ELSEVIER

Effects of Scutellariae Radix Extract and its Components (Baicalein, Baicalin, and Wogonin) on the Experimental Elevation of Aqueous Flare in Pigmented Rabbits

Yasunori Nagaki, Seiji Hayasaka,
Chiharu Kadoi, Nobuo Nakamura and Yoriko Hayasaka

Department of Ophthalmology, Toyama Medical and Pharmaceutical, University, Toyama, Toyama, Japan

Purpose: To evaluate the possible inhibitory effects of hot water extract of Scutellariae radix and its major components (baicalein, baicalin, and wogonin) on experimental elevation of aqueous flare in pigmented rabbits.

Methods: To produce aqueous flare elevation in rabbits, prostaglandin E_2 (PGE_2), 25 μg/mL, was applied to the cornea with the use of a glass cylinder, or lipopolysaccharides (LPS), 0.5 μg/kg, were injected into an ear vein. Animals were pretreated by the oral administration of 150 g/day of food containing 0.02%, 0.07%, or 0.2% (w/w) extract of Scutellariae radix for 5 days, or by intravenous injection of baicalein, baicalin, or wogonin, 60 μg/kg or 600 μg/kg, 30 minutes before experimental uveitis was induced. Aqueous flare was measured with a laser flare-cell meter. Aqueous flare intensity was expressed as the area under the curve (AUC) in arbitrary units.

Results: The AUC of PGE_2- and LPS-induced aqueous flare elevation was 1,343 and 5,066 arbitrary units, respectively. Pretreatment by oral administration of 0.07% or 0.2% extract of Scutellariae radix did not inhibit PGE_2-induced aqueous flare elevation (AUC: 1,252 and 1,210, respectively), but it did inhibit LPS-induced aqueous flare elevation (AUC: 2,248 and 1,973, respectively). Pretreatment by intravenous injection of 600 μg/kg of baicalein, baicalin, or wogonin inhibited LPS-induced aqueous flare elevation (AUC: 2,289, 2,163, and 1,509, respectively). Pretreatment with 60 μg/kg of wogonin also inhibited LPS-induced aqueous flare elevation (AUC: 1,980).

Conclusion: Hot water extract of Scutellariae radix may have an inhibitory effect on experimental anterior uveitis induced by LPS in pigmented rabbits. **Jpn J Ophthalmology 2001;45:216–220** © 2001 Japanese Ophthalmological Society

Key Words: Baicalein, baicalin, experimental anterior uveitis, Scutellariae radix extracts, wogonin.

Introduction

Traditional Sino-Japanese herbal medicines have been used clinically in east Asia for 3,000 years. Several herbal medicines containing Scutellariae radix (Ogon in Japanese, Huangin in Chinese) extract, such as Sairei-to, Orengedoku-to, and Unsei-in, have anti-inflammatory activity.[1–3] Lin and Shieh[4] reported that in the extracts of Scutellariae radix, three major flavonoids (baicalein, baicalin, and wogonin) have strong anti-inflammatory activity against carrageenan-induced paw edema in rats. We previously reported that intraveous injection of lipopolysaccharide (LPS) or transcorneal diffusion of prostaglandin E_2 (PGE_2) produced aqueous flare elevation in pigmented rabbits.[1] We therefore examined the effects of Scutellariae radix extract, baicalein, baicalin, and wogonin, on aqueous flare elevation induced by LPS or PGE_2 in pigmented rabbits.

Received: May 15, 2000
Correspondence and reprint requests to: Yasunori NAGAKI, MD, Department of Ophthalmology,2630 Sugitani, Toyama 930-0194, Japan

Jpn J Ophthalmol 45, 216–220 (2001)

Published by Elsevier Science Inc.

0021-5155/01/$–see front matter
PII S0021-5155(01)00330-6

Materials and Methods

Animals

Japanese mongrel pigmented male rabbits weighing 2.5 to 3.5 kg (mean = 3 kg) were used. The animals were cared for strictly following the recommendations of the Association for Research in Vision and Ophthalmology, USA. Each animal was housed in a separate cage, and was given food (140–160 g/day, mean = 150 g/day) and water ad libitum. The weight of food consumed was measured every day.

Following PGE_2 administration, the right eye of each animal was used for one experiment. Following LPS injection, both eyes of each animal were measured to obtain the mean value for one experiment.

Chemicals

PGE_2 was purchased from Funakoshi Company, Ltd (Tokyo), dissolved in 100% ethanol, and stored at −70°C. PGE_2 solutions were diluted to 5% ethanol with 0.9% NaCl aqueous solution just before use. LPS from *Escherichia coli*, serotype 055:B5, was obtained from Sigma (St Louis, MO, USA) and was dissolved in 0.9% NaCl aqueous solution just before use. Powdered Scutellariae radix extract was a gift from Tsumura & Co, Ltd (Tokyo). This powdered extract was prepared as follows: Scutellariae radix was boiled in water, and the aqueous extract was lyophilized to obtain powder. Baicalein, baicalin, and wogonin (Figure 1) were purchased from Wako Pure Chemical Industries (Osaka), and were dissolved in 0.01 M Na_2CO_3 solution just before use.

Pretreatment with Scutellariae radix extract Food containing 0.2%, 0.07%, or 0.02% (w/w) extract powder of Scutellariae radix for rabbit consumption was prepared by Sankyo Labo Service Corporation (Tokyo). Each animal was fed the treated food (average consumption, 150 g/day) ad libitum for 5 days. Thereafter, the rabbit was treated to induce experimental elevation of aqueous flare. Animals not undergoing pretreatment with the herbal medicine served as controls.

Pretreatment with baicalein, baicalin, and wogonin A total of 1.5 mL of baicalein, baicalin, or wogonin, 60 μg/kg or 600 μg/kg, was administered into an ear vein of an unanesthetized rabbit 30 minutes before LPS injection. Animals that received an intravenous injection of 1.5 mL of 0.01 M Na_2CO_3 solution served as controls.

PGE_2 Administration

To produce the experimental elevation of aqueous flare, transcorneal diffusions of PGE_2 were performed, as described previously.[1,5] A glass cylinder, 11 mm in diameter, was placed on the right cornea of an unanesthetized rabbit. Then, 600 μL of PGE_2, 25 μg/mL, solution was delivered into the cylinder, and 4 minutes later the solution was pipetted out. The cylinder was removed, and the corneal surface and conjunctival sac were rinsed with 20 mL of 0.9% NaCl aqueous solution.

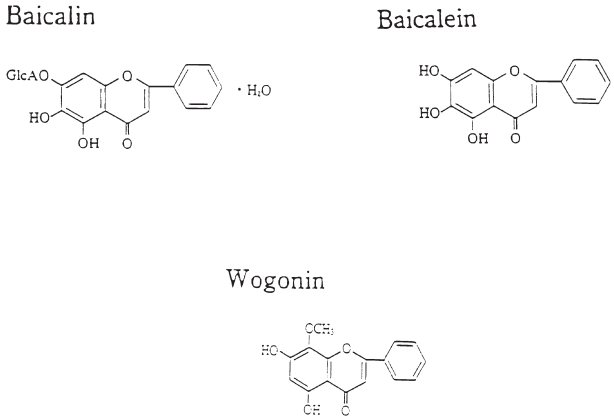

Figure 1. Chemical structures of baicalein, baicalin, and wogonin.

LPS Injection

To produce the experimental elevation of aqueous flare, LPS, 0.5 μg/kg, was injected into an ear vein of an unanesthetized rabbit, as described previously.[1]

Aqueous Flare Measurement

Aqueous flare was measured using a laser flare-cell meter (FC-1000; Kowa, Tokyo), according to the method of Sawa et al.6 With this instrument, the content of intracameral proteins could be determined. Five measurements were performed at each time point to calculate the mean value in the midportion of the anterior chamber. The sampling area was 0.075 mm^3. Aqueous flare elevation was expressed as the area under the curve (AUC) in arbitrary units, as described previously.[1]

Statistical Analysis

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

Results

The animals consumed 140–160 g/day (mean = 150 g/day) of the food with or without herbal medi-

Jpn J Ophthalmol
Vol 45: 216–220, 2001

cines. No apparent abnormality of body weight and behavior was noted in animals pretreated with Scutellariae radix extract (food containing 0.2% extract for 5 days, per os), baicalein (600 μg/kg, intravenous [IV]), baicalin (600 μg/kg, IV), or wogonin (600 μg/kg, IV).

Aqueous flare following topical application of PGE_2 increased up to 60 minutes, gradually decreased, and then returned to baseline level at 8 hours. The mean AUC of PGE_2-induced aqueous flare elevation was 1,343 arbitrary units (Table 1). Pretreatment with 0.07% and 0.2% Scutellariae radix extract did not inhibit flare elevation: the AUC was 1,252 and 1,210 arbitrary units, respectively. After intravenous injection of LPS, aqueous flare increased up to 3 to 4 hours, gradually decreased, and then returned to baseline levels at 24 hours. The mean AUC of LPS-induced aqueous flare elevation was 5,066 arbitrary units (Table 2). The AUCs (1,973 and 2,248 arbitrary units) in animals pretreated with 0.2% and 0.07% Scutellariae radix extract, respectively, were significantly ($P < .001$) smaller than those in animals not undergoing pretreatment. The mean AUC of LPS-induced aqueous flare elevation 30 minutes after injection of 0.01 M Na_2CO_3 solution was 5,021 arbitrary units (Table 3). This value was quite similar to those without pretreatment. The AUCs (2,289 and 2,163 arbitrary units, respectively) in animals pretreated by injection of baicalein and baicalin, 600 mg/kg, were significantly ($P < .01$) smaller than those in animals treated with the vehicle. Baicalein and baicalin, 60 μg/kg, did not inhibit flare elevation. The AUCs (1,509 and 1,980) in animals pretreated by injection of wogonin, 600 μg/kg and 60 μg/kg, respectively, were significantly ($P < .001$ and $P < .01$) smaller than those in animals that received the vehicle. Figure 2 shows the inhibition of LPS-induced aqueous flare elevation by baicalein, baicalin, and wogonin (600 μg/kg).

Discussion

In our present study, rabbits weighing 2.5–3.5 kg (mean = 3 kg) ate 150 g of the treated food daily.

Table 1. Effects of Scutellariae Radix Extract on PGE_2-induced Aqueous Flare Elevation

Pretreatment of Food Containing Aqueous Extract for 5 Days	No. of Rabbits	AUC of PGE_2-induced Aqueous Flare Elevation (arbitrary unit)[a]	P value
None	23	1343 ± 313	
Scutellariae radix			
0.07%	6	1252 ± 142	> .05
0.2%	6	1210 ± 260	> .05

[a]mean ± standard deviation; P value, compared with no pretreatment.

Table 2. Effects of Scutellariae Radix Extract on LPS-induced Aqueous Flare Elevation

Pretreatment of Food Containing Aqueous Extract for 5 Days	No. of Rabbits	AUC of LPS-induced Aqueous Flare Elevation (arbitrary unit)[a]	P Value
None	23	5066 ± 1408	
Scutellariae radix			
0.02%	5	4012 ± 808	> .05
0.07%	5	2248 ± 336	> .001
0.2%	5	1973 ± 408	> .001

[a]mean ± standard deviation; P value, compared with no pretreatment.

Pretreatment by oral administration of 0.07% aqueous extract of Scutellariae radix daily, which was estimated to contain roughly 35 mg of the extract/kg per day, inhibited LPS-induced aqueous flare elevation. Baicalein, baicalin, and wogonin are flavonoids in Scutellariae radix extract. The total amount of flavonoids contained in Scutellariae radix is reportedly about 20%.[7] The ordinary daily dosage of Orengedoku-to for human use is 1.5 g, which contains about 500 mg of the extract of Scutellariae radix or 100 mg of flavonoids. The dose of 35 mg of Scuteralliae radix extract/kg per day in the present study may be about 3.5-fold higher than the ordinary clinical dosage for human use. The doses of 600 μg/kg of baicalein, baicalin, or wogonin used in the present study may be considerably higher than the clinically administered daily dosage for human use. Wakui et al[8] reported that after oral administration of baicalin

Table 3. Effects of Baicalein, Baicalin, and Wogonin on LPS-induced Aqueous Flare Elevation

Pretreatment by Intravenous Injection	No. of Rabbits	AUC of LPS-induced Aqueous Flare Elevation (arbitrary unit)[a]	P Value
None	23	5066 ± 1408	0.95
Vehicle (0.01 M Na_2CO_3)	10	5021 ± 1351	
Baicalein			
60 μg/kg	5	4528 ± 1581	> .05
600 μg/kg	5	2289 ± 461	< .01
Baicalin			
60 μg/kg	5	4630 ± 2893	> .05
600 μg/kg	5	2163 ± 691	< .01
Wogonin			
60 μg/kg	5	1980 ± 1131	< .01
600 μg/kg	5	1509 ± 684	< .001

[a]mean ± standard deviation; P value, compared with the unit pretreated with vehicle.

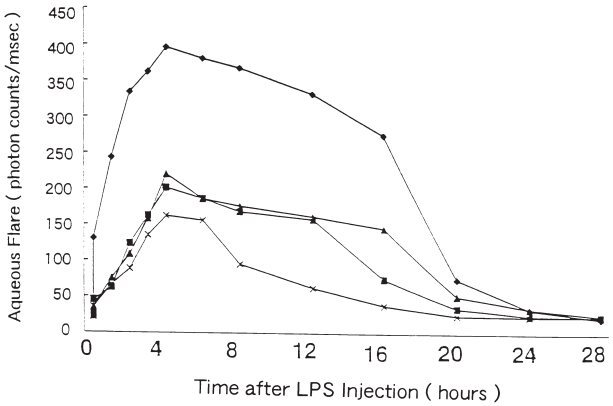

Figure 2. Effects of baicalein, baicalin, and wogonin on lipopolysaccharide (LPS)-induced aqueous flare elevation. One-and-one-half milliliters of 0.01 M Na_2CO_3 (◆), or 1.5 mL of baicalein (■), baicalin (▲), or wogonin (×), in 0.01 M Na_2CO_3, 600 μg/kg, was intravenously injected into rabbit ear vein 30 minutes before LPS administration.

and baicalein in rats, these substances were found in the blood. It is possible that these flavonoids of Scutellariae radix may be directly absorbed from the gastrointestinal tract into the plasma.

Nitric oxide, produced by constitutive and inducible nitric oxide synthases, has been implicated in the development of inflammation.[9–11] Hiraki et al[5] reported that pretreatment with N^G-nitro-L-arginine, a nonselective inhibitor of constitutive and inducible nitric oxide synthases, reduced PGE_2-induced aqueous flare elevation in pigmented rabbits. Nitric oxide is also thought to be a mediator of LPS-induced uveitis in rats and albino rabbits.[12–14] Mandai et al[13] reported that the constitutive form of nitric oxide synthase plays a critical role in the onset of development of endotoxin (LPS)-induced uveitis in rats. Fukuda[15] demonstrated that Scutellariae radix inhibited nitric oxide production in LPS-stimulated macrophages. Wakabayashi[16] showed that baicalein and wogonin attenuated LPS-stimulated nitric oxide synthase induction in macrophages.

In our present study, 0.07% Scutellariae radix extract inhibited LPS-induced aqueous flare elevation, but did not suppress PGE_2-induced aqueous flare elevation. It is likely that nitric oxide is synthesized mainly by inducible nitric oxide synthase instilled in the rabbit eyes following transcorneal diffusion of PGE_2, while nitric oxide is synthesized by constitutive nitric oxide synthase in the early phase following intravenous injection of LPS. Although the mechanism of action of the extract remains unclear, it is likely that the Scutellariae radix extract may reduce constitutive nitric oxide synthase activity, resulting in inhibition of LPS-induced aqueous flare elevation, while the extract may not reduce inducible nitric oxide synthase, resulting in a lack of suppression of PGE_2-induced aqueous flare elevation.

In our present study, Scutellariae radix extracts, baicalein, baicalin, and wogonin inhibited LPS-induced aqueous flare elevation in pigmented rabbits. The dosages of baicalein and baicalin, 600 μg/kg, were higher than the dosages of these components contained in 0.2% Scutellariae radix extract. It is possible that wogonin or some other flavonoids in Scutellariae radix play a role in the inhibition of LPS-induced inflammation.

The authors would like to thank Professor K. Terasawa and the staff of the Department of Japanese Oriental Medicine at Toyama Medical and Pharmaceutical University for their advice, and Tsumura & Ltd for providing herbal medicines. This work was supported in part by a grant-in-aid for scientific research (no. 10671636), and a grant-in-aid for encouragement of young scientists (no. 11771041) from the Ministry of Education, Science, Sports and Culture of Japan, and the research fund of the Institute of Kampo Medicine, Japan.

References

1. Yano H, Hiraki S, Hayasaka S. Effects of Kakkon-to and Sairei-to on experimantal elevation of aqueous flare in pigmented rabbits. Jpn J Ophthalmol 1999;43:279–84.
2. Mizukawa H, Yoshida K, Honmura A, et al. The effect of Orengedokuto on experimentally inflamed rat. Am J Chin Med 1993;21:71–8.
3. Wang LM, Mineshita S. Preventive effects of Unsei-in and Oren- gedoku-to, Chinese traditional medicines, against rat paw edema and abdominal constriction in mice. J Pharm Pharmacol 1996;48:327–31.
4. Lin CC, Shieh DE. The anti-inflammatory activity of Scutellaria rivularis extracts and its components, baicalin, baicalein and wogonin. Am J Chin Med 1996;24:31–6.
5. Hiraki S, Zhang XY, Hayasaka S. Effects of a nitric oxide synthase inhibitor on prostaglandin-induced aqueous flare elevation in pigmented rabbits. Ophthalmic Res 1996;28:260–4.
6. Sawa M, Tsurimaki Y, Shimizu H. New quantitative method to determine protein concentration and cell number in aqueous in vivo. Jpn J Ophthalmol 1988;32:132–42.
7. Takido M. Chemical constituents of Scutellaria root. Gendai Toyo Igaku. 1993;14:247–56.
8. Wakui Y, Yanagisawa E, Ishibashi E, et al. Determination of baicalin and baicalein in rat plasma by high-performance liquid chromatography with electrochemical detection. J Chromatogr 1992;575:131–6.
9. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43:109–42.
10. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002–12.
11. Gad MZ, KKhattab M. Modulation of nitric oxide synthase in inflammation. Arznein Forsch/Drug Res 2000;50:449–55.

Jpn J Ophthalmol
Vol 45: 216–220, 2001

12. Parks DJ, Cheung MK, Chan CC, Roberge FG. The role of nitric oxide in uveitis. Arch Ophthalmol 1994;112:544–6.
13. Mandai M, Mittag TW, Kogishi J, Iwaki M, Handai M, Yoshimura N. Role of nitric oxide synthase isozymes in endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 1996;37:826–32.
14. Bellot JL, Palmero M, Alcoriza N, et al. Concomitant treatment with a 5-lipoxygenase inhibitor improves the anti-inflammatory effect of the inhibition of nitric oxide synthase during the early phase of endotoxin-induced uveitis in rabbit. Ophthalmic Res 1997;29:227–36.
15. Fukuda K. Modulation of nitric oxide production by crude drugs and Kampo medicines. J Trad Med 1998;15:22–32.
16. Wakabayashi I. Inhibitory effects of baicalein and wogonin on lipopolysaccharide-induced nitric oxide production in macrophages. Pharmacol Toxicol 1999;84:288–91.