Mean ± standard deviations are plotted. Statistical analysis was performed using Dunn multiple comparisons procedure. ***$P <$ .001.

were significantly lower than those in the controls (the seventh group) ($P < .001$).

Discussion

In the present study, aqueous flare and IOP were elevated following transcorneal diffusion of PGE_2 into the rabbit eye pretreated with the vehicle (the first group). The present findings were quite similar to those in eyes without pretreatment that were previously reported by our laboratory.[15,17] To our knowledge, the median lethal dose (LD_{50}) of an intravenous injection of tetramethylpyrazine in rabbit is not known. Chiou et al[9] reported that intravenous injection of tetramethylpyrazine, 10 mg/kg, increased retinal and choroidal blood flow but did not affect systemic blood pressure or heart rate in rabbits. In the present study, therefore, intravenous injection of tetramethylpyrazine, 10 mg/kg and 30 mg/kg, was used.

Kaji et al[15,18] and Zhang et al[17] previously reported that intravenous injection of nicardipine or nilvadipine (a calcium channel blocker) suppressed aqueous flare elevation induced either by PGE_2 or laser photocoagulation in the iris. Pang et al[8] reported that tetramethylpyrazine acted as a calcium channel blocker in vascular smooth muscle cells. The inhibition of PGE_2-induced aqueous flare elevation by tetramethylpyrazine may be partly due to its action as a calcium channel blocker. Jager et al[19] reported that topical application of PGE_2 to the rabbit eye significantly elevated the concentration of platelet-activating factor in the anterior chamber. Platelet-activating factor has been shown to be an important mediator of PGE_2-mediated ocular inflammation in conditions such as anterior chamber paracentesis.[20] It is possible that tetramethylpyrazine may inhibit the production of platelet-activating factor induced by transcorneal PGE_2 application.

In the present study, topical instillation of 0.1% tetramethylpyrazine solution (eight applications) suppressed the aqueous flare elevation induced by PGE_2. If the drug form can be changed to penetrate into the eye more readily than the present form, the modified drug may be useful for treatment of some types of ocular inflammation, such as laser-induced uveitis, in which PGE_2 plays a major role.[10]

The IOP elevation induced by PGE_2 was inhibited by intravenous injection of tetramethylpyrazine, 30 mg/kg. It was difficult to prepare solutions at concentrations higher than 18 mg/mL for intravenous use because of this drug's poor solubility in saline. It therefore remains unclear whether or not IOP elevation can be inhibited by tetramethylpyrazine in a dose-dependent manner. In the present study, IOP in the rabbit was determined using a noncontact tonometer, which was intended for human use. A nictitating membrane in rabbits sometimes disturbed the measurement of the IOP using a contact-type tonometer. We therefore used a noncontact tonometer. The baseline IOPs of the rabbits in our present study were lower than the levels reported by Rusbell et al.[21] The low pressure in the present study may have been due to the use of a noncontact tonometer that was not adjusted for use in rabbits. Further investigation of the IOP with a tonometer intended for use in the rabbit eye should be carried out.

In the present study, transcorneal diffusion of 11-deoxy PGE_1 (EP_2 agonist) into the rabbit eye induced aqueous flare elevation, but 17-phenyl trinor PGE_2 and sulprostone (EP_1 and EP_3 agonists) caused no disruption of the blood-aqueous barrier. These results were similar to the findings previously described by Protzman and Woodward.[22] It is therefore likely that EP_2 receptors may play an important role in the disruption of the blood-aqueous barrier. Protzman and Woodward further reported that topical instillation of 0.001% to 0.1% PGE_2 in the rabbit eye induced aqueous flare elevation 10-fold more potently than 11-deoxy PGE_1. Bhattacherjee et al[23] studied PG receptors coupled to adenylyl cyclase in the rabbit, and reported that the concentrations of PGE_2 and 11-deoxy PGE_1 eliciting 50% of the maximal response were 0.37 and 1.03 μM, respectively. Thus, the potencies of these prostanoids may be different. In the present study transcorneal diffusion of 11-deoxy PGE_1, 25 μg/mL (AUC = 2,173 ± 141 units), induced higher aqueous flare elevation than PGE_2 at the same dose (AUC = 1,809 ± 101 units). The difference in findings may have been due to the dissimilarity of application methods. Pretreatment by intravenous injection of tetramethylpyrazine, 10 mg/kg and 30 mg/kg, suppressed aqueous flare elevation induced by transcorneal application not only of PGE_2, but also of 11-deoxy PGE_1. Thus, this drug may have an EP_2 receptor antagonistic effect.

In conclusion, tetramethylpyrazine showed a protective effect against the disruption of the blood-aqueous barrier induced by transcorneally applied PGE_2 and EP_2 agonist. The mechanism of the anti-inflammatory effect of tetramethylpyrazine should be further investigated.

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