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Endothelin-1 and Intraocular Inflammation in Pigmented Rabbit Eyes

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Abstract: We assessed the function of Endothelin-1 (ET-1) in the development of anterior chamber inflammation in pigmented rabbit eyes. After the injection of ET-1 solution (10^{-13}, 10^{-11}, 10^{-9}, or 10^{-7} M, diluted with 300 μL of artificial aqueous humor) into the anterior chamber, the aqueous protein concentration (APC) increased significantly in a dose-dependent fashion. Peak effects were observed 1–2 hours posttreatment. The APC returned to normal 12 hours after the injection. Pretreatment with antiprostaglandin agents, topical indomethacin, or intravenous diclofenac sodium suppressed the increase in APC. In an endotoxin-induced experimental uveitis model, the ET-1 concentration in the aqueous humor was significantly higher than in normal controls, as was the plasma ET-1 level. These results suggest that ET-1 is an important mediator in ocular inflammatory reactions via arachidonic acid cascade. **Jpn J Ophthalmol 1997;41:150–153** © 1997 Japanese Ophthalmological Society

Key Words: Anti-prostaglandin agents, aqueous protein concentration, arachidonic acid cascade, endothelin-1.

Introduction

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide isolated from a conditioned medium of cultured porcine aortic endothelial cells.[1] In ocular tissues, endothelin receptors occur in the iris, ciliary body, retina, and choroid and corneal endothelium.[2,3] ET-1 is reported to regulate the chorio-retinal circulation,[4–7] functions as a growth factor in the corneal endothelium[8] and epithelium,[9] and influences the intraocular pressure, pupil size, and aqueous protein concentration (APC).[7,10,11] The pattern of changes in the APC after injection of ET-1 into the anterior chamber, however, has not been studied. It is also reported that ET-1 is a potent agonist for arachidonic acid and eicosanoid synthesis, and that arachidonic acid is released from phosphoinositides mainly through activation of phospholipase A_2 in the rabbit iris sphincter[12]; however, its influence in the arachidonic acid cascade in anterior chamber inflammation is not clear.

In this study, we documented the pattern of APC response to the injection of ET-1 to analyze the effect of this peptide in anterior chamber inflammation. The influence of antiprostaglandin agents on the effects of ET-1 was also assessed. In addition, we measured the concentration of ET-1 in the aqueous humor in an endotoxin-induced experimental uveitis model.

Materials and Methods

Experimental Animals

Normal pigmented rabbits of either gender were used (body weight: 1.5–2.5 kg). All experimental procedures were in accord with the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research. Rabbits were anesthetized with an injection of a solution of 5% pentobarbital sodium: 0.5% chlorpromazine hydrochloride = 1:2) 1 mL/kg body weight intravenous 30–60 minutes before the procedures.

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Experimental Procedure

Artificial aqueous humor (Opegard® MA, Senju Pharmaceutical, Osaka) was used to dissolve the ET-1. The concentration of the ET-1 solution was adjusted to 10^{-7}, 10^{-9}, 10^{-11}, and 10^{-13} mol/L when 10 μL solution was diluted with aqueous humor to a volume of 300 μL. After topical anesthesia with 0.4% oxybuprocaine hydrochloride ophthalmic solution (Santen Pharmaceutical, Osaka), 10 μL ET-1 solution was injected intracamerally into one eye using a microsyringe with a 30 gauge needle. The same amount of vehicle was used in the other eye.

Experiment 1

In 10 rabbits, the APC was measured with a laser flare-cell meter (FC-1000, Kowa, Tokyo) and the photon count was converted to an albumin concentration.[13] Measurements were recorded before the injection of ET-1 and 0.5, 1, 2, 4, 8, 12, and 24 hours thereafter. If aqueous humor leaked from the injection site, or the iris or lens was damaged by the needle, the animal was excluded from the study.

Experiment 2

The influence of pretreatment with antiprostaglandin agents on the effects of ET-1 was investigated in 15 rabbits. Group A (n = 5) received an injection of ET-1 only. Group B (n = 5) were pretreated with topical indomethacin before the ET-1 injection. Group C (n = 5) were pretreated with an intravenous injection of diclofenac sodium before the ET-1 injection. In group B, the application of topical indomethacin (Indomelol®, Senju Pharmaceutical, Osaka) to both eyes was begun 2 hours before the injection of ET-1 or vehicle solution and repeated every 30 minutes until 30 minutes before ET-1 injection. In group C, the diclofenac sodium (20 mg/kg body weight) solution was injected into a vein 30 minutes before the injection of ET-1 or the vehicle solution. Three different concentrations of ET-1 (10^{-7} mol/L, 10^{-9} mol/L, or 10^{-11} mol/L) were used. Changes in APC were measured as in experiment 1.

Experiment 3

Experimental uveitis was induced in 10 rabbits by injecting lipopolysaccharide W.S. Thphimurium solution (2.5 μg/kg body weight) into the vein. Two hours later, the APC was measured with the laser flare-cell meter and aqueous humor was collected for measurement of ET-1 concentration. Aqueous humor collected from both eyes of a rabbit was mixed and the ET-1 concentration was determined by a sensitive sandwich enzyme immunoassay.[14] Another 15 rabbits served as controls. In four randomly selected rabbits from each group, the plasma ET-1 concentration was also determined just after collecting the aqueous humor.

Results

There was no fibrin reaction seen on slit lamp examination in any eyes; flare intensity was uniform in all anterior chambers.

Experiment 1

The conjunctiva was slightly hyperemic in the 10^{-7} and 10^{-9} mol/L groups. Injection of ET-1 (10^{-7}, 10^{-9}, 10^{-11}, and 10^{-13} mol/L) increased the APC in a dose-dependent fashion, with peak effects observed 1–2

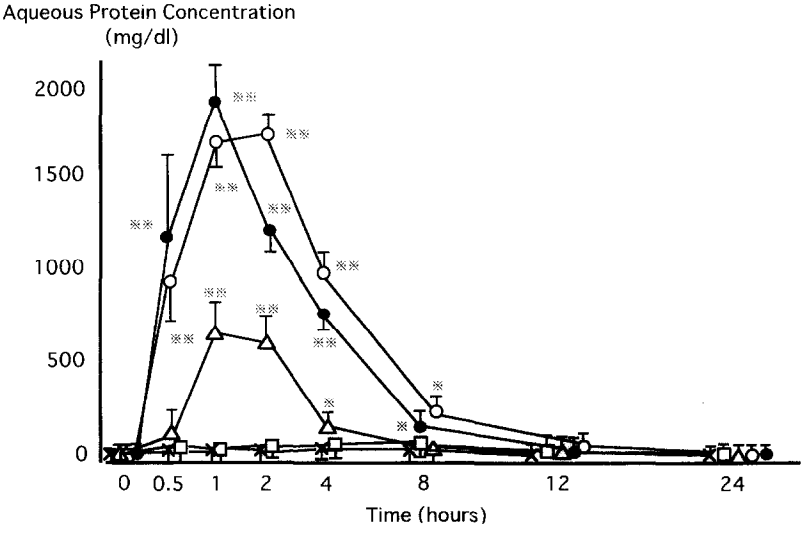

Figure 1. Changes in APC after the injection of ET-1 into the anterior chamber. APC increased significantly with peak effects 1–2 hours after ET-1 injection ($P < 0.01$** or $P < 0.05$*). ET-1 effect was dose-dependent. APC returned to normal 12 hours posttreatment. n = 10 each. Mean ± SE.
● 10^{-7} mol/L ET-1.
○ 10^{-9} mol/L ET-1.
△ 10^{-11} mol/L ET-1.
□ 10^{-13} mol/L ET-1.
× vehicle.

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hours posttreatment (Figure 1). The APC returned to normal 12 hours after the injection. Peak values of APC were 1909.4 ± 221.7 (mean ± standard error) mg/dL in the 10^{-7} mol group; 1744.4 ± 64.6 mg/dL in the 10^{-9} mol/L group; and 654.6 ± 98.5 mg/dL in the 10^{-11} mol/L group. In the 10^{-13} mol/L group, there was no significant increase compared to the vehicle-treated group.

Experiment 2

No hyperemia of the conjunctiva was observed on slit lamp examination in any rabbits. Both topical indomethacin and intravenous injection of diclofenac sodium almost completely suppressed the ET-1 effect on APC (Figure 2).

Experiment 3

The APC prior to collection of the aqueous humor in the endotoxin-induced uveitis (EIU) group (1502.2 ± 141.1 mg/dL, mean ± standard error) was significantly higher than in the normal group (27.5 ± 1.5 mg/dL) ($P < 0.01$, Student's t-test). The aqueous ET-1 concentration in the EIU group (3.74 ± 0.15 × 10^{-12} mol/L) was significantly higher than in the normal group (2.35 ± 0.09 × 10^{-12} mol/L) ($P < 0.01$). Plasma ET-1 concentration was 9.1 ± 0.8 × 10^{-13} mol/L in the EIU group and 6.3 ± 0.9 × 10^{-13} mol/L in the normal group. Aqueous ET-1 concentrations were 1.5–3.9 times higher than the corresponding plasma levels.

Discussion

In the current study, we determined the pattern of change in the aqueous protein concentration (APC) after the injection of ET-1 into the anterior chamber and found that the APC increased significantly in a dose-dependent fashion. Peak effects were observed 1–2 hours posttreatment and values returned to normal at 12 hours. MacCumber et al[2] reported that the APC elevated 48 hours after injection of 2.5 μg of ET-1 into the anterior vitreous. Granstam et al[11] reported that the intracameral injection of ET-1 at pmol doses caused a dose-dependent rise in intraocular pressure and an increase in protein concentration in the aqueous humor, indicating a breakdown of the blood–aqueous barrier. They also reported that injection of 4 pmol ET-1 into the anterior chamber increased prostaglandin E_2 (PGE_2) concentration in the aqueous humor, and the effects of ET-1 were to a large extent mediated by arachidonic acid metabolites.[10] In the current study, APC increasing effect of ET-1 was blocked by the antiprostaglandin agent, suggesting participation of ET-1 in anterior segment inflammation. Abdel-Latif et al[12] reported that ET-1 was a potent agonist for arachidonic acid release and eicosanoid synthesis in the rabbit iris sphincter and that arachidonic acid was released from phosphoinositides mainly through activation of phospholipase A_2. In our current study, we assessed only anticyclooxygenase agents, topical indomethacin, and intravenous diclofenac sodium. The influence of antilipoxygenase agents on the effects of ET-1 should be investigated in future.

The normal concentration of aqueous ET-1 in the rabbit eye has not been documented. In our experiment 3, the aqueous ET-1 concentration in the endotoxin-induced uveitis (EIU) group (3.74 ± 0.15 × 10^{-12} mol/L) was significantly higher than in normal group (2.35 ± 0.09 × 10^{-12} mol/L). It was 1.5–3.9

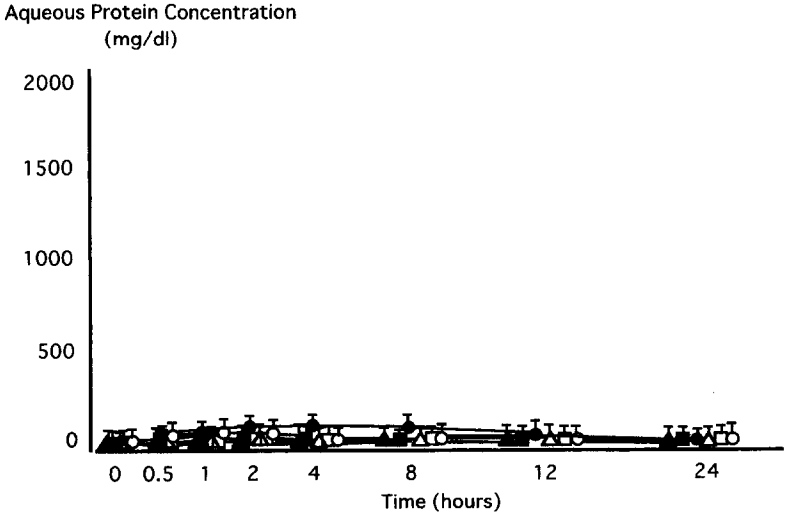

Figure 2. Pretreatment with antiprostaglandin agent and APC changes. Effects of ET-1 on APC were blocked by pretreatment with antiprostaglandin agents. IND: topical indomethacin. DF: intravenous diclofenac sodium. n = 5 each. Mean ± SE.
▲ IND + 10^{-11} mol/L ET-1.
■ IND + 10^{-9} mol/L ET-1.
● IND + 10^{-7} mol/L ET-1.
△ DF + 10^{-11} mol/L ET-1.
□ DF + 10^{-9} mol/L ET-1.
○ DF + 10^{-7} mol/L ET-1.

times higher than the corresponding plasma level in both the EIU and normal groups. Lepple-Wienhues[15] measured endothelin-like immunoreactivity in the human and bovine aqueous humor and reported concentrations 2–3 times higher than the corresponding plasma levels. These reports indicate that the increase of aqueous ET-1 concentration was not due to leakage from a vessel, but to the increased production of ET-1 in the ocular tissues. This study suggests that ET-1 is an important mediator in the ocular inflammatory reaction via arachidonic acid cascade.

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