ELSEVIER

Effects of α_2-Adrenergic Agonists on Lipopolysaccharide-induced Aqueous Flare Elevation in Pigmented Rabbits

Kazuhiko Watanabe, Seiji Hayasaka, Shigeyoshi Hiraki, Masayuki Matsumoto, Chiharu Kadoi and Yasunori Nagaki

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

Purpose: To evaluate the effects of the α_2-adrenergic agonists (clonidine, apraclonidine, and guanfacine) on lipopolysaccharide (LPS)-induced aqueous flare elevation in pigmented rabbits.

Methods: Anterior uveitis was induced with an intravenous injection of LPS (0.5 μg/kg) in an ear vein. The reproducibility of experimental uveitis induced by LPS (0.5 μg/kg) was also determined. Clonidine (0.01, 0.05, 0.25, or 1%), apraclonidine (1%), or guanfacine (1%) was topically instilled in the right eye 30 and 5 minutes before and 30 minutes after LPS application (N = 6 animals, respectively). Clonidine (0.25%) was topically administered three times at 30-minute intervals from 240 or 120 minutes before, or 120 or 240 minutes after LPS application (N = 6 animals, respectively). Then 1 mg/kg of yohimbine was injected into an ear vein 30 minutes before each topical three-time instillation of clonidine 1%, apraclonidine 1% or guanfacine 1% (N = 6 animals, respectively). Aqueous flare was measured with a laser flare-cell meter. Aqueous flare elevation was expressed as the area under the curve (AUC) in arbitrary units. Rabbits received the first LPS intravenous injection, and the control values of the AUC were obtained. Three months later, the α_2-agonist and the second LPS administration were given to the same animals.

Results: The AUCs (5,184 ± 1,255 units) after the first application of LPS were similar to those (5,033 ± 1,290) after the second application 3 months after the first administration. Topical instillation of clonidine inhibited LPS-induced aqueous flare elevation in a dose-dependent manner (0.01–0.25%). Topical instillation of clonidine 1%, apraclonidine 1% or guanfacine 1% inhibited LPS-induced aqueous flare elevation by 98 ± 2.0% (mean ± SD), 86 ± 14% and 94 ± 5.7%, respectively. Pretreatment with intravenous yohimbine prevented the inhibitory effect on flare elevation induced by each agent.

Conclusion: The present findings suggested that topical instillation of some α_2-agonists may have an inhibitory effect on ocular inflammation, which is mediated in part by α_2-receptors.
Jpn J Ophthalmol 2001;45:221–226

Key Words: Apraclonidine, aqueous flare elevation, clonidine, guanfacine, lipopolysaccharide.

Introduction

Clonidine [2-(2,6-dichlorophenylamino) 2-imidazoline], apraclonidine (p-aminoclonidine), and guanfacine [N-amidino-2-(2,6-dichlorophenyl) acetamide] are known α_2-adrenergic agonists, and yohimbine [(16α,17α)-17-hydroxyyohimban-16-carboxy acid methyl ester] is an α_2-adrenergic antagonist.[1] Clonidine and apraclonidine have an imidazoline component, and guanfacine has a guanidine moiety. The α_2-adrenergic agonists lower intraocular pressure when applied topically to the eyes of rabbits, healthy humans and patients with glaucoma.[2–4] These agents prevented an acute rise in intraocular pressure following anterior segment laser surgery and cataract operation.[5–7] The anti-inflammatory actions of clonidine and guanfacine on rat paw edema induced by various inflammatory agents have been reported previously.[8,9] Pretreatment with apraclonidine 1% eye-

Received: March 24, 2000
Correspondence and reprint requests to: Kazuhiko WATANABE, MD, Department of Ophthalmology, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama-shi, Toyama-ken, 930-0194, Japan

Published by Elsevier Science Inc.

0021-5155/01/$–see front matter
PII S0021-5155(00)00386-5

Jpn J Ophthalmol
Vol 45: 221–226, 2001

drops inhibited ocular inflammation induced by YAG laser to the albino rabbit iris, argon laser to the pigmented rabbit iris, and cyclocryotherapy in albino rabbits.[10–12] We previously reported that transcorneal diffusion of prostaglandin E_2 (PGE_2) administered using a glass cylinder produced aqueous flare elevation in the eyes of pigmented rabbits, and that topical clonidine inhibited PGE_2-induced aqueous flare elevation, mediated through the α_2-receptor peripherally.[13,14] In the present study, we investigated the effects of topical α_2-agonists, clonidine, apraclonidine, and guanfacine, on lipopolysaccharide (LPS)-induced aqueous flare elevation.

Materials and Methods

Animals

Eighty-six Japanese mongrel pigmented male rabbits, weighing 2.0–3.5 kg each, were used. All animal procedures conducted in the study followed the tenets of the U.S. Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Chemicals

Clonidine hydrochloride and yohimbine hydrochloride were purchased from Wako Pure Chemical (Osaka). Lipopolysaccharide from *Escherichia coli*, serotype 055: B5, was obtained from Sigma (St. Louis, MO, USA). Apraclonidine hydrochloride 1% was provided by Alcon Japan (Tokyo). Guanfacine hydrochloride was supplied by the Mochida Pharmaceutical Company (Tokyo). Clonidine was dissolved in 1/15 M phosphate buffer (pH 7.4). Yohimbine and guanfacine were dissolved in distilled water. LPS was dissolved in 0.9% NaCl immediately before use.

Induction of Uveitis by LPS

Uveitis was induced by an injection of LPS (0.5 μg/kg) into an ear vein without anesthesia. The rabbits that initially received LPS were given a second dose of the same agent after an interval of 3 months.

Influence of LPS on Aqueous Flare Elevation (Preliminary Experiment)

To determine the changes in flare intensity following the first and the second administrations of LPS, of the 16 rabbits that initially received LPS (0.5 μg/kg), 6 rabbits were given the same agent at an interval of 1 month and 10 rabbits were given the same agent at an interval of 3 months, as a preliminary experiment. After determining that the rabbits seemed to develop a tolerance to LPS between the 1-month and the 3-month administrations, we decided that at 3 months was the best time for the second LPS administration, and this timing was adopted in our present experiments.

Topical Instillation of Clonidine

Twenty-four rabbits received the first intravenous injection of LPS (0.5 μg/kg). Three months later, 50 μL of clonidine (0.01%, 0.05%, 0.25%, or 1%) was topically instilled in the right eye 30 and 5 minutes before, and 30 minutes after, the second LPS application in the same animals. The same volume of vehicle solution was instilled in the left eye. To determine the optimum time for administration, 0.25% clonidine was topically instilled in 30 rabbits from 240, 120, or 30 minutes before, or 120 or 240 minutes after LPS application. This dose was administered three times at 30-minute intervals.

Topical Instillation of α_2-Adrenergic Agonists

Eighteen rabbits received the first intravenous injection of LPS (0.5 μg/kg). Three months later, 50 μL of clonidine 1%, apraclonidine 1%, or guanfacine 1% was topically instilled in the right eye 30 and 5 minutes before and 30 minutes after, the second LPS application in the same animals. The 0.9% NaCl solution was instilled in the left eye in a similar manner.

Administration of Yohimbine

Eighteen rabbits received the first intravenous injection of LPS (0.5 μg/kg). Three months later, yohimbine injection, an α_2-agonist, and the second LPS administration were given to the same animals. Yohimbine (1 mg/kg) was injected into an ear vein, 30 minutes before the topical three-time instillation of clonidine 1%, apraclonidine 1%, or guanfacine 1%. The second LPS injection was administered 60 minutes after yohimbine application.

Aqueous Flare Measurement

Aqueous flare was measured with a laser flare-cell meter (FC-1000; Kowa, Tokyo), according to the method described by Sawa et al.[15] No mydriatics were instilled. A laser flare-cell meter indicated the presence of intracameral proteins. Aqueous flare was measured immediately before and 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, and 28 hours after LPS administration. Five measurements were taken in the midportion of the anterior chamber at each time point to obtain the mean value. The sampling area was 0.075 mm^3.

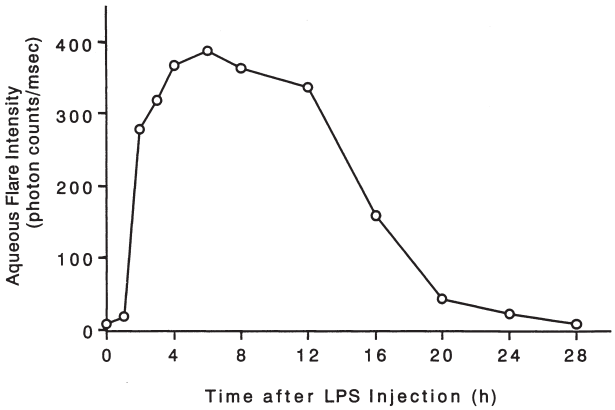

Figure 1. Changes in aqueous flare after intravenous lipopolysaccharide (LPS) (0.5 μg/kg) injection into an ear vein of rabbits.

Aqueous flare elevation is expressed as the area under the curve (AUC). The AUC of the first LPS-induced aqueous flare in the untreated rabbits served as control. After an interval of 3 months, the AUC of the second LPS-induced aqueous flare in the same animals with pretreatment (α_2-agonist or vehicle) was obtained. Inhibition was estimated using the following equation:

$$\text{Inhibition (\%)} = [1 - (\text{AUC by } \alpha_2\text{-agonist or vehicle}) / (\text{AUC of untreated rabbit})] \times 100 \quad (1)$$

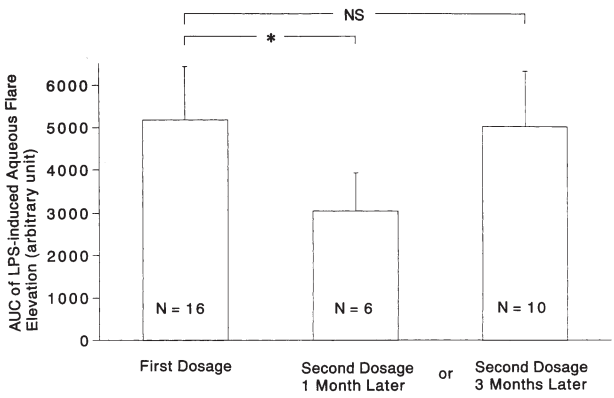

Figure 2. Changes in area under curve (AUC) of lipopolysaccharide (LPS)-induced aqueous flare elevation following first and second administrations of LPS (0.5 μg/kg). Of 16 rabbits that initially received LPS, 6 were given the same agent at an interval of 1 month and 10 were given the same agent at an interval of 3 months, as a preliminary experiment. Mean ± SD is shown. *$P < .05$. NS: not significant, N: number of rabbits used for data.

Statistics

Results are presented as mean ± SD. Differences were determined using Dunn's multiple comparisons procedure, with $P < .05$ accepted as significant.

Results

No systemic conditions, including body weight, were affected by the three-time topical instillation of clonidine 1%, apraclonidine 1%, or guanfacine 1%, or intravenous injection of LPS (0.5 μg/kg) or yohimbine (1 mg/kg).

After intravenous injection of LPS (0.5 μg/kg) the aqueous flare increased to a peak between 4–8 hours, then gradually decreased and returned to baseline levels 28 hours later (Figure 1). The mean ± SD of the AUC of 0.5 μg/kg LPS-induced aqueous flare was 5,184 ± 1,255 arbitrary units.

Changes in flare intensity following the first and second intravenous injections of LPS are shown in Figure 2. The AUCs (5,033 ± 1,290 units) after the

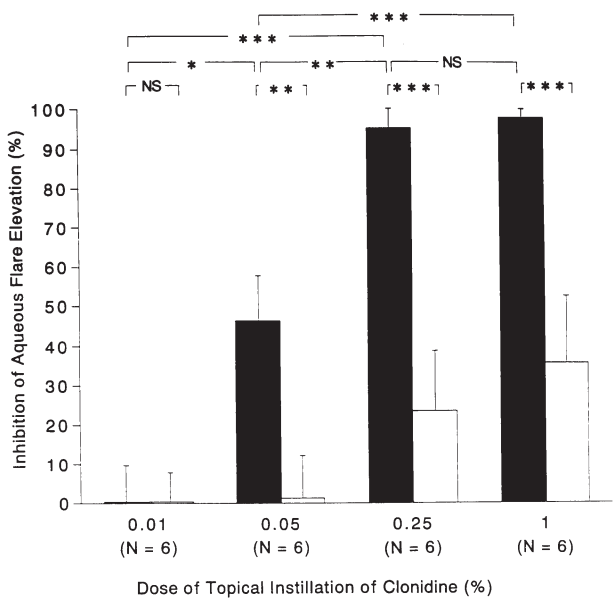

Figure 3. Inhibition of lipopolysaccharide (LPS)-induced aqueous flare elevation by topical instillation of clonidine. Twenty-four rabbits received first intravenous injection of LPS (0.5 μg/kg) and control values of area under curve were obtained. Three months later, α_2-agonist and second LPS administration were given to same animals. Thirty and 5 minutes before and 30 minutes after second LPS application, 0.01%, 0.05%, 0.25%, or 1% clonidine (50 μL) was topically administered to right eyes. Vehicle was instilled in left eyes. Mean ± SD of data from 6 rabbits is shown. *$P < 0.05$, **$P < .01$, ***$P < .001$. NS: not significant. ■ α_2-agonist-administered eye, □ vehicle-administered eye.

Jpn J Ophthalmol
Vol 45: 221–226, 2001

second application of LPS 3 months after the initial administration were similar to those (5,184 ± 1,255 units) after the first application, although those (3,030 ± 903 units) after the second application 1 month after the initial administration (in our preliminary experiment) were significantly smaller than those after the first application ($P < .05$).

Topical instillation of 0.01%, 0.05%, 0.25%, or 1% clonidine inhibited the LPS-induced aqueous flare elevation by 1.0 ± 9.1%, 46 ± 11%, 94 ± 5.9% and 98 ± 2.0%, respectively (Figure 3). In the vehicle-administered eyes, 0.25% and 1% clonidine inhibited aqueous flare elevation by 23 ± 15% and 35 ± 17%, respectively.

The relationship between the administration time of clonidine and LPS-induced aqueous flare elevation is shown in Figure 4. Clonidine 0.25% showed maximum inhibition of flare elevation when instilled three times at 30-minute intervals before LPS application. The inhibition was insignificant when clonidine was instilled 240 minutes before and after LPS treatment.

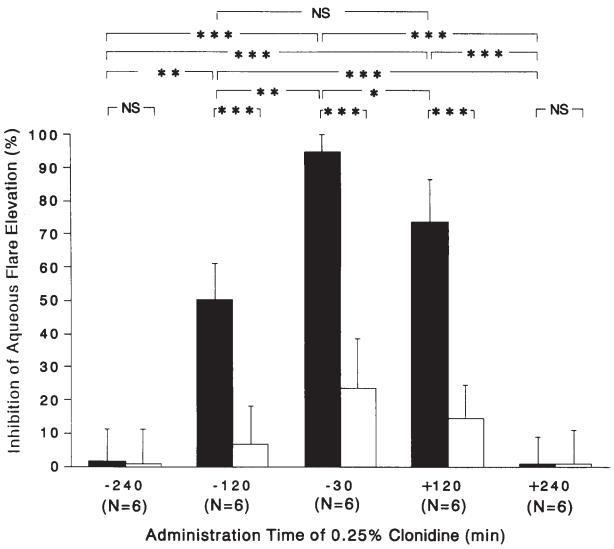

Figure 4. Inhibition of lipopolysaccharide (LPS)-induced aqueous flare elevation by topical instillation of clonidine. Thirty rabbits received first LPS (0.5 μg/kg) intravenous injection and control values of area under curve were obtained. Three months later, α_2-agonist and second LPS administration were given to same animals. Clonidine 0.25% (50 μL), was topically instilled from 240, 120, or 30 minutes before (−) or 120 or 240 minutes after (+) second LPS application. This dose was administered three times every 30 minutes. Mean ± SD of data from 6 rabbits is shown. $*P < .05$, $**P < .01$, $***P < .001$. NS: not significant. ■ α_2-agonist-administered eye, □ vehicle-administered eye.

Topical instillation of clonidine 1%, apraclonidine 1%, or guanfacine 1% inhibited LPS-induced aqueous flare elevation by 98 ± 2.0%, 86 ± 14% and 94 ± 5.7%, respectively (Figure 5). In the vehicle-treated eyes, clonidine 1% and apraclonidine 1% inhibited aqueous flare elevation by 35 ± 17% and 27 ± 16%, respectively.

The effects of intravenous yohimbine on the inhibition of LPS-induced aqueous flare elevation induced by topical clonidine, apraclonidine, or guanfacine are shown in Figure 6. Pretreatment with yohimbine diminished significantly the inhibition of the flare elevation by clonidine 1% (65 ± 13%, $P < .05$), apraclonidine 1% (45 ± 13%, $P < .05$), or guanfacine 1% (60 ± 12%, $P < .05$), when compared with the findings in Figure 5.

Discussion

In the present study, the AUCs after the first application of LPS were similar to those reported by Yano et al.[16] After the second application of LPS, 1 month after the first application, the AUCs were diminished, but the AUCs at 3 months were almost the same as after the first application. This phenomenon may possibly be attributed to LPS tolerance, as reported by Howes et al.[17] Because the rabbits seemed

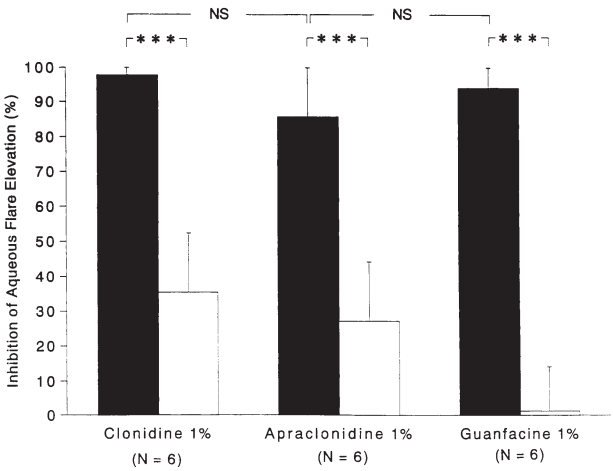

Figure 5. Inhibition of lipopolysaccharide (LPS)-induced aqueous flare elevation by topical instillation of clonidine, apraclonidine, or guanfacine. Eighteen rabbits received first LPS (0.5 μg/kg) intravenous injection and control values of area under curve were obtained. Three months later, α_2-agonist and second LPS administration were given to same animals. Each 1% agent (50 μL) was topically administered 30 and 5 minutes before and 30 minutes after second LPS application. Mean ± SD of data from 6 rabbits is shown. $***P < .001$. NS: not significant. ■ α_2-agonist-administered eye, □ vehicle-administered eye.

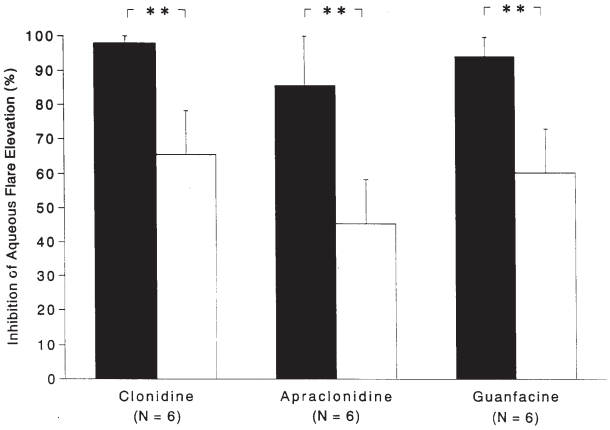

Figure 6. Effect of intravenous yohimbine on inhibition of lipopolysaccharide (LPS)-induced aqueous flare elevation by topical clonidine 1%, apraclonidine 1%, or guanfacine 1%. Eighteen rabbits received the first LPS intravenous injection (0.5 μg/kg) and control values of area under curve were obtained. Three months later, yohimbine injection, α_2-agonist instillation and second LPS administration were given to same animals. No yohimbine pretreatment (■), intravenous yohimbine, 1 mg/kg (□). Yohimbine was intravenously administered 30 minutes before topical three-time instillation of clonidine, apraclonidine, or guanfacine. LPS was administered 60 minutes after yohimbine application. Mean ± SD of data from 6 rabbits is shown. $^{**}P < .01$.

to develop a tolerance to LPS between the 1-month and 3-month administration, we decided that 3 months was the best time for the second LPS administration in the present experiments. Accordingly, the α_2-agonists and the second LPS administration were given to rabbits that had initially received LPS 3 months before.

Changes in intraocular pressure following LPS application were not determined in the present study. The relationship of the changes between aqueous flare elevation and intraocular pressure following administration of LPS or α_2-agonists remains unclear.

A preliminary study showed that a single instillation of clonidine 0.25% inhibited LPS-induced aqueous flare elevation by approximately 30%. In the present study, therefore, α_2-agonist was topically instilled three times at 30-minute intervals.

Lipopolysaccharide-induced uveitis has been used as a model for some human diseases and to evaluate the pharmacologic efficacy of potential anti-inflammatory agents.[18,19] The present findings showed that clonidine, apraclonidine, and guanfacine inhibited the aqueous flare elevation induced by intravenous LPS. The pretreatment with intravenous yohimbine prevented the inhibition of LPS-induced aqueous flare elevation by clonidine, apraclonidine, or guanfacine. Therefore, it is possible that the inhibitory effects of these agents on LPS-induced aqueous flare elevation may be mediated, in part, by α_2-receptors.

We previously reported that clonidine inhibited PGE_2-induced aqueous flare elevation, partly mediated peripherally by α_2-receptors.[14] Kulkarni et al[9] previously demonstrated that pretreatment with yohimbine prevented the anti-inflammatory effect of clonidine, guanfacine, and B-HT 920 on rat paw edema induced by various inflammatory agents including carrageenan. Topical instillation of apraclonidine inhibited a blood aqueous-barrier breakdown following argon laser burning of pigmented rabbit iris, and this effect was diminished by pretreatment with yohimbine.[11] These findings[9,11] were quite similar to the present observations. Holsapple et al[8] reported that an imidazoline component played an important role in anti-inflammatory activity. However, guanfacine, which does not have the imidazoline moiety, inhibited flare elevation in the present study. It is likely that the activation of α_2-receptors may be important for the anti-inflammatory action, as reported by Kulkarni et al.[9]

In animals that underwent topical instillation of clonidine or apraclonidine in the right eye, 35% or 27% inhibition of aqueous flare elevation was found in the left eye. This inhibition was possibly produced by clonidine or apraclonidine absorbed into the vessels of the conjunctiva and nasal mucosa of the drug-treated animals.

Both the previous and the present findings have indicated that some α_2-agonists may have the potential to become new anti-inflammatory agents in the treatment of some kinds of uveitis, although the exact mechanism involved remains unclear.[10–12,14]

The authors would like to thank Alcon Japan and Mochida Pharmaceutical Company for providing α_2-agonists.

References

1. Jasper JR, Lesnick JD, Chang LK, et al. Ligand efficacy and potency at recombinant α_2 adrenergic receptors. Biochem Pharmacol 1998;55:1035–43.
2. Allen RC, Langham ME. The intraocular pressure response of conscious rabbits to clonidine. Invest Ophthalmol 1976; 15:815–23.
3. Yüksel N, Güler C, & Çaglar Y, Elibol O. Apraclonidine and clonidine: a comparison of efficacy and side effects in normal and ocular hypertensive volunteers. Int Ophthalmol 1992; 16:337–42.
4. Schwartzenberg GWS, Buys YM. Efficacy of brimonidine 0.2% as adjunctive therapy for patients with glaucoma inade-

Jpn J Ophthalmol
Vol 45: 221–226, 2001

quately controlled with otherwise maximal medical therapy. Ophthalmology 1999;106:1616–20.

5. Chevrier RL, Assalian A, Duperré J, Lesk MR. Apraclonidine 0.5% versus brimonidine 0.2% for the control of intraocular pressure elevation following anterior segment laser procedures. Ophthalmic Surg Lasers 1999;30:199–204.
6. Araie M, Ishi K. Effects of apraclonidine on intraocular pressure and blood-aqueous barrier permeability after phacoemulsification and intraocular lens implantation. Am J Ophthalmol 1993;116:67–71.
7. Sterk CC, Renzenbrink-Bubberman AC, van Best JA. The effect of 1% apraclonidine on intraocular pressure after cataract surgery. Ophthalmic Surg Lasers 1998;29:472–5.
8. Holsapple MP, Malave A, Lowy MT, Yim GKW. Central and peripheral anti-inflammatory actions by clonidine and a structurally related imidazoline analog. J Pharmacol Exp Ther 1984;229:399–403.
9. Kulkarni SK, Mehta AK, Kunchandy J. Anti-inflammatory actions of clonidine, guanfacine and B-HT920 against various inflammagen-induced acute paw oedema in rats. Arch Int Pharmacodyn 1986;279:324–34.
10. Sugiyama K, Kitazawa Y, Kawai K. Apraclonidine effects on ocular responses to YAG laser irradiation to the rabbit iris. Invest Ophthalmol Vis Sci 1990;31:708–14.
11. Bonomi L, Bellucci R, Pagliarusco A, Stefani L. Apraclonidine protection of the blood-aqueous barrier from traumatic break-down. J Ocul Pharmacol 1995;11:25–35.
12. Sobaci G, Mutlu FM, Söyler M, Tatar T, Yildirim E. Effect of apraclonidine on blood-aqueous barrier. Ann Ophthalmol 1999;31:176–9.
13. Hirata H, Hiraki S, Kaji Y, Takeda N, Fukuo Y, Tachinami K. The effects of transcorneal administration of prostaglandin E2 on rabbit eyes. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1994;98:927–34.
14. Watanabe K, Hayasaka S, Hiraki S, et al. Effects of topical clonidine on prostaglandin E2-induced aqueous flare elevation in pigmented rabbits. Ophthalmic Res 2000;32:210–4.
15. Sawa M, Tsurimaki Y, Tsuru T, Shimizu H. New quantitative method to determine protein concentration and cell number in aqueous in vivo. Jpn J Ophthalmol 1988;32:132–42.
16. Yano H, Hiraki S, Hayasaka S. Effects of Kakkon-to and Sairei-to on experimental elevation of aqueous flare in pigmented rabbits. Jpn J Ophthalmol 1999;43:279–84.
17. Howes EL Jr, Goldyne ME, Perez HD, Goldstein IM, Rosenbaum JT. Lipopolysaccharide tolerance inhibits eye inflammation. Arch Ophthalmol 1985;103:257–60.
18. Rosenbaum JT, McDevitt HO, Guss RB, Egbert PR. Endotoxin-induced uveitis in rats as a model for human disease. Nature 1980;286:611–3.
19. Bhattacherjee P, Williams RN, Eakins KE. An evaluation of ocular inflammation following the injection of bacterial endotoxin into the rat foot pad. Invest Ophthalmol Vis Sci 1983;24:196–202.