12(R)-Hydroxyeicosatetraenoic Acid, an Endogenous Corneal Arachidonate Metabolite, Lowers Intraocular Pressure in Rabbits

Jaime L. Masferrer, Michael W. Dunn, and Michal Laniado Schwartzman

12(R)-hydroxyeicosatetraenoic acid (12(R)HETE) is an endogenous corneal epithelial arachidonic acid metabolite formed by the cytochrome P450 system and a potent inhibitor of Na⁺-K⁺-ATPase activity. We studied the effect of topically applied 12(R)HETE, either derived endogenously from corneal epithelium or synthetically prepared, on the IOP of the rabbit eye and compared it to its stereoisomer 12(S)HETE. Topical application of 1 μg of biologically derived 12(R)HETE to both eyes of rabbits resulted in a marked reduction in IOP: a reduction of 4-7 mmHg occurred within 30-120 min. The IOP reduction effect of a single application of 12(R)HETE was long-lasting (9 days), whereas no effect on IOP was found for the vehicle control. Using synthetic compound, we demonstrated that the effect of 12(R)HETE on IOP is dose-dependent. Single topical application of 1, 10, and 50 μg of 12(R)HETE caused a reduction in IOP of 4, 6, and 12 mmHg, respectively. The stereoisomer, 12(S)HETE, did not have any effect on IOP at doses up to 5 μg. The IOP reduction effect of 12(R)HETE was not associated with hyperemia, appearance of flare, miotic response, or increased protein concentration of the aqueous humor. This study was the first to demonstrate that an endogenous inhibitor of Na⁺-K⁺-ATPase generated by the corneal epithelium potently and specifically lowers IOP in rabbits. Further studies are needed to elucidate the mechanism by which 12(R)HETE lowers IOP. Invest Ophthalmol Vis Sci 31:535-539, 1990

12(R)-hydroxyeicosatetraenoic acid is one of the major cytochrome P450-dependent arachidonate metabolites in the bovine, rabbit, and human corneal epithelium.1-2 Microsomal preparation of corneal epithelium in the presence of NADPH converts arachidonic acid to 12(R)HETE, the formation of which is not affected by indomethacin but is inhibited by carbon monoxide and SKF-525A; both are inhibitors of cytochrome P450 enzymes. These findings clearly demonstrate that the 12(R)HETE is a cytochrome P450 metabolite of arachidonic acid, a system that is present in the corneal epithelium.1-3

12(R)HETE is a potent inhibitor of Na⁺-K⁺-ATPase activity in the cornea, kidney, heart, and blood vessels, a property not shared by its stereoisomer, 12(S)HETE.2-5 An endogenous inhibitor of Na⁺-K⁺-ATPase may have fundamental importance in the transport epithelia of the eye that rely on this pump mechanism. Deturgescence of the cornea mediated by the corneal endothelium and secretion of aqueous humor by the ciliary epithelium are examples of basic physiologic functions that are dependent upon Na⁺-K⁺-ATPase-based metabolic pumps and that are readily inhibited by digitalis glycosides, such as ouabain. Therefore, ouabain has been shown to reduce intraocular pressure (IOP) by decreasing the rate of aqueous humor formation.6-8

The current study was undertaken in order to evaluate the effects of topically applied 12(R)HETE, derived from bovine corneal epithelium or synthetically prepared, on IOP in the rabbit eye.

Materials and Methods

Animals

New Zealand albino rabbits (2–3 kg; Hare Merland, Hewitt, NJ) were placed in rabbit boxes until
they became accustomed to handling and restraint before applying topical anesthesia. The investigation conformed to the ARVO Resolution on the Use of Animals in Research.

**Preparation of Biologically Derived 12(R)HETE**

Corneal epithelial microsomes were prepared from freshly obtained cow eyes as described previously. The microsomes (3 mg/ml) were incubated with a mixture of 14C-arachidonic acid (Amersham, Arlington Heights, IL) diluted with cold arachidonic acid (approximately 30,000 cpm/µg) in the presence of a nicotinamide adenine dinucleotide phosphate (NADPH)-generating system composed of glucose-6-phosphate (0.1 mM), NADP+ (0.4 mM), and glucose-6-phosphate dehydrogenase (1 unit; Sigma, St. Louis, MO). The reaction was terminated by acidification at pH 3.5-4.0, and the arachidonate metabolites were extracted with ethyl acetate. The final extract was resuspended in 100 µl of methanol, and the arachidonate metabolites were separated by reverse-phase high-pressure liquid chromatography (HPLC) as described previously. The fractions containing 12(R)HETE were concentrated and submitted to further purification by reverse-phase HPLC. The final compound was resuspended in 500 µl of ethanol and stored at -70°C. The amount of 12(R)HETE recovered was calculated from the specific activity of arachidonate added.

**Preparation of Synthetic 12(R)- and 12(S)HETE**

Synthetic 12(R)HETE was obtained from Dr. J. Falck (Department of Molecular Genetics, University of Texas, Dallas, TX), and 12(S)HETE was obtained from Cayman Chemical Company (Ann Arbor, MI). The appropriate amounts were taken from the stock solution, and the ethanol was dried out with N2. The compounds were resuspended in saline to yield a concentration of 1, 10, or 50 µg in 25 µl.

**Measurements of IOP**

After topical application of one drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon Laboratories, Fort Worth, TX), the IOP of the conscious rabbits was measured with a floating-tip pneumatic tonometer (Digilab model 30R Pneuma-Tonometer; Digilab, Cambridge, MA) calibrated for use on the rabbit eye. IOP measurements were made on each eye once a day for 3 days prior to drug administration, and two times before the experiment started. IOP was measured after a single application of topical drug treatment at 30, 60, 120, 180, 240 min, and once daily for 15 days. In one set of experiments, both eyes of the experimental rabbits were treated with 1 µg of either 12(R)HETE or 12(S)HETE dissolved in a 25-µl drop of saline. The control rabbits received in both eyes a 25-µl drop of saline.

In another set of rabbits, a dose-response experiment was conducted in which 1, 10, or 50 µg of the synthetic 12(R)HETE in 25 µl of saline was applied onto the cornea of the experimental eye, and the contralateral eye received 25 µl of saline.

**Measurement of Ocular Parameters**

Pupillary diameters were measured with a millimeter ruler in normal room light. Aqueous flare and cellular response in the anterior chamber were assessed by slit-lamp examination 180 min after drug application and once a day during the 12-day study.

**Measurement of Aqueous Humor Protein**

New Zealand white rabbits weighing about 2.5 kg were anesthetized with a mixture of ketamine (50 mg/kg body weight) and Xylazine (10 mg/kg body weight). In addition, 1 drop of proparacaine-HCl 0.5% was applied to each eye. Varying doses of 12(R)HETE dissolved in phosphate buffered saline, pH = 7.4, were injected with a 27-gauge needle through the cornea into the anterior chamber of one eye in a final volume of 10 µl. The other eye received an equal volume of buffer. Extreme care was taken to ensure that during injections the needle did not touch the iris and that no aqueous humor was lost upon withdrawal of the needle. Samples of aqueous humor were removed after 15 min from the anterior chamber of both eyes, and the protein content was measured according to the method of Lowry et al.

**Statistical Analysis**

The mean IOP values of the experimental and control groups were compared by a one-way analysis of variance (ANOVA). Differences among groups were analyzed by a Bonferroni test. A P value of less than 0.05 was considered significant.

**Results**

In the first set of experiments, we used experimental and control rabbits, i.e., both eyes of each rabbit received either 12(R)HETE or vehicle. Topical administration of 1 µg of 12(R)HETE derived from bovine corneal epithelium to both eyes of the rabbits resulted in a reduction of IOP within 30 min by 4 mmHg (Fig. 1). The maximal effect, a reduction of 7...
mmHg in IOP, was obtained within 90–120 min. The IOP in the experimental group remained significantly low for 9 days, and IOP returned to normal within 11 days after a single application of the biologically derived 12(R)HETE at a dose of 1 μg (Fig. 1).

In the following experiments, the tested compound was administered to one eye, and a drop of vehicle was administered to the fellow eye, as a control. Fig. 2 shows the effect of topically applied doses of 1, 10, and 50 μg of synthetic 12(R)HETE. It is clear that the reduction observed in IOP after topical application of 12(R)HETE is dose-dependent. The mean IOP was significantly lower after 60 min in eyes treated with 12(R)HETE (1, 10, and 50 μg), and the reduction persisted for at least 24 hr. The maximum decrease in IOP was found to be between 2 and 3 hr after a single topical application of 1, 10, or 50 μg, with reductions in IOP of 4, 6, and 12 mmHg, respectively. At all concentrations tested, 12(R)HETE produced a significant decrease in IOP (approximately 2–4 mmHg) in experimental as compared to control eyes. The contralateral, control eyes showed small reductions of IOP 30–60 min after the administration of 12(R)HETE to the experimental eyes. Conjunctival hyperemia occurred only after the administration of 50 μg of 12(R)HETE, but even at this concentration, slit-lamp examination showed no cells or flare in the anterior chamber. 12(R)HETE did not produce a pupillary response at any dose tested. In addition, topical application as well as intracameral injection of 12(R)HETE at concentrations of 0.01–1.0 μg did not increase the aqueous humor protein concentration: changes of 30 ± 6 and 16 ± 5 mg/dl were observed for control and 12(R)HETE (1 μg)-treated rabbits, respectively, indicating that 12(R)HETE did not produce a breakdown of the blood–aqueous barrier.

When 12(S)HETE was applied onto the eyes of rabbits in the same manner as its stereoisomer 12(R)HETE, no effect on IOP was observed with either 1 or 5 μg (Fig. 3). However, 12(S)HETE at a higher concentration (50 μg) produced an immediate and severe inflammation in the eye, such that we were unable to measure IOP.
Discussion

The current study is the first to demonstrate that an endogenous corneal inhibitor of Na⁺-K⁺-ATPase, 12(R)HETE, potently and specifically lowers IOP in rabbits after topical administration. The effect of 12(R)HETE on IOP as well as on Na⁺-K⁺-ATPase is specific for the R configuration; the stereoisomer 12(S)HETE is inactive. This compound is a cytochrome P450-dependent metabolite of arachidonic acid. Among ocular tissues, the corneal epithelium possesses by far the highest activity of this enzyme system: it is the main site for 12(R)HETE generation. Retinal pigment epithelium ciliary body and retina are additional sites in the eye that produce small but detectable levels of 12(R)HETE. Thus, 12(R)HETE purified from incubates of corneal epithelium with arachidonic acid, as well as synthetically prepared 12(R)HETE, similarly lowers IOP in rabbits. However, the biologically derived 12(R)HETE seems slightly more potent than the synthetic 12(R)HETE: maximal IOP reduction at 1 μg was 7 mmHg for biologically derived, versus 4 mmHg for 1 μg synthetic 12(R)HETE. This difference was due probably to a possible error in quantitating the amount of bovine corneal 12(R)HETE generated from a mixture of labeled and unlabeled arachidonic acid. However, a 5–10% contamination of the synthetic 12(R)HETE with its stereoisomer 12(S)HETE also may account for its lower potency relative to that of the biologic 12(R)HETE.

The observation that a low dose of topically applied 12(R)HETE can reduce IOP by as much as 7 mmHg and that this effect is prolonged for several days, without apparent side effects, suggests that 12(R)HETE may be useful in the therapy of ocular hypertension. This investigation did not determine the mechanism by which the 12(R)HETE reduces IOP. However, the known role of 12(R)HETE as a potent Na⁺-K⁺-ATPase inhibitor in several tissues including the corneal epithelium suggests a decrease in the rate of aqueous humor formation at the ciliary body through inhibition of the ciliary epithelial metabolic pump. In fact, cardiac glycosides such as ouabain decrease the rate of aqueous humor formation in rabbits and cats, and humans. These agents, in order to be effective, must be administered intravitreally or systemically and in potentially toxic higher doses. Another Na⁺-K⁺-ATPase inhibitor that lowered IOP by topical application is vanadate, but its IOP-reduction effect can be related also to changes in adenylyl cyclase activity or with prevention of cyclic AMP effects.

Another possibility for the mechanism of the IOP-reduction effect of 12(R)HETE is that it enhances aqueous outflow facility or uveoscleral outflow, as has been shown for PGF2α. These results together with our previous observation of an inhibition of Na⁺-K⁺-ATPase suggest that 12(R)HETE from one or more sources in the eye is a potent endogenous modulator of IOP in the rabbit. Further studies are needed to elucidate the mechanism of this pressure-reduction effect as well as to determine the therapeutic potential of this new compound.

During the revision of this manuscript, Socci et al reported at the 1989 ARVO meeting that 12(R)HETE, but not 12(S)HETE, reduced IOP in rabbits and inhibited the ciliary epithelial Na⁺-K⁺-ATPase.

Key words: arachidonic acid, 12(R)HETE, IOP, cytochrome P450, cornea

Acknowledgments

The authors thank Dr. P. Kaufman and Dr. H. F. Edelhauser for their critical review, Dr. J. R. Falck for the 12(R)HETE, and J. Jones for typing the manuscript.

References

12(R)-HETE LOWERS IOP IN RABBITS / Masferrer et al


