In Vitro Hydrolysis of Latanoprost by Human Ocular Tissues

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Purpose

Latanoprost is an isopropyl ester prostaglandin F₂α analogue prodrug indicated for the treatment of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. Latanoprost is hydrolyzed in the eye to the biologically active metabolite, latanoprost acid. The objective of this study was to assess the extent of latanoprost hydrolysis in various ocular tissues dissected from fresh human eye globes. Insight into the enzymatic activity of these tissues is useful for the interpretation of human data and the design and testing of new drugs for ophthalmology.

Methods

Whole human eyes were obtained from tissue banks, from five donors. None of the donors had clinical history of glaucoma or treatment with agents that may affect intraocular pressure. Each eye, with attached conjunctiva, was enucleated within 15 hours of donor’s death, placed in a moistened chamber, and kept on cold packages for transportation to the lab. The tissues were carefully dissected immediately upon arrival; the aqueous humor (0.2-0.3 mL) was withdrawn using a 27-gauge needle and syringe. Then, the conjunctiva was carefully removed and the eye globe sectioned to separate the cornea, ciliary body, retina, choroid, and sclera. Each tissue was blotted, weighed, and small pieces were transferred to silanized incubation tubes containing 2 mL of Glutathione-bicarbonate Ringer’s (GBR) buffer, pH 7.4. Latanoprost was dosed at a concentration of 20 µM (final DMSO content was 0.2% v/v); the tubes were incubated at 37°C in a shaking water bath (50 rpm). Samples (0.1 mL) were collected into 96-well plates prefilled with acetonitrile (1:4 ratio) at the onset of the incubation and at pre-determined time points up to 4 hours. Plates were shaken and centrifuged; the concentrations of latanoprost and latanoprost acid were determined by LC-MS/MS.

Results

Latanoprost was readily metabolized in the preparations containing retina, ciliary body, choroid and conjunctival tissue, where 45% or less of the dosed latanoprost remained after 30 minutes of incubation (Figure 1). The concentration of the acid in the same tissues approached a plateau after 2 hours of incubation and represented 65-85% of the dosed concentration of latanoprost. Moderate rates of degradation of latanoprost were observed in cornea and sclera, where 60% of the dosed concentration remained after 30 min of incubation (Figure 2). The compound was highly stable in the aqueous humor. The rate of formation of latanoprost acid correlated with the rate of degradation of the parent compound (Figure 3). When normalized for tissue weight, the rate of hydrolysis of latanoprost was ranked as follows: choroid > ciliary body > retina > conjunctiva > sclera = cornea >> aqueous humor. Similar results were obtained with ocular tissues from pigmented rabbits under similar experimental conditions, the major difference being that choroid was relatively less active in the rabbit eye than in the human eye (Figure 4).

Conclusions

Latanoprost is extensively hydrolyzed in the majority of human ocular tissues. A good correlation was found between the disappearance of the parent compound and formation of latanoprost acid, indicating a single dominant metabolism pathway, i.e. hydrolysis. The profiles of latanoprost degradation are similar between human and pigmented rabbit ocular tissues.