ELSEVIER

Effect of Cyclosporin A Eyedrops on Tear Secretion in Rabbit

Hiroshi Toshida, Kiyoo Nakayasu, and Atsushi Kanai

Department of Ophthalmology, Juntendo University School of Medicine, 3-1-3 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

Abstract: We investigated the tear secretion-stimulating effect of cyclosporin A (CyA) eyedrops in rabbits using Schirmer's method. The following findings were obtained: (1) CyA induced tear secretion in a concentration-dependent manner. CyA in 0.1% concentration showed the strongest effect in accelerating lacrimation, and this effect persisted from 3 to 8 hours after instillation. (2) CyA-induced lacrimation was inhibited by atropine sulfate, a muscarinic blocker. (3) CyA-induced lacrimation was also inhibited in a concentration-dependent manner by (D-Pro^2, D-Trp^{7,9})-Substance P, which is a tachykinin-receptor–selective antagonist. (4) CyA-induced lacrimation was also inhibited by capsaicin, which is a stimulator that releases and depletes neurotransmitters from sensory nerve endings. We conclude from these pharmacological studies that, in the rabbit, CyA-induced tear secretion is mediated by both cholinergic and tachykinergic nerves. **Jpn J Ophthalmol 1998;42:168–173** © 1998 Japanese Ophthalmological Society

Key Words: Antagonist, atropine, capsaicin, CyA, cyclosporine, pharmacological study, rabbit, Schirmer's tear test, tachykinin, tear secretion.

Introduction

Cyclosporin A (CyA), a cyclic undecapeptide of fungal origin,[1] is currently being used for immunosuppression in solid organ transplantation.[2] There is also reliable evidence that CyA improves tear production in dogs with keratoconjunctivitis sicca[3] and in patients with Sjögren's syndrome or dry eye syndrome.[4,5] Recently, CyA has been reported to accelerate lacrimation in transplant recipients[6] and experimental rabbits.[7] Although these effects of CyA are believed to be the result of improvement in autoimmunological conditions in the lacrimal gland of treated subjects and animals, there is no evidence that explains the causes of the same effect on lacrimation in normal subjects and animals.

It has been reported that after administration of CyA eyedrops in rabbits, CyA concentrations in both the lacrimal gland and Harderian gland tissues were found to be below the detectable limit (50 ng/g tissue) on radio immunoassay (RIA), and that the effect of CyA on tear secretion was inhibited by atropine sulfate.[7] These findings indicate that enhancement of lacrimation by CyA in rabbits is not attributable to direct actions on the lacrimal and Harderian glands, but is caused by the parasympathomimetic nerves. In addition to the efferent parasympathomimetic nerves, the afferent trigeminal nerves, which are involved in reflex tear secretion, may play a role in enhancement of lacrimation in human eyes.[8] Possible neurotransmitters of the trigeminal nerve are neuropeptides, including substance P (SP), and other tachykinins, such as neurokinin A and B.[9,10]

The present study, therefore, was designed to investigate the effect of CyA eyedrops on tear secretion and its innervation, especially, pharmacological involvement of the afferent tachykinergic nerves and efferent parasympathomimetic nerves.

Materials and Methods

Experimental Animals

All animals were treated in accordance with the ARVO Resolution on Use of Animals in Research.

Received: August 6, 1997

Address correspondence and reprint requests to: Hiroshi TOSHIDA, MD, Juntendo University School of Medicine, Department of Ophthalmology, 3-1-3 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

Jpn J Ophthalmol 42, 168–173 (1998)

Published by Elsevier Science Inc.

0021-5155/98/$19.00
PII S0021-5155(98)00003-3

A total of 51 Japanese albino rabbits of both sexes (bodyweight: 2.5–3.5 kg) were examined by slit-lamp biomicroscopy and Schirmer's Tear Test (STT). Only animals without ocular abnormalities were used.

Drug Preparation

The CyA eye drops (0.1%) and the vehicle, nonionic surfactant, were gifts from the Santen Pharmaceutical Company (Osaka). The CyA solutions were diluted with the vehicle to concentrations of 0.01% and 0.03%. Atropine sulfate (Sigma, St. Louis, MO, USA) was dissolved in sterile isotonic saline for systemic administration. Commercially available (D-Pro^2, D-Trp^{7,9})-Substance P (Sigma), which is a tachykinin-receptor–selective antagonist,[11,12] was dissolved in sterile isotonic saline to make a final concentration of 1% (6.6 mmol/L) or 0.3% (2.0 mmol/L) solutions. Capsaicin (Sigma), which is known to excite some sensory neurons and to release neurotransmitters from their nerve endings, resulting in depletion of the neurotransmitters within them,[13,14] was dissolved in saline solutions of 1.5% ethanol and 8.5% Tween 80 to make final concentrations of 1% (33 mmol/L) or 0.3% (9.9 mmol/L).

Experimental Procedure

Only one drop of CyA eyedrops (about 40 μL) of various concentrations was instilled to the right eye and the vehicle, to the left eye. We determined the tear volume of each animal using the Schirmer's tear test strip (Alcon, Fort Worth, TX, USA) under topical anesthesia, because animals tend to remove the strip by themselves due to the resulting foreign-body sensation.[15] One drop of 0.4% oxybuprocaine hydrochloride ophthalmic solution (Santen Pharmaceutical Company, Osaka) (40 μL) was administered to both eyes of each animal. The Schirmer's strips were placed over the lower lid about 5 minutes after instillation, and the length of wetting was determined after another 5 minutes. This was performed before and at 1, 3, 5, 8, 24, and 48 hours after administration of CyA eyedrops or the vehicle in all the following experiments.

Experiment 1. Various concentrations of CyA eyedrops (0.01%, 0.03%, and 0.1%) or the vehicle were instilled, and STT was conducted as described above.

Experiment 2. Atropine sulfate (0.8 mg/kg), a muscarinic blocker, was administered systemically via a marginal ear vein 1 hour before and 6 hours after CyA eyedrops or its vehicle were instilled. We determined the effect of atropine sulfate on the eyes by measuring the diameter of the pupil. Since mydriasis disappeared within a few hours, an additional identical dose of atropine sulfate was injected 6 hours after instillation of CyA eyedrops.

Experiment 3. (D-Pro^2, D-Trp^{7,9})-SP, a tachykinin receptor-selective antagonist, was administered topically to both eyes 1 hour before administration of CyA eyedrops or its vehicle.

Experiment 4. Capsaicin, a substance that eliminates neurotransmitters in the sensory nerves, was administered topically to both eyes 1 hour before administration of CyA eyedrops or its vehicle.

Statistical Analysis

All results were presented as the mean ± standard error (SE). Student's paired *t*-test was used to evaluate the difference between the STT values of CyA and vehicle in Experiment 1. Nonpaired *t*-test was applied to evaluate the difference between without and with pretreatment of atropine, (D-Pro^2, D-Trp^{7,9})-SP or capsaicin. Significance levels of $P < 0.05$ were considered statistically significant.

Results

Experiment 1

Hyperemia of the conjunctiva was not observed in any of the rabbits on slit-lamp examination. Figure 1 shows the time course of STT values after instillation of CyA eyedrops or vehicle. CyA eyedrops increased the secretion of tear fluid in a concentration dependent manner. CyA eyedrops (0.1%) induced an effect on lacrimation 3 hours after administration ($P < 0.05$). It took 5 hours for the effects of 0.1% CyA eyedrops to reach the maximum ($P < 0.01$). The peak STT value was 24.83 ± 2.70 mm, this action persisted until 24 hours after instillation. Significant differences were observed in the effects of the 0.1% CyA eyedrops and its vehicle between 3 to 8 hours after instillation ($P < 0.05$ or $P < 0.01$). CyA eyedrops exerted no effect on the contralateral control eye in which only the vehicle had been instilled. There were significant differences between the CyA-treated eyes and contralateral control eyes after administration of 0.1% CyA eyedrops. The peak value of STT after 0.03% CyA eyedrops was 18.75 ± 0.52 mm at 3 hours after instillation. Data on the vehicle are not shown except for the control side, when 0.1% CyA eyedrops were used. Since the strongest effect in enhancing lacrimation was noted with the concentra-

Jpn J Ophthalmol
Vol 42: 168–173, 1998

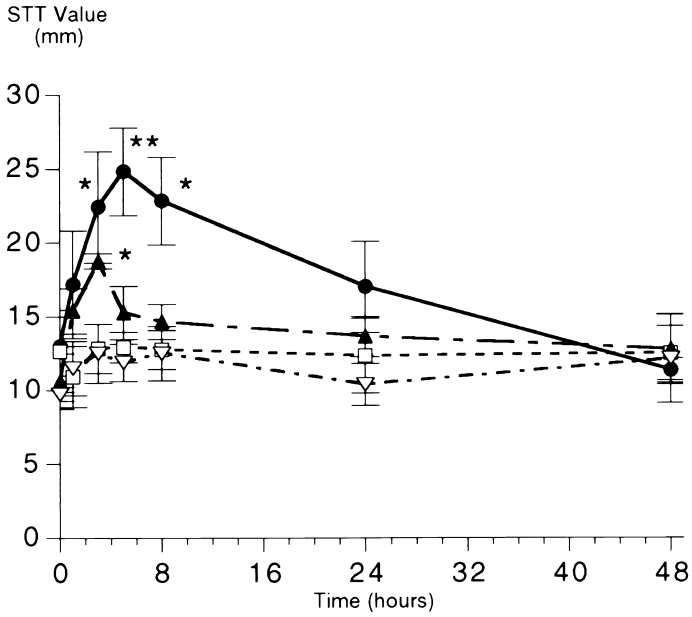

Figure 1. Time course of Schirmer's Tear Test (STT) values after instillation of Cyclosporin A (CyA) eyedrops. STT values increased significantly, with peak effects 3 to 5 hours after 0.1% CyA eyedrop instillation (**$P < 0.01$ or *$P < 0.05$). Tear secretion-stimulating effect of CyA eyedrops was concentration-dependent. Mean ± SE. ● 0.1% CyA (n = 6); ▲ 0.03% CyA (n = 4); ▽ 0.01% CyA (n = 4); □ vehicle (n = 6).

tion of 0.1%, the 0.1% CyA eyedrop was used in the subsequent experiments.

Experiment 2

Mydriasis of the pupil was observed by slit-lamp examination after systemic injection of atropine sulfate. At first, not only 0.1% CyA eyedrops but also the vehicle with atropine sulfate pretreatment enhanced STT value slightly until 1 to 3 hours after instillation. Next, administration of additional atropine sulfate 6 hours after instillation of CyA eyedrops almost completely suppressed the effect of 0.1% CyA eyedrops in enhancing tear secretion compared with 0.1% CyA eyedrops without atropine pretreatment. Significant difference was observed at 8 hours after instillation ($P < 0.05$) (Figure 2).

Experiment 3

Hyperemia of the conjunctiva was not observed in any of the rabbits by slit-lamp examination. (D-Pro^2, D-Trp^{7,9})-SP significantly inhibited the effect of 0.1% CyA eyedrops in enhancing tear secretion in a concen-

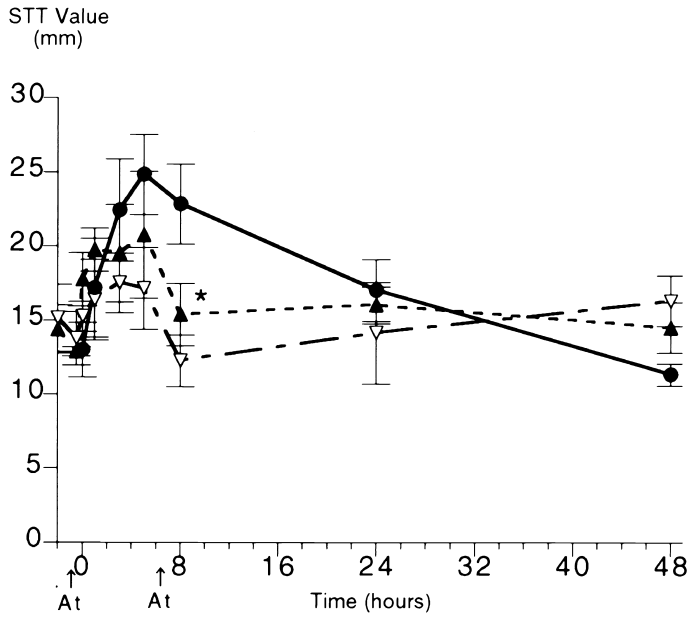

Figure 2. Time course of Schirmer's tear test (STT) values following instillation of 0.1% Cyclosporin A (CyA) eyedrops after pretreatment with atropine sulfate, which inhibits effects of CyA. Additional intravenous atropine sulfate 6 hours after eyedrop instillation almost completely blocked effects of CyA eyedrops (*$P < 0.05$). Mean ± SE. At: intravenous atropine sulfate; ▲ At + 0.1% CyA (n = 4); ▽ At + vehicle (n = 4); ● 0.1% CyA (n = 6).

tration dependent manner, as compared with the STT values in the control eyes in which CyA eyedrops were instilled without pretreatment (Figure 3). Significant differences were observed between the 0.1% CyA eyedrops with preinstillation of 1% (D-Pro^2, D-Trp^{7,9})-SP and 0.1% CyA eyedrops without pretreatment between 5 to 8 hours after instillation ($P < 0.05$).

Experiment 4

Hyperemia of the conjunctiva was not observed in any of the rabbits by slit-lamp examination. Pretreatment with concentrations of either 1% or 0.3% capsaicin significantly inhibited the effect of 0.1% CyA eyedrops in enhancing tear secretion, as compared with the STT values in the control eyes to which only 0.1% CyA drops had been instilled (Figure 4). Significant differences were observed between the 0.1% CyA eyedrops with preinstillation of 1% capsaicin and 0.1% CyA eyedrops without pretreatment between 5 to 8 hours after instillation, and between the 0.1% CyA eyedrops with preinstillation of 0.3% capsaicin and 0.1% CyA eyedrops without pretreatment at 5 hours after instillation of CyA eyedrops ($P < 0.05$).

Discussion

In this study, we investigated the concentration-dependent acceleration of lacrimation by CyA eyedrops, and the possible innervation of this action. The effects of CyA eyedrops were noted over a prolonged period from 3 to 8 hours after a single instillation of CyA eyedrops with significant differences (Figure 1). These effects do not appear to be a direct, simple inflammatory stimulation, because tear secretion increases immediately after instillation of such irritants as prostaglandins in response to the irritation.[15] Since the present study was designed so that STT was measured under topical anesthesia, it can be denied that CyA eyedrops were irritating to the ocular surface. A past report suggested that CyA eyedrops did not directly affect secretion from the lacrimal gland.[7] Among adverse reactions to CyA, effects on the nervous system have been reported in the brain[16] and vessels.[17,18] Accordingly, we investigated the effects of CyA on the nerves involved in lacrimation.

In our study, acceleration of lacrimation by CyA eyedrops was inhibited by pretreatment with atropine sulfate, (D-Pro^2, D-Trp^{7,9})-SP, which is an SP antagonist, and capsaicin, which depletes tachykinin in nerve endings (Figures 2–4). These findings indicate that both the parasympathomimetic and trigeminal nerves are involved in the action. In the present pretreatment with atropine sulfate study, not only 0.1% CyA eyedrops but also the vehicle enhanced STT values slightly until 1 to 3 hours after instillation. We cannot explain why atropine sulfate had this effect. One possibility is that this is an unknown side effect of atropine sulfate. At the moment, the roles of lacrimation, including both basal tear secretion and reflex tear secretion, have not been clarified. Recently, the cloning of the cDNAs that encode muscarinic receptors has identified five distinct

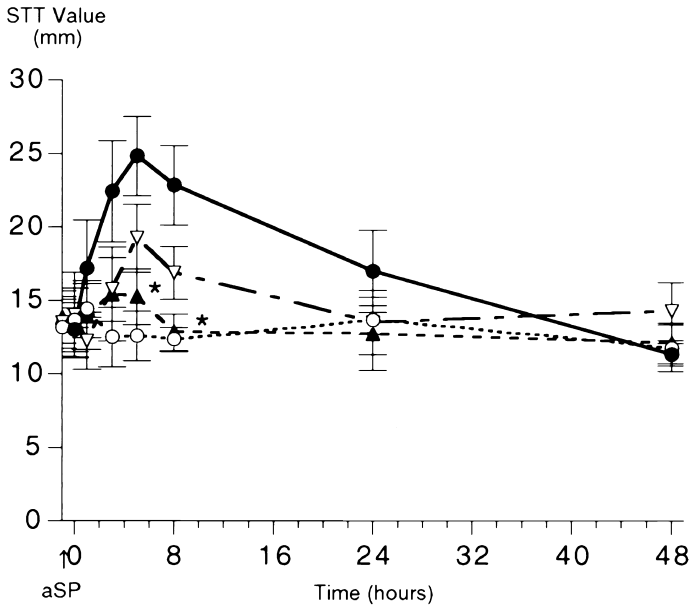

Figure 3. Time course of Schirmer's Tear Test (STT) values following instillation of 0.1% Cyclosporin A (CyA) eyedrops after pretreatment with tachykinin antagonist (D-Pro^2, D-Trp^{7,9})-SP, which inhibited effects of CyA on STT values in a concentration dependent manner. Three to 5 hours after instillation significant differences were observed between effects of 0.1% CyA eyedrops with and without tachykinin antagonist pretreatment. (*$P < 0.05$). Mean ± SE. aSP: topical (D-Pro^2, D-Trp^{7,9})-SP. ▲ 1% aSP + 0.1% CyA (n = 6); ▽ 0.3% aSP + 0.1% CyA (n = 4); ○ 1% aSP + vehicle (n = 6); ● 0.1% CyA (n = 6).

Jpn J Ophthalmol
Vol 42: 168–173, 1998

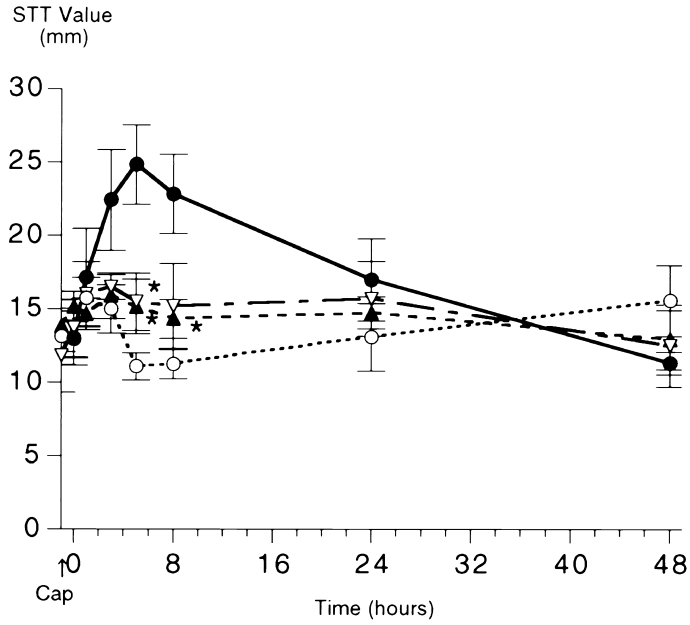

Figure 4. Time course of Schirmer's tear test (STT) values following instillation of 0.1% Cyclosporin A (CyA) eyedrops with and without pretreatment with capsaicin, which completely blocked effects of CyA. Five to 8 hours after instillation, significant differences were observed between effects of 0.1% CyA eyedrops with and without 1% capsaicin pretreatment. Effects also differed between 0.1% CyA eyedrops with and without 0.3% capsaicin pretreatment 5 hours after instillation. (*$P < 0.05$). Mean ± SE. Cap: topical capsaicin. ▲ 1% Cap + 0.1% CyA (n = 6); ▽ 0.3% Cap + 0.1% CyA (n = 5); ○ 1% Cap + vehicle (n = 6); ● 0.1% CyA (n = 6).

gene products, m_1 to m_5. Their cellular properties correspond to pharmacologically designated properties M_1, M_2, M_3, and M_4 (M_5 is not clarified).[19] A previous study by our group reported that pirenzepine, which is an M_1 selective antagonist, was not effective in inhibiting the acceleration of lacrimation by CyA eyedrops, but atropine sulfate,[7] which antagonized all types of muscarinic receptors, did inhibit it. Further studies are necessary to clarify the subtype most closely related to lacrimation. In the present study, we used atropine sulfate to antagonize the parasympathomimetic effect completely. Based upon this, we can consider that it may be possible to substitute pilocarpine or SP for CyA to enhance lacrimation. However, the effect of accelerating tear secretion after instillation of pilocarpine lasted a much shorter time than the effect of 0.1% CyA eyedrops.[20] A larger dose of pilocarpine would cause miosis and insufficiency of accommodation, even in lower concentrations. In the present study of pretreatment with (D-Pro^2, D-Trp^{7,9})-SP or capsaicin, since SP itself has been reported not to have a direct action in accelerating tear secretion,[21] it seems possible that endogenous tachykinins present in the trigeminal nerves, other than exogenously applied SP, may be involved.[22] If there are few side effects, CyA will become one of the drugs of choice to enhance lacrimation.

The actions of CyA are generally thought to be activated for the first time when it becomes bound to intracellular receptors or binding proteins called cyclophilin or immunophilin. Hasel et al[23] reported that cyclophilin is universally present in various organs of mice and humans in a study using cDNA cloning. They detected manifestation of cyclophilin in the entire eyeball. The site of action, however, remains unknown, because examinations of different tissues of the eye have not been conducted. In a previous study, we found that CyA-induced miosis was atropine resistant and suggested its mechanism was very complicated.[24] It may be possible that cyclophilin is involved in both the acceleration of lacrimation and the miosis caused by CyA eyedrops.

In our study, CyA eyedrops accelerated tear secretion in a concentration-dependent manner after a single instillation. The effect was prolonged from 3 to 8 hours by a single instillation of 0.1% CyA eyedrops. Both the parasympathomimetic and trigeminal nerves were indicated in involvement in the mechanism of action. These findings suggest that CyA eyedrops could be applied as a clinical therapeutic drug, not only to decrease tear secretion due to autoimmune diseases, but also to decrease tear secretion in conditions not related to autoimmune diseases (e.g., conjunctivitis sicca, dry eye syndrome). Some adverse reactions, however, have not been sufficiently investigated, and the possibility of species-related differences between man and rabbit remains. Further studies, including clinical administration, are necessary before CyA eyedrops can be applied as a safe therapeutic drug for dry eye and conjunctivitis sicca.

An abstract of this study was presented at the 100th General Conference of the Japanese Ophthalmological Society, May 1996, Kyoto.

References

1. Borel JF. Pharmacology of Cyclosporine (Sandimmun). IV. Pharmacologic properties in vivo. Pharmacol Rev 1989;41: 260–351.
2. Kahan BD. Cyclosporine. N Eng J Med 1989;321:1725–36.
3. Kaswan RL, Salisbury ML, Ward DA. Spontaneous canine keratoconjunctivitis sicca. A useful model for human keratoconjunctivitis sicca. Treatment with cyclosporine eye drops. Arch Ophthalmol 1989;107:1210–6.
4. Gündüz K, Özdemir Ö. Topical cyclosporin treatment of keratoconjunctivitis sicca in secondary Sjögren's syndrome. Acta Ophthalmol 1994;72:438–42.
5. Laibovitz RA, Solch S, Andriano K, O'Connell M, Silverman MH. Pilot trial of cyclosporine 1% ophthalmic ointment in the treatment of keratoconjunctivitis sicca. Cornea 1993;12:315–23.
6. Palmer SL, Bowen PA, Green K. Longitudinal tear study after cyclosporine in kidney transplant recipients. Ophthalmol 1996;103:670–3.
7. Ichikawa T, Kanai A, Yamazaki Y. Tear secretion-stimulating effect of cyclosporin A eyedrops in rabbits. Atarachii Ganka [J Eye] 1995;12:983–7.
8. Walcott B. Anatomy and innervation of the human lacrimal gland. In: Daniel MA, Frederick AJ, eds. Principles and practice of ophthalmology. Basic Sciences. Philadelphia: WB Saunders, 1994:454–8.
9. Otsuka M, Yoshioka K. Neurotransmitter functions of mammalian tachykinins. Physiol Rev 1993;73:229–308.
10. Butler JM, Hammond BR. The effects of sensory denervation on the responses of the rabbit eye to prostaglandin E_1,bradykinin and substance P. Br J Pharmacol 1980;69:459–502.
11. Leander S, Håkanson R, Rosell S, Folkers K, Sundlers F, Tornqvist K. A specific substance P antagonist blocks smooth muscle contractions induced by non-cholinergic, non-adrenergic nerve stimulation. Nature 1981;294:467.
12. Håkanson R, Horig J, Leander S. The mechanism of action of a substance P antagonist (D-Pro^2, D-$Trp^{7,9}$)-SP. Br J Pharmacol 1982;77:697–700.
13. Theriault E, Otsuka M, Jessell T. Capsaicin-evoked release of substance P from primary sensory neurons. Brain Res 1979;170:209–13.
14. Harti G, Sharkey KA, Pierau Fr-K. Effects of capsaicin on peripheral nerves containing substance P and calcitonin gene-related peptide. Cell Tissue Res 1989;256:465–74.
15. Toshida H, Kogure N, Kimura T, Nakayasu K, Kanai A. Effects on tear secretion of isopropyl unoprostone eyedrops in rabbits. Nippon Ganka Kiyo [Folia Ophthalmol Jpn] 1996;47:1323–8.
16. Nussmaum ES, Maxwell RE, Bitterman PB, Hertz MI, Bula W, Latchaw RE. Cyclosporine A toxicity presenting with acute cerebellar edema and brainstem compression. J Neurosurg 1995;82:1068–70.
17. Mayer-Lehnert KH, Schrier RW. Potential mechanism of cyclosporine A-induced vascular smooth muscle contraction. Hypertension 1989;13:352–60.
18. Bokemeyer D, Friedrichs U, Bäcker A, Meyer-Lehnert KH. Cyclosporine A enhances total cell independent of Na-K-ATPase in vascular smooth muscle cells. Clin Invest 1994; 72:992–5.
19. Brown JH, Taylor P. Muscarinic receptor agonists and antagonists. In: Hardman JG, Gilman AG, Limbird LE, eds. Goodman & Gilman's the pharmacological basis of therapeutics. 9th ed. New York: McGraw-Hill, 1995:141–160.
20. Goldstein AM, de Palau A, Botelho SY. Inhibition and facilitation of pilocarpine-induced lacrimal flow by norepinephrine. Invest Ophthalmol 1967;6:498–511.
21. Sullivan DA, Sato EH. Immunology of the lacrimal gland. In: Daniel MA, Frederick AJ, eds. Principles and practice of ophthalmology. Basic Sciences. Philadelphia: WB Saunders, 1994;479–86.
22. Nikkinen A, Lehtosalo JJ, Unisital H, et al. The lacrimal glands of the rat and guinea pig are innervated by nerve fibers containing immunoreactivities for substance P and vasoactive intestinal peptide. Histochemistry 1984;81:23–7.
23. Hasel KW, Glass JR, Godbout M, Sutcliffe G. An endoplasmic reticulum-specific cyclosporin. Mol Cell Biol 1991;11: 3484–91.
24. Toshida H, Ichikawa T, Nakayasu K, Kanai A. The effect of cyclosporin eyedrops on the rabbit pupil. Atarashii Ganka [J Eye] 1995;12:1935–9.