Reports

Intraocular Hypotensive Effect of a Topically Applied Cortisol Metabolite: 3α, 5β-Tetrahydrocortisol*

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3α, 5β-tetrahydrocortisol, previously considered an inactive metabolite of cortisol, was found to lower significantly the intraocular pressure (IOP) of rabbits made ocular hypertensive with dexamethasone alone or with threshold levels of dexamethasone plus 5β-dihydrocortisol. The ocular hypotensive effect appeared within 3–7 days after the metabolite was started and persisted through the duration of the experiments. The metabolite did not lower the IOP of ocular normotensive untreated animals. Thus, 3α, 5β-tetrahydrocortisol is a naturally occurring steroid antagonist, which may be of use in the treatment of primary open-angle glaucoma. Invest Ophthalmol Vis Sci 28:901–903, 1987

Recent studies have shown that young rabbits are consistently sensitive to the ocular hypertensive effect of topical glucocorticoids.1,2 The authors have found that the young rabbits are more sensitive during the fall and winter months. The accumulation of 5α- and 5β-dihydrocortisol in cultured cells derived from the outflow region of the human eye in primary open-angle glaucoma (POAG)3 and the finding that 5β-dihydrocortisol can potentiate the intraocular pressure (IOP) raising effect of topically applied dexamethasone in the rabbit2 makes this rabbit model particularly appropriate in evaluating antiglaucoma agents.

The authors now report that 3α, 5β-tetrahydrocortisol, a cortisol metabolite previously considered to be biologically inactive, lowers the IOP of rabbits made ocular hypertensive with dexamethasone alone or with dexamethasone plus 5β-dihydrocortisol. Materials and Methods. Young New Zealand white rabbits weighing about 1 kg were used. The animals were treated by placing 25 μl of the test solution on each eye four times a day, 7 days a week. IOP was measured several times a week in both eyes between 8–10 AM with an Alcon pneumatonometer (OCVM, from Digilab Division of BioRad, Cambridge, MA) after addition of a topical anesthetic (tetracaine). A single mean value was used for each animal. The steroids (Steraloids, Inc., Cambridge, MA) were suspended in phosphate buffered saline (PBS) by homogenization with a Teflon pestle. This produced a fine suspension of the steroids that minimized corneal irritation. The experiments were carried out in a masked fashion and conformed to the ARVO Resolution of the Use of Animals in Research. Significance levels for IOP readings between groups of animals were determined for parametric data by analysis of variance followed by a Bonferroni post-test and for nonparametric data by the Mann Whitney rank sum test (an equivalent to the Wilcoxon rank sum).

Results. Figure 1 shows the average IOP data of a group of nine animals receiving 0.06% dexamethasone plus 0.1% 5β-dihydrocortisol and a group of six animals

Fig. 1. The effect of 3α, 5β-tetrahydrocortisol on the IOP of rabbits made ocular hypertensive with topical application of 0.06% dexamethasone (threshold level) plus 0.1% 5β-dihydrocortisol. Each point shown represents the mean intraocular pressure (IOP) of all eyes treated in each group. The closed circles (●●●) represent the IOP of nine animals treated with dexamethasone plus 5β-dihydrocortisol. The filled squares (■■■) represent six animals treated with phosphate buffered saline (vehicle control). On day 23 (indicated with an arrow) five ocular hypertensive animals (○○○) were treated with 0.1% 3α, 5β-tetrahydrocortisol in addition to the original formulation of dexamethasone plus 5β-dihydrocortisol. The other four ocular hypertensive animals were continued on dexamethasone plus 5β-dihydrocortisol. Analysis of variance using the Bonferroni post-test showed that the addition of tetrahydrocortisol led to a significant (P < 0.01) decrease in IOP.

* Patent pending--864,610.
Fig. 2. The effect of 3α,5β-tetrahydrocortisol on the intraocular pressure (IOP) of rabbits made ocular hypertensive with 0.1% dexamethasone. The mean IOP is plotted as a function of days after beginning the tetrahydrocortisol therapy. Each point shown represents the mean IOP of all eyes treated in each group. The filled circles (●) represent the IOP of four rabbits continuing to receive 0.1% dexamethasone. The open circles (○) represent the IOP of four animals treated with 0.1% dexamethasone plus 0.1% 3α,5β-tetrahydrocortisol. The filled squares (□) represent five animals receiving phosphate buffered saline (vehicle control). Analysis by the Mann Whitney test indicates that tetrahydrocortisol caused a significant ($P < 0.01$) decrease in IOP.

Animals made ocular hypertensive with 0.1% dexamethasone also responded with a statistically significant ($P < 0.01$) decrease in IOP after the addition of 0.1% 3α,5β-tetrahydrocortisol (Fig. 2).

Animals treated with 1% 3α,5β-tetrahydrocortisol alone showed no change in IOP during 19 days of treatment (Fig. 3). Steroid sensitivity of these animals was demonstrated by the fact that parallel groups of animals treated with (1) 0.1% dexamethasone or (2) 0.06% dexamethasone plus 0.1% 5β-dihydrocortisol responded with the expected increase in IOP of 5–8 mm Hg in 7–10 days.

Discussion. 3α,5β-tetrahydrocortisol has been shown to significantly lower the IOP in rabbits made ocular hypertensive with dexamethasone alone or with threshold levels of dexamethasone plus 5β-dihydrocortisol. The ocular hypotensive effect occurred in 3–7 days after 3α,5β-tetrahydrocortisol was started and persisted for the duration of the experiments (up to 17 days). Although the IOP did not reach the levels of the vehicle controls during the period of observation, there was no indication of tachyphylaxis during the experiment. The authors' data suggests that 3α,5β-tetrahydrocortisol may be a glucocorticoid antagonist in the target cells of the outflow region of the rabbit eye that are responsible for the glucocorticoid induced elevation of IOP. Progesterone, which is a partial glucocorticoid antagonist, has been reported to be acutely ocular hypotensive in the rabbit.* More recently, the glucocor-
Bullous Pemphigoid Autoantibodies Are Markers of Corneal Epithelial Hemidesmosomes

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Sera from patients with bullous pemphigoid (BP) contain autoantibodies that bind to the BP antigen, which is a component of the epithelial–stromal junction of the cornea. Previous studies, employing direct immunoelectron microscopy (IEM) on perilesional skin of patients have localized the BP antigen to the lamina lucida. On this basis, studies of corneal epithelial–stromal adhesion and wound healing have employed BP antigen as a marker of the lamina lucida of the corneal basement membrane zone (BMZ). The authors used indirect IEM with BP autoantibodies on frozen sections of cornea and found that the majority of the BP antigen is intracellular and is closely associated with the corneal epithelial hemidesmosome. Only a small amount of BP antigen appears to be extracellular, limited to the portion of the lamina lucida directly beneath individual hemidesmosomes. When rabbit corneal epithelium is extracted and analyzed by Western immunoblotting, BP autoantibodies recognize two polypeptides of molecular weights of 240 and 180 kilodaltons, which comigrate with BP antigens extracted from epidermis. BP autoantibodies are a specific marker of corneal epithelial hemidesmosomes and can be used as a probe to identify and study the role of hemidesmosomes in epithelial–stromal adhesion. Invest Ophthalmol Vis Sci 28:903–907, 1987