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Effects of Topical Instillation of Traditional Herbal Medicines, Herbal Extracts, and Their Components on Prostaglandin E_2-induced Aqueous Flare Elevation in Pigmented Rabbits

Yasunori Nagaki*, Seiji Hayasaka*, Xue-Yun Zhang*,
Yoriko Hayasaka*, Nobuo Nakamura* and Katsutoshi Terasawa†

*Department of Ophthalmology, Toyama Medical and Pharmaceutical University, Toyama, Japan;
†Japanese Oriental Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan

Purpose: To evaluate the effect of topical instillation of traditional herbal medicines, herbal extracts, and their components on the elevation of aqueous flare induced by prostaglandin E_2 (PGE_2) in pigmented rabbits.

Methods: Transcorneal diffusion of 25 μg/mL of PGE_2 was carried out through a glass cylinder placed on the cornea to induce aqueous flare elevation in pigmented rabbits. Traditional herbal medicines, herbal extracts, and their components were topically instilled before the PGE_2 application. Aqueous flare was measured with a laser flare-cell meter.

Results: Two instillations, 60 and 30 minutes before PGE_2, of Kakkon-to, Sairei-to, Orengedoku-to, Senkanmeimoku-to, Scutellariae radix extract, Coptidis rhizoma extract, Gardeniae fructus extract, Phellodendri cortex extract, baicalein, baicalin, wogonin, crocetin, berberine, or glycyrrhizine did not inhibit the elevation induced by PGE_2. Two instillations, 60 and 30 minutes before PGE_2, of a Ligusticum wallichii extract (100 mg/mL) inhibited the elevation by 20%. Two instillations (5 and 3 hours before PGE_2) of baicalein (1 mg/mL) or baicalin (5 mg/mL) inhibited the elevation by 16% and 24%, respectively. Two instillations, 5 and 3 hours before PGE_2, of wogonin, crocetin, berberine, or glycyrrhizine did not inhibit the elevation.

Conclusion: Two instillations of Ligusticum wallichii extract 60 and 30 minutes before the PGE_2, and two instillations of baicalein or baicalin, 5 and 3 hours before the PGE_2, inhibited the PGE_2-induced aqueous flare elevation in pigmented rabbits. **Jpn J Ophthalmol 2003;47:249–253** © 2003 Japanese Ophthalmological Society

Key Words: Aqueous flare, baicalein, baicalin, herbal medicines, topical instillation.

Introduction

Traditional herbal (Kampo) medicines have been used clinically in east Asia for 3,000 years. The hot water extract of an herbal mixture is administered orally for several days to treat human disorders. In Japan, several herbal medicines are approved as therapeutic drugs, and we previously reported that the oral administration of Kakkon-to decreased the aqueous flare elevation observed after surgery for age-related cataract and complicated cataract.[1,2]

Other studies from our laboratory demonstrated that the transcorneal diffusion of prostaglandin E_2 (PGE_2), introduced by placing the PGE_2 in a glass cylinder, induced aqueous flare elevation in pigmented rabbits, and the elevation was reproducible when PGE_2 was reapplied after an interval of more than 1 week.[3,4] However, the aqueous flare elevation decreased if the repeated applications of PGE_2 were within a short time (hourly or daily). Weekly applications of PGE_2 did not change the aqueous flare elevation in pigmented rabbits.[4] We also reported that oral administration of Kakkon-to, Sairei-to, and Scutellariae radix extract did not suppress the PGE_2-induced aqueous flare elevation,[5,6] but oral administration of Orengedoku-to and Senkanmeimoku-to inhibited the elevation in pigmented rabbits.[7] We further reported that

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Correspondence and reprint requests to: Yasunori NAGAKI, MD, PhD, Department of Ophthalmology, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

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intravenous injection or topical instillation of tetramethylpyrazine, a component of Ligusticum wallichii (Chungchong in Chinese, and Senkyu in Japanese), inhibited the PGE_2-induced and EP_2 agonist-induced elevation of aqueous flare.[8,9]

In the present study, we examined the effects of topical instillation of traditional herbal medicines (Kakkon-to, Sairei-to, Orengedoku-to, and Senkanmeimoku-to), herbal extracts (Scutellariae radix, Coptidis rhizoma, Gardeniae fructus, Phellodendri cortex, and Ligusticum wallichii), and their components (baicalein, baicalin, wogonin, crocetin, berberine, and glycyrrhizine) on the PGE_2-induced flare elevation. We have reported that a single instillation of 0.1% betamethasone phosphate (corticosteroid) 5 hours before PGE_2 application inhibited the PGE_2-induced aqueous flare elevation by 70%, and that a single instillation of 0.1% diclofenac sodium (nonsteroidal anti-inflammatory drug) 1 hour before PGE_2 application inhibited PGE_2-induced aqueous flare elevation by 23%.[10] Therefore, the instillation of the agents used in this study was performed twice, either 60 and 30 minutes, or 5 and 3 hours, before the PGE_2 application.

Materials and Methods

Animals

A total of 53 pigmented male rabbits (Japanese mongrel) that weighed 2.5–3.5 kg each were used. The animals were housed and treated according to the Association for Research in Vision and Ophthalmology Resolution on Use of Animals in Research. The study was approved by the Institutional Animal Care and Utilization Committee, Toyama Medical and Pharmaceutical University, Toyama, Japan.

One eye of each animal was used to determine the effect of one drug. The eyes received two transcorneal applications of PGE_2 at 1- or 2-week intervals. Three months later, the other eye of the animal was used to determine the effect of another drug.

Chemicals

Kakkon-to (Ge-Gen-Tang in Chinese), Sairei-to (Cai-Ling-Tang in Chinese), Orengedoku-to (Huang-lian-Jie-Du-Tang in Chinese), and Senkanmeimoku-to (Xygan-Ming-Mu-Tang in Chinese) were gifts from Tsumura & Company, Ltd. (Tokyo). Kakkon-to is a mixture of the extracts of 7 medicinal herbs (Table 1), and Sairei-to is a mixture of the extracts of 12 medicinal herbs. Orengedoku-to is a mixture of the extracts of 4 medicinal herbs, and Senkanmeimoku-to is a mixture of the extracts of 19 medicinal herbs.

Table 1. Herbs Used in Kampo Medicines*

Herbs	Kakkon-to	Sairei-to	Orengedoku-to	Senkan-meimoku-to
Puerariae radix	4.0			
Zizyphi fructus	3.0	3.0		
Ephedrae herba	3.0			
Glycyrrhizae radix	2.0	2.0		1.0
Cinnamomi cortex	2.0	2.0		
Paeoniae radix	2.0			1.5
Zingiberis zhizoma	2.0	1.0		
Bupleuri radix		7.0		
Alismatis rhizoma		5.0		
Pinelliae tuber		5.0		
Scutellariae radix		3.0	3.0	1.5
Atraotylodis lanceae rhizoma		3.0		
Polyporus		3.0		
Ginseng radix		3.0		
Hoelen		2.0		
Coptidis rhizoma			2.0	1.0
Gardeniae fructus			2.0	1.5
Phellodendri cortex			1.5	
Saposhnikoviae radix				1.5
Cassiae torae semen				1.5
Forsy thiae fructus				1.5
Cnidii rhizoma				1.5
Angelicae radix				1.5
Rehmanniae radix				1.5
Schizonepetae spica				1.0
Viticis fructus				1.0
Tribuli fructus				1.0
Menthae herba				1.0
Notopterygii rhizoma				1.0
Chrysanthemi flos				1.0
Platycodi radix				1.0
Gypsum fibrosum				3.0

*The indicated amounts (grams) of each gram of herb is mixed and boiled water.

The extracts of Scutellariae radix, Coptidis rhizoma, Gardeniae fructus, Phellodendri cortex, and Ligusticum wallichii were also gifts from Tsumura & Company, Ltd. The extracts were boiled in water to obtain the ophthalmic solution. Scutellariae radix extract contains baicalein, baicalin, and wogonin (Table 2). Coptidis rhizome contains

Table 2. Components in Herbs

Herb	Component
Scutellariae radix	Baicalein
	Baicalin
	Wogonin
Coptidis rhizoma	Crocetin
Ligusticum wallichii	Tetramethylpyrazine

crocetin and berberine. Glycyrrhizae radix contains glycyrrhizine, and Lingusticum wallichii contains tetramethylpyrazine.

Baicalein, baicalin, and wogonin were purchased from Wako Pure Chemical Industries (Osaka, Japan), dissolved in 1 mM Na_2CO_3 solution, and diluted with 20 mM phosphate buffer (pH 7.4). Crocetin was obtained from Sigma Chemical Company (St. Louis, MO, USA), dissolved in 1 mM Na_2CO_3 solution, and diluted with 20 mM phosphate buffer (pH 7.4). Berberine ophthalmic solution was a gift from Saga Pharmaceutical Company (Saga). Glycyrrhizine ophthalmic solution was a gift from Santen Pharmaceutical Company (Osaka).

The traditional herbal medicine was boiled in water to obtain a maximally concentrated solution. A small amount of water was added to the solution, and the mixture was used as the ophthalmic solution. The concentrations (200 mg/mL) of traditional herbal medicines were estimated from the dry weight in the solution. The concentrations (100 mg/mL) of herbal extract used were one half of the maximal concentrated solution of traditional herbal medicine. The maximal concentrated solutions of the components were made and then a small amount of 20 mM phosphate buffer (pH 7.4) was added to the solutions to obtain the ophthalmic solutions. The pH range of the ophthalmic solutions in the present study was 5.5–8.0, and the relative osmotic pressure ranged from 0.9 to 1.1 compared to that of 0.9% NaCl.

PGE_2 was obtained from Funakoshi Chemicals (Tokyo), dissolved in 100% ethanol, and stored at −70ºC. The PGE_2 solution was diluted to 5% ethanol with 0.9% NaCl just before use.

Topical Instillation of Agent or Placebo

The placebo (0.9% NaCl) or 50 μL of the agent was instilled topically into one eye. Two instillations were performed either 30 and 60 minutes, or 3 and 5 hours, before the PGE_2. The bottles were masked, and the person administering the eye drops had no preliminary knowledge of the contents.

Transcorneal Diffusion of PGE_2

For transcorneal diffusion of PGE_2, a glass cylinder (11 mm in diameter) was attached to the cornea as described by Hirata et al.[3] Next, 600 μL of the PGE_2 solution, 25 μg/mL or 7.09×10^{-2} mmol/L, was placed in the cylinder and pipetted out 4 minutes later. The cylinder was removed, and the corneal surface and conjunctival sac were rinsed with 20 mL of 0.9% NaCl. The eyes received two transcorneal applications of PGE_2 at 1- or 2-week intervals (Figure 1).

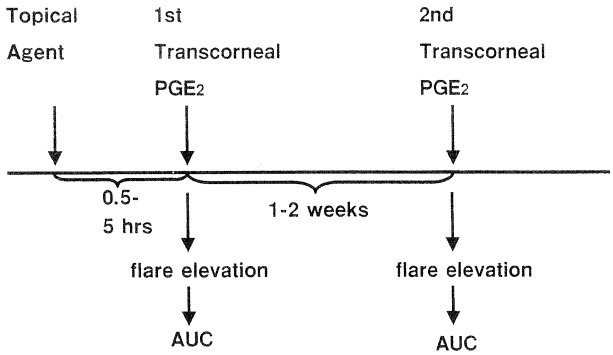

Figure 1. Two transcorneal diffusions of prostaglandin E_2 (PGE_2).

An initial measurement of PGE_2-induced flare elevation was performed in the eye pretreated with an agent or the placebo (0.9% NaCl). After the interval, the same eye received only the PGE_2 solution.

Aqueous Flare Measurements

Aqueous flare was measured with a laser flare-cell meter (FC 1000; Kowa, Tokyo) as described by Sawa et al.[11] The laser flare-cell meter measures the intracameral protein, and five measurements were taken at each time point to obtain a mean value. The measurements were made in the mid-portion of the anterior chamber. The sampling area was 0.075 mm^2. The aqueous flare was expressed as the area under the curve (AUC) for each eye. The degree of inhibition was estimated from the AUCs in the same eye using the following equation:

$$\text{Inhibition (\%)} = [1 - \text{AUC with the treatment}) / (\text{AUC without the treatment})] \times 100.$$

The measurer had no preliminary knowledge of the treatment.

Statistics

Statistical analysis was performed using the Scheffe multiple comparisons procedure. A probability (P) value less than .05 was considered significant.

Results

No apparent abnormality of body weight or behavior was noted in animals treated with the topical instillation of traditional herbal medicines, herbal extracts, or their

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components. No elevation of aqueous flare was found in eyes treated with the topical instillation of such agents.

After PGE_2 was administered, aqueous flare increased, reached a maximum (482 photon counts/ms) at 60–90 minutes, and then gradually decreased and returned to baseline level at 7-8 hours (Figure 2).

When 5 mg/mL of baicalin was topically instilled 5 and 3 hours before the PGE_2 application, the aqueous flare increased, reached a maximum (350 photon counts/ms) at 60–90 minutes, and then gradually decreased and returned to the baseline level at 7–8 hours. Two instillations (60 and 30 minutes before PGE_2) of Kakkon-to, Sairei-to, Orengedoku-to, or Senkanmeimoku-to did not inhibit the elevation. Extracts of Scutellariae radix, Coptidis rhizoma, Gardeniae fructus, or Phellodendri cortex also did not affect the elevation. The effects of topical instillation of herbal medicines, herbal extracts, and their components on the PGE_2-induced aqueous flare elevation are shown in Table 3.

Two instillations (60 and 30 minutes before PGE_2) of Ligusticum wallichii extract (100 mg/mL) inhibited the elevation by 20%. Two instillations (60 and 30 minutes before PGE_2) of baicalein, baicalin, wogonin, crocetin, berberine or glycyrrhizine did not inhibit the elevation. Two instillations (5 and 3 hours before PGE_2) of baicalein (1 mg/mL) or baicalin (5 mg/mL) inhibited the elevation by 16% and 24%, respectively. Two instillations (5 and 3 hours before PGE_2) of wogonin, crocetin, berberine, or glycyrrhizine did not inhibit the elevation.

Table 3. Effects of Herbal Medicines and Their Components on Prostaglandin E_2 (PGE_2)-induced Aqueous Flare Elevation

	Concentration (mg/ml)	Inhibition of Flare Elevation* (%) Double Instillation (60, 30 min)	Double Instillation (5, 3 h)
NaCl	9	6 ± 5	7 ± 5
Traditional herbal medicine			
Kakkon-to	200	7 ± 2	
Sairei-to	200	5 ± 4	
Orengedoku-to	200	9 ± 6	
Senkanmeimoku-to	200	8 ± 5	
Herbal extract			
Scutellariae radix	100	9 ± 3	
Coptidis rhizoma	100	7 ± 5	
Gardeniae fructus	100	9 ± 5	
Phellodendri oortex	100	5 ± 4	
Ligusticum wallchii	100	20 ± 5†	
	75	15 ± 4†	
	50	11 ± 5	
Components			
Baicalein	1	8 ± 6	16 ± 6†
	0.75	7 ± 4	12 ± 6
	0.5	7 ± 4	10 ± 4
Baicalin	5	8 ± 5	24 ± 6†
	2.5	7 ± 4	17 ± 6†
	1	6 ± 4	12 ± 5
Wogonin	5	9 ± 4	11 ± 5
Crocetin	5	6 ± 4	2 ± 4
Berberin	30	6 ± 2	6 ± 3
Glycyrrhizine	10	11 ± 5	5 ± 5

*Four eyes used in each experiment.
†$P < .05$, compared to the value of 0.9% NaCl.

Discussion

In this study, the eyes received two transcorneal applications of PGE_2 at 1- or 2-week intervals, and the agent was topically instilled before the first application of PGE_2.

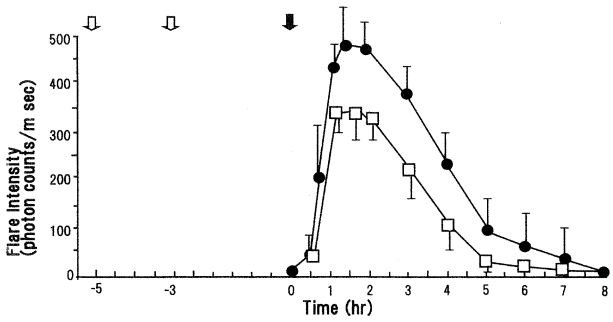

Figure 2. Changes in flare intensity after prostaglandin E_2 (PGE_2) instillation. ●: PGE_2 (25 μg/mL) was applied through a glass cylinder attached to the cornea (⇩). □ Baicalin (5 mg/mL) was topically instilled 5 and 3 hours (⇩) before PGE_2, and then PGE_2 was delivered into the cylinder.

In our preliminary study, the inhibition of flare elevation that was estimated from the values after the instillation of baicalin before the first application of PGE_2 was quite similar to that of the agent before the second application of PGE_2 1 week later. Therefore, it is likely that the agent may be washed out after 1 week.

We previously reported that oral administration of Orengedoku-to and Senkanmeimoku-to inhibited the PGE_2-induced elevation of aqueous flare in pigmented rabbits.[7] In the present study, however, topical instillation of these herbal medicines did not inhibit the elevation. It is possible that these different effects may have been due to the different doses of the active components in the iris-ciliary body due to the dissimilar routes of administration.

In our previous paper, topical instillation of tetramethylpyrazine inhibited the PGE_2-induced aqueous flare elevation.[8] In the present study, Ligusticum wallichii extract also inhibited the elevation. It is possible that tetramethylpyrazine is the active anti-inflammatory component in the Ligusticum wallichii extract. In the present study, two

instillations (60 and 30 minutes before PGE_2) of baicalein and baicalin did not inhibit the elevation, but two instillations (5 and 3 hours before PGE_2) of these compounds inhibited the elevation in pigmented rabbits.

We previously reported that a single topical instillation of 0.42% epinephrine or 0.03% iganidipine 30 minutes before PGE_2 inhibited the elevation of aqueous flare in pigmented rabbits.[12,13] We also showed that when instilled 1 hour before PGE_2, 0.1% diclofenac inhibited flare elevation more strongly (23%) than 0.1% betamethasone (12%), and that when instilled 6 hours before PGE_2, 0.1% diclofenac did not inhibit flare elevation but 0.1 betamethasone inhibited PGE_2-induced aqueous flare elevation by 88%.[10] Corticosteroids have been reported to bind to a specific protein receptor, subsequently enter into the cytoplasm and nucleus, and induce the synthesis of specific proteins.[14] Diclofenac sodium directly inhibits cyclooxygenase,[15] and it is possible that the difference between the times required to detect the effects of betamethasone and that of diclofenac may be due to the dissimilar mechanisms of action of these agents. PGE_2-like activity has been detected in the aqueous humor after paracentesis in rabbits,[16] and it may be involved in traumatic iridocyclitis in rabbits. The mechanism of PGE_2-induced aqueous flare elevation may be complex because the elevation is inhibited by several substances including corticosteroids,[10] nonsteroidal anti-inflammatory drugs,[10] calcium channel blocking agents,[12] and nitric oxide synthase inhibitor.[17] Chen et al[18] reported that baicalin and baicalein inhibited lipopolysaccharide-induced nitric oxide production and decreased inducible nitric oxide synthase gene expression in mouse macrophages. It is thus likely that some traditional herbal medicines decrease nitric oxide synthase gene expression, thereby inhibiting flare elevation.

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