4803 - B996
8-iso Prostaglandins and Aqueous Humor Dynamics in Monkeys - Effect of 17-Phenyl Trinor 8-iso Prostaglandin E2

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**Purpose:** To evaluate the effects of 17-phenyl trinor 8-iso PGF, on intraocular pressure (IOP) in monkeys with laser-induced glaucoma, and on aqueous humor dynamics in normal monkeys.

**Methods:** Two single-dose tests were performed on 8 monkeys with unilateral laser-induced glaucoma at concentrations of 0.1% and 0.2%. One 25µl drop of 17-phenyl trinor 8-iso PGF, was topically applied to the glaucomatous eye at 9:30 a.m. IOP was measured hourly for 6 hrs beginning at 9:30 a.m. Following one baseline day and one vehicle-treated day, a multiple-dose study was carried out in 8 glaucomatous monkeys with 0.1% 17-phenyl trinor 8-iso PGF, applied to the glaucomatous eye at 9:30 a.m. for 5 consecutive days. Tonometric outflow facility (C) and fluorophotometric flow rates (F) were measured in 6 normal monkeys before and after a single dose of 0.2% 17-phenyl trinor 8-iso PGF, to one eye.

**Results:** A single dose of either 0.1% or 0.2% 17-phenyl trinor 8-iso PGF, reduced IOP (p<0.05) by 5% for 5 hrs with the maximum reduction of 4.5 ± 0.6 mmHg (15%) in 8 glaucomatous monkeys, and 5.7 ± 1.4 mmHg (19%) in 6 glaucomatous monkeys, respectively. The ocular hypotensive effect was maintained for 5 days with once daily administration of 0.1% 17-phenyl trinor 8-iso PGF, in 8 glaucomatous monkeys. In 6 normal monkeys, C was increased (p<0.05) by 59% in the drug-treated eyes compared with the treated control eyes and by 69% compared with baseline measurements. F was unchanged (p>0.90).

**Conclusions:** 17-phenyl trinor 8-iso PGF, reduces IOP in both normal and glaucomatous monkey eyes. An increase in tonographic outflow facility accounts for most if not all of the IOP reduction in normal monkeys. This PG analog appears to lower IOP primarily by increasing trabecular outflow facility.

**CR:** R. Wang, None; K. Segal, None; T.W. Mittag, Patent application, P; S.M. Podos, Pfizer Inc., C; Patent application, P.

**Support:** NIH grant EY03867

4804 - B997
Upregulation of NTPDase 1 in an Experimental Monkey Glaucoma Model


**Purpose:** ATP is released in numerous tissues to signal a change in flow, pressure or other mechanical perturbation. The increased intraocular pressure that can accompany glaucoma may also trigger the release of ATP. Retinal ganglion cells die in glaucoma, and the stimulation of the P2X, receptors for ATP can kill ganglion cells. Elevated pressure increases the concentration of ATP in the vitreous chamber of the bovine eye. Direct measurements of ATP levels in the extracellular space of the retina are not presently possible. However, we have recently found that the enzyme ecto-nucleoside triphosphate diphosphohydrolase 1 (NTPDase1; aka CD39) is upregulated after prolonged exposure to extracellular ATP. This study asked whether expression of the NTPDase1 was increased in the retinas of primates with elevated intraocular pressure (IOP).

**Methods:** Elevation of IOP was induced in one eye of 15 monkeys by laser photoocoagulation of the trabecular meshwork. The IOP was monitored weekly; IOP values were the mean measurement throughout the experiment. For immunoblot experiments, eyes were fast frozen, retinal proteins purified using standard techniques and run on a SDS-PAGE. Gels were blotted with antibodies to NTPDase1 (BU61) and stained quantified with Image Pro Plus software. For immunohistochemical studies, tissue was perfused with 2% paraformaldehyde, sectioned at 12 µm and processed for immunohistochemistry.

**Results:** The IOP of the lasered eyes was significantly higher than the non-lasered controls, at 21.8 ± 1.2 mm Hg vs. 15.6 ± 0.4 mm Hg, respectively (p<0.01). Expression of NTPDase1 was also increased in the lasered eyes. Western blot analysis gave the mean ratio of protein in lasered vs non-lasered (L/NL) eye as 2.00 ± 0.28 (Mean ±SE). There was a significant correlation between the IOP and the increase in NTPDase1 protein in the glaucomatous retina (r = 0.714). Immunochemical analysis indicated that the upregulation of NTPDase1 occurred both in the inner nuclear layer and the optic nerve.

**Conclusions:** We have demonstrated that NTPDase1 levels are increased in the inner nuclear layer and optic nerve layer of eyes with increased intraocular pressure. This result provides indirect evidence for chronic exposure to extracellular ATP in experimental glaucoma.

**CR:** W. Lu, None; C. Rasmussen, None; B. Gabell, None; B. Hennes, None; P. Kaufman, None; A.M. Laties, Penn; C.H. Mitchell, Penn; P.

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4805 - B998
Ocular Hypotensive and Neuroprotective Effects of K-115, a Novel Rho-Kinase Inhibitor


**Purpose:** In this study, we evaluated the kinase inhibition property in vitro, ocular hypotensive effect, distribution pattern, and neuroprotective effect in vivo of K-115.

**Methods:** This study was conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Inhibition potency of K-115 on some kinase enzymes including Rho-kinase were assayed. Ocular hypotensive effect of K-115 was evaluated by the topical instillation into normal rabbit (0.0625% to 0.5%, n=10) and monkey (0.1% to 0.4%, n=8) eyes. In addition, the effect of K-115 was compared with 0.05% Xalatan in monkey. The distribution pattern of K-115 was examined by the whole head autoradiography with [14C]K-115 in rabbits. The neuroprotective effect of K-115 (10 4 to 10 6 M, 5 µL injection into the vitreous) was assayed by using the survival ratio of retinal ganglion cells (GCs) in rat retinal ischemia-reperfusion model (n=5-7).

**Results:** K-115 inhibited Rho-kinase (IC50=0.031 µM) with a competitive manner, which was highly selective and approximately 8 times more potent than Y-27632 (0.23 µM). Topical instillation of K-115 showed dose-dependent ocular hypotensive effect in rabbit (p<0.001) and monkey (p<0.001) eyes. In the monkey, the maximal reduction was obtained at 2 hour after the instillation, and the values were 2.3±0.3, 3.3±0.3, and 4.4±0.3 mmHg for 0.1%, 0.2%, and 0.4% of K-115, respectively. The ocular hypotensive effect of 0.4% K-115 was more potent than that of 0.05% Xalatan (2.5±0.2 mmHg, p>0.01). Whole head autoradiography revealed that the topical instillation of K-115 penetrated into the posterior part of the eye including the retina or choroid on the ipsilateral side. Ischemia-reperfusion injury reduced GC numbers to the 57.3±3.9% of normal (p<0.001) and over, and 10 -6 M K-115 almost abolished the reduction of GCs (98±1.3% of control, p<0.001).

**Conclusions:** These results suggest that K-115 has a potent ocular hypotensive and neuroprotective effects, and may be a useful therapeutic agent for the treatment of glaucoma.

**CR:** K. Mizuno, Kowa Company Ltd, E; T. Koide, Kowa Company Ltd, E; Y. Fujieda, Kowa Company Ltd, E; J. Mori, Kowa Company Ltd, E; S. Kondo, Kowa Company Ltd, E; J. Matsumoto, Kowa Company Ltd, E; Y. Hattori, Kowa Company Ltd, E.

**Support:** None

4806 - B999
Glaucoma-Causing Myocilin Mutants Require the Peroxisomal Target Signal-1 Receptor (PTS1R) to Elevate Intraocular Pressure

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**Purpose:** Glaucoma is a leading cause of worldwide irreversible visual impairment and blindness and is a clinically and genetically heterogeneous group of optic neuropathies. Intraocular pressure (IOP) is the major risk factor for glaucoma and myocilin (MYOC) gene cause primary open angle glaucoma (POAG) with varying age-of-onset and degree of severity.

**Methods:** Interaction of the C-terminal half of myocilin with PTS1R was determined using N-ethylmaleimide-sensitive and co-immunoprecipitation. Visualization of intracellular mutant myocilin localization was done by confocal microscopy analysis. Effects of adenoviral expressed myocilin on IOP were determined in mice.

**Results:** We show a mutation-dependent, gain-of-function association between human myocilin and PTS1R. There was correlation between the glaucoma phenotype and the specific MYOC mutations, with the more severe early-onset POAG mutations (e.g. Y437H and G384V) having a higher degree of association with PTS1R. Expression of human myocilin glaucomatous mutants in mouse eyes causes significantly (p<0.001) elevated intraocular pressure (IOP), which is a major phenotype of MYOC glaucoma.

**Conclusions:** This is the first demonstration of a disease resulting from mutation-induced exposure of a cryptic signaling site that causes mislocalization of mutant protein to peroxisomes and the first disease-gene-based animal model of human POAG.

**CR:** A.R. Shepard, Alcon Research Ltd, E; N. Jacobson, Alcon Research Ltd, E; J.C. Miller, Alcon Research Ltd, E; L.-H. Pang, Alcon Research Ltd, E; H.T. Steel, Alcon Research Ltd, E; C.C. Searby, None; V.C. Shefford, None; E.M. Stone, None; P.A. Clark, Alcon Research Ltd, E.

**Support:** Alcon Research Ltd, E.
Additive Effects of Timolol and Cannabinoids on Intraocular Pressure in a Rat Glaucoma Model

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Purpose: Half of all glaucoma patients cannot be maintained on single drug therapy e.g., timolol alone; most require use of two or even three drugs to control their IOP. Cannabinoids are known to reduce IOP. Since cannabinoids and timolol have different mechanisms of action, there is potential for synergistic effect. In the present study, we examined the combination of timolol and a synthetic cannabinoid (WIN55212-2, O-1812, or O-2545) to reduce IOP in a chronic rat model of ocular hypertension.

Methods: Surgical ligation of three of the four episcleral veins in one eye of Sprague-Dawley rats caused a long-lasting (34±4 wk) IOP increase. IOP was measured by Goldmann tonometry at baseline (+30, +30, 60 and 120 min, as was heart rate and blood pressure. For combination therapy, after baseline IOP measurement, timolol 0.5% was applied topically followed 30 min later by WIN55212-2 1.0%, O-1812 1.0%, or O-2545 1.0%. In another experiment, O-1812 1.0% and O-2545 1.0% were administered simultaneously. An analysis for ocular irritation was performed by slit lamp examination (SLE) at baseline and 150 min.

Results:

<table>
<thead>
<tr>
<th>Drug</th>
<th>IOP at 120 min (% Baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timolol 0.5%</td>
<td>91.5 ± 5.2</td>
</tr>
<tr>
<td>WIN55212-2 1.0%</td>
<td>48.1 ± 2.8</td>
</tr>
<tr>
<td>O-1812 1.0%</td>
<td>62.0 ± 5.5</td>
</tr>
<tr>
<td>O-2545 1.0%</td>
<td>65.5 ± 3.1</td>
</tr>
<tr>
<td>Timolol 0.5% + WIN55212-2</td>
<td>56.9 ± 12.2</td>
</tr>
<tr>
<td>O-1812 1.0% + O-2545 1.0%</td>
<td>41.1 ± 3.1</td>
</tr>
<tr>
<td>O-1812 1.0% + O-2545 1.0%</td>
<td>41.1 ± 8.8</td>
</tr>
</tbody>
</table>

* significantly lower than baseline, p<0.05
† significantly lower than O-2455 1.0% alone, p<0.05
‡ significantly lower than O-2455 1.0% alone, p<0.05

Within 30 min all combinations significantly decreased IOP. Unlike timolol alone, after the addition of the cannabinoids, IOP reduction was maintained for over 120 min. The combination of O-1812 and O-2545 had the greatest effect. No combination caused ocular irritation or systemic effects.

Conclusions: Compared to timolol alone, combination therapy with timolol and cannabinoids prolonged both the duration and magnitude of their effect on IOP. However, the combination of two synthetic cannabinoids also had synergistic effects. The potential for multidrug therapy using cannabinoids may provide a greater benefit.

CR: M.H. Oltmanns, None; S.S. Samudre, None; F.A. Lattanzio, None; B.R. Martin, None; J. Pintor, B. Williams. None.

Support: Supported in part by Richmond Eye & Ear Foundation, Richmond, VA.

Effect of Several siRNA in the Treatment of Ocular Hypertension and Glaucoma

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Purpose: To demonstrate that a MT1 receptor which is founded in ciliary processes. Melatonin agonist as a potential novel therapy for glaucoma.

Methods: We have designed several siRNAs for the following ATPase genes: Na+/K+ ATPases as potential therapeutic targets to control IOP levels. The siRNAs were designed according to the guidelines of the siRNA design algorithm (http://www.ambion.com/si.swap.html). The siRNAs were synthesized and tested in vitro for their ability to silence the expression of the target ATPase genes. The effects of the siRNAs on IOP were measured in vivo by means of the MT1 receptor.

Results: The siRNAs targeting the ATPases Na+/K+ and β2 were similar to those reached with carbonic anhydrases and β-adrenergic receptors. Moreover, siRNA treatment showed a synergistic effect on IOP reduction when compared with control.

Conclusions: The siRNAs tested in this study showed two different behaviours: siRNAs targeting Angiotensin receptors did not cause any hypotensive effect, while the rest of the siRNAs, tested against Renin, Cochin, ELAM and HSD, produced a reduction of IOP similar to that produced by the control product (Xalatan). Moreover, the siRNAs have the advantage of presenting a long-lasting effect compared to commercial pharmaceutical products.

CR: A. Perälä, Sylenis; F. P. Loma, None; A. Mediero, None; A. Sesto, Sylenis; E. J. Pintor, Sylenis; F. A. J. Jimenez, Sylenis, E. None; Support: None.

Effect of Several siRNA in the Treatment of Ocular Hypertension and Glaucoma

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Purpose: To demonstrate that a MT1 receptor is involved in the reduction of IOP by means of the MT1 receptor agonist IK7, and to determine the presence of MT1 receptors in the ciliary body in New Zealand white rabbits.

Methods: Melatonin and IK7 were formulated in isotonic saline containing 1% DMSO and were tested at a final concentration of 100µM. The compounds were applied unilaterally to the corneas at a fixed volume of 10 µL in New Zealand white rabbits eyes. The contralateral eye received the same volume of saline + 1% DMSO (vehicle). Corneas were anaesthetized by applying 10 µl of 1% (w/v) oxibuprocaine / tetracaine (4:1) (mg respectively). IOP was measured by means of a TONOPEN XL twice before the application of the solution and after that for once every hour for at least 8 hours. Crystal sections of ciliary body were used for immunohistochemical experiments. Sections were incubated with MT1, melatonin receptor antibody and subsequently incubated with a secondary IgG mouse labelled with TRITC, then, the tissues were observed under confocal microscopy.

Results: While the melatonin inhibited a sharp reduction in IOP of 20.2 ± 5.3% compared to control (n=8), followed by a gradual return to control values 4 hours after the beginning of the experiment, 100 µM IK7 reduced IOP 36.5 ± 3.2% compared to control (n=8) and IOP remained low for almost 7 hours, returning to basal values after this period of time. Also, the non-selective melatonin antagonist luzindole partially returned the effect of IK7 towards the control values (80.5 ± 2.6%, n=8) and the MT1 receptor antagonist 4PDDP and DH 97 returned the effect of IK7 to control values (92.6 ± 6.3% and 97.4 ± 3.3% respectively, p<0.05). On the other hand, immunohistochemical studies showed the presence of MT2 melanin receptors in the ciliary processes.

Conclusions: The results demonstrate that the main target of IK7 in the control of IOP is the MT1 receptor, which is founded in ciliary processes. Melatonin agonist as IK7 may have clinical potential for treating elevated IOP.

CR: M. Alarma-Estrany; None; T. Felaez; None; A. Perälä; None; A. Crooke; None; A. I. Guzman; None; J. Pintor. None.

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4813 - B1004
Sucinimides as Topical Ocular Hypotensive Agents
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**Purpose:** To evaluate the anti- Petit Mal epilepsy sucinimides as potential topical ocular hypotensive agents. The hypothesis is that Absence epilepsy and glaucoma share similar pathophysiology, reflecting in secretion and/or defective outflow facility (of CSF and aqueous humor, respectively). Sucinimides modulate chloride secretion in the choroidal plexus, and thus will be able to exert their effect at the posterior chamber of the eye.

**Methods:** Ocular pharmacodynamic studies were conducted at the Tel-Aviv University animal facility. Six Dark Agouti (DA) pigmented rats (250-300g in weight) were slightly sedated with 1.5-2mg intraperitoneal xylazine. One eye was randomly selected to receive 50 microliters of gelatinous topical 250mg/0.2ml ethosuximide (Petlandan, Desitin, Germany), while the control eye received a viscoelastic tear substitute (Viscotears, Novartis, Switzerland). At 2 and 1/2, 1, 2, 4 and 6 hours post administration, intraocular pressure (IOP) was measured repeatedly by Tonopen XL tonometer (Medtronics, USA) and eyes assessed for local toxicity. In a second experiment, using the same protocol, the topical compound was tested on five awake albino rats (250-g in weight). Results were recorded and analyzed, using two-tailed Student’s t-test for paired data as well as analysis of variance.

**Results:** In the DA rats the IOP showed a biphasic response, with an increase of 0.5 mmHg at 1 1/2 and 1 hour post instillation, followed by a significant 2.6 mmHg decrease in the treated eyes versus control (or 18% from baseline, p<0.005) at 2 hours. In the rabbit eye, the effect was even more impressive, with a decrease of 4.8 mmHg (12%) vs. control at 4-5 hours (p<0.005). Two of the rat treated eyes, three of the rabbit treated eyes and one control eye showed mild conjunctival injection, which subsided an hour after the treatment. In the rabbit drops, the IOP was then measured over 12 hours, with no additional drops administered.

**Conclusions:** Ethosuximide, an effective anti-epileptic medication, can be efficacious as a topical ocular hypotensive agent. The effect measured in the studies is comparable to that of beta-adrenoceptor antagonists and prostaglandin analogues. Ethosuximide’s topical effect should be due to its low molecular weight and favorable physicochemical properties, being both lipid (alcohol or ether) and water soluble.

**CR:** M. Sharir. None.

**Support:** None.

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4814 - B1007
Effects of Topical Anti-Glaucoma Medications on Ocular Surface as Evaluated by Gene Expression Pattern

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**Support:** None.

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4812 - B1005
A Potential of Rho-Associated Coiled-Coil-Forming Kinase Inhibitor in Reduction of Murine IOP

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**Purpose:** Rho-associated coiled-coil-forming kinase (ROCK) inhibitors are reported to decrease intraocular pressure (IOP) in rabbit eyes and supposed to regulate IOP in monkey and human eyes through relaxation of trabecular meshwork and ciliary muscle. In this study, IOP-lowering effect of a ROCK inhibitor and its synergistic effect with latanoprost are investigated using mouse eyes.

**Methods:** A ROCK inhibitor (K115) was prepared as 0.5% solution. C57BL/6 mice were anesthetized under the 12-hour light-dark cycle (6:00 am 18:00 off) for at least 2 weeks before experiments. Three micro liters of the solution was topically applied once into one of two eyes in a blind manner at 18:00. The fellow eyes were untreated. IOP was measured by a microneedle method. At 0.5, 1, 2, and 4 hours after the administration, the IOP-lowering effect was calculated as the difference between IOP in the treated eye and that in the contralateral untreated eye. (n=5 for each time point) 0.125, 0.25, and 0.5% K115 were used to examine a dose-dependent response in IOP reduction. (n=710 for each dose) Next, the additional IOP-lowering effect of K115 to latanoprost was examined. 0.25% K115 was applied 2 hours after the administration of 0.005% latanoprost, then further 30 minutes later IOP was measured. (K115/latanoprost group) As a control group, IOP was measured 2.5 hours after the administration of latanoprost without K115 (latanoprost group) The IOP reductions in the two groups were compared. (n=6 for each group)

**Results:** 0.5% K115 solution showed maximum IOP reduction by 29.6±4.3% in 30 minutes after the administration. 0.125, 0.25, and 0.5% K115 revealed significant IOP reduction (p<0.005) in a dose-dependent manner by 17.9±6.7%, 22.1±2.7% and 30.5±3.7%, respectively. IOP reduction in K115/latanoprost group (37.3±6.4%) was significantly higher than that in latanoprost group (24.8±4.1%) (p<0.005)

**Conclusions:** A ROCK inhibitor (K115) significantly reduced mouse IOP and also showed a significant additive effect on latanoprost-induced IOP reduction. K115 may have a therapeutic potential for glaucoma.

**CR:** T. Saeki; None; H. Tsuruga; None; M. Aihara; None; M. Araie; None; K. Mizuno; company, F. Y. Hattori, company, F.

**Support:** None.

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4801 - B1006
The Effect of Travoprost Z, Latanoprost and Their Individual Components on the Ocular Surface (Corneal and Conjunctival Epithelium).

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**Purpose:** Prostaglandin analogues (PGAs) are the most common treatment for glaucoma. Most PGA formulations, like travoprost and latanoprost, contain the preservative benzalkonium chloride (BAK). Travoprost Z is preserved with a new ionic buffered preservative system, disodium ethylene-diamine-tetra-acetate (EDTA). To evaluate the potential toxicity of travoprost Z, latanoprost, latanoprost and their components to the ocular surface, a tissue culture model utilizing immortalized corneal and conjunctival epithelial cells was utilized.

**Methods:** Human conjunctival (CCC: P61 HCK) and corneal epithelial cells (HCE: P61 HCK) were cultured. At confluency, media was replaced with 100 µl of the commercial PGA formulations or their major components: travoprost Z (0.004%), travoprost 0.004% with benzalkonium chloride (BAK) 0.015%; latanoprost 0.005% with 0.02% BAK; as well as viable (cell media) and dead (formalin) controls. After 1 hour, the solution was removed and 150 µl of MTI were added to each well and incubated for 4 hours at 37°C. After decanting, 100 µl of acid isopropanol was added. The absorbance was then determined at 570 nm and normalized, relative to the controls.

**Results:** Travoprost Z showed 37.3% (CCC: 37.5±2.5, HCE: 37.1±6.8) < travatran 74.0% (CCC: 74.3±1.7, HCE: 73.6±1.5) < latanoprost 80.4% (CCC: 80.2±1.5, HCE: 80.6±1.8) toxicity. The active agents of the formulations were more toxic than controls [travoprost: 36.6% (CCC: 37.0±3.2, HCE: 36.2±1.5%); latanoprost: 43.9% (CCC: 47.5±5.4, HCE: 40.2±1.4)] BAK demonstrated the majority of the toxicity exhibited by the PGA formulations exhibiting an average toxicity of 72.1% at a concentration of 0.015% [that in travoprost: (CCC: 74.2±2.1, HCE: 73.6±1.5) and 78.9% at 0.02% [that in latanoprost: (CCC: 76.1±2.2, HCE: 81.7±0.9]. Increasing concentrations of BAK caused increased toxicity and BAK proved more toxic than the active agent.

**Conclusions:** Preservatives exhibited the majority of the toxicity; travoprost Z exhibited the lowest: Travoprost Z < travoprost < latanoprost. BAK exhibited dose dependent toxicity and was more toxic than the active agents. Use of the non-BAK-protected PGA, travoprost Z seems to lead to less surface toxicity as evaluated in this model.


**Support:** Supported in part by a research grant from Alcon Laboratories, Inc., NEI/NIH/NEI/NIH/NIH, Research to Prevent Blindness, Inc. & The Martin and Toni Sosnoff Foundation.

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4811-4814
Distribution of Aggrecanase-1 and Aggrecanase-2 in the Primate Uveoscleral Outflow Pathway

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Purpose: Aggrecan is a major proteoglycan in the sclera and may contribute to normal resistance to uveoscleral outflow. Aggrecanase-1 (ADAMTS-4) and aggrecanase-2 (ADAMTS-5) are enzymes that can cleave specific sites within the aggrecan core protein. Hence, the present study was undertaken to determine the distribution of aggrecanase-1 and aggrecanase-2 in uveoscleral outflow pathway tissues of primate eyes.

Methods: Normal cynomolgus monkey eyes (n=3) were fixed in formaldehyde, embedded in a modified paraffin, sectioned, and immunostained using specific polyclonal antibodies to aggrecanase-1 and aggrecanase-2. Secondary antibody was conjugated to horseradish peroxidase, immunoreactivity was visualized using diaminobenzidine. The staining within the ciliary muscle, ciliary nonpigmented epithelium, scleral stroma, scleral fibrils, blood vessel endothelium and a thin smooth muscle were evaluated by light microscopy and stain intensity was graded.

Results: Aggrecan immunoreactivity was observed in each of the eyes throughout the anterior segment. Intense staining for aggrecanase-1 and aggrecanase-2 were observed in ciliary muscle and ciliary nonpigmented epithelium. Light-to-moderate staining for aggrecanase-1 and moderate-to-intense staining for aggrecanase-2 were observed in blood vessel endothelium and arterial smooth muscle. Light-to-moderate staining for aggrecanase-1 and for aggrecanase-2 were also observed in scleral stroma and scleral fibrils. Aggrecanase-2 staining consistently was less in the inferior rectus and temporal than in the inferior rectus and temporal stroma.

Conclusions: Aggrecanase-1 and aggrecanase-2 were identified in regions of the anterior segment of normal primate eyes which are associated with the uveoscleral outflow pathway. These data indicate that aggrecanase-1 and aggrecanase-2 activity are well positioned to contribute to the regulation of uveoscleral outflow facility.

Support: NIH Grant EY05990 (RNW)

Aggrecanase activity in the uveoscleral outflow pathway

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4819 - B1012
Protective Effect of Estron on Glaucoma
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Purpose: In the pathogenesis of glaucoma different changes in cellular and biomechanical metabolism have been discussed. Estron seems to interfere in these cellular mechanisms. Several epidemiological studies already suspected a connection between a modified hormone level and glaucoma but so far only few studies underlined this causality. Aim of this study was to prove a hormonal effect of estron on the development of glaucoma by using blood samples of glaucoma patients and healthy subjects.

Method: 28 female patients were enclosed in this study. 20 patients belonged to the control group and showed clinically no glaucoma. 21 patients had a glaucoma which was defined by means of the visual field, intraocular pressure and the excavation of the optic nerve head. Exclusion criterion was the intake of additional hormone medication (e.g. birth control pill). Blood samples were taken from all participants on the 18th day of menstruation cycle. The mean of estradiol concentration E2 was compared in both groups using the t-test.

Results: Age of participants of the control group was 46, 4 +/- 8, 2 (range: 29-60), the age of patients with glaucoma was 50, 4 +/- 7, 3 (range: 36-60). Age showed no statistical significance between the two groups (P=0.112). The estradiol concentration in the control group was 242, 6 +/- 305, 9 pmol/l and the estradiol concentration in the patients’ group about more than the half less (94, 8 +/- 110, 8 pmol/l). This difference was statistically significant (P= 0.004).

Conclusions: This study presented that the estrogen concentration in glaucoma patients in contrast to the control group was significantly diminished. Therefore a possible protective effect of estrogen on the cellular metabolism of glaucoma patients could be postulated. A substitution of estradiol in glaucoma patients may as a consequence be a valuable therapeutic option and could influence the course of disease positively.

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4821 - B1014
Right versus Left Asymmetry of 24 Hour Intraocular Pressure Response to Medical Treatment

Purpose: To assess the asymmetry in right versus left intraocular pressure (IOP) responses to monotherapy of travoprost or timolol over a 24-hour period.

Methods: Thirty-four patients (ages 41-78 years) with untreated open-angle glaucoma or ocular hypertension were included. Patients were housed in a sleep laboratory for 24 hours and baseline IOP profiles were created. Measurements of IOP were taken in both eyes using a pneumatonometer every two hours in the sitting position during the diurnal period (7 AM to 11 PM) and in the supine position during the nocturnal period (11 PM to 7 AM). Seventeen patients then initiated the treatment with 0.004% travoprost in both eyes once before bedtime (Group I) and seventeen patients with the 0.5% timolol treatment in both eyes once in the morning (Group II). Upon completion of at least four weeks of treatment, patients were housed in the sleep laboratory again for collecting the second 24-hour IOP data. The mean baseline IOPs and mean IOP changes in response to the monotherapy were calculated. The strength of association between the right and left IOP responses to the monotherapy were calculated for the right and left eyes during the office-hour (9 AM to 5 PM), diurnal, nocturnal, and 24-hour periods. The strengths of association between right and left IOP prior to initiation of therapy and IOP responses to the treatment were evaluated using the coefficient of determination (R²).

Results: For the group I prior to treatment, coefficients of determination between baseline IOPs in the two eyes were 0.413, 0.349, 0.438, and 0.360 during the office-hour, diurnal, nocturnal, and 24-hour periods, respectively. Under the travoprost treatment, coefficients of determination between IOP reductions in the two eyes were 0.173, 0.048, 0.413, and 0.197 during the office-hour, diurnal, nocturnal, and 24-hour periods. For the group II, pre-treatment coefficients of determination between baseline IOPs in the two eyes during the office-hour, diurnal, nocturnal, and 24-hour periods were 0.425, 0.453, 0.597, and 0.442, respectively. Under the timolol treatment, the corresponding coefficients of determination between IOP reductions in the two eyes were 0.372, 0.269, 0.727, and 0.423.

Conclusions: The strengths of association between the right and left IOP responses to travoprost or timolol treatment were weak to moderate. This suggests that the monocular therapeutic trial may not necessarily predict the response of the fellow eye to topical therapy. The strengths of association for the IOP responses to the timolol treatment were better than those for the IOP responses to the travoprost treatment, which may reflect the fact that there was a crossover effect on IOP under the timolol treatment.

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4820 - B1013
Elevated ATP in Aqueous Humor of Patients With Acute Glaucoma

Purpose: Glaucoma is typically characterized by an increase in intraocular pressure (IOP). Although an elevated pressure can lead to multiple changes in ocular tissues, the initial signals are not well understood. Throughout the body, the release of ATP transduces mechanical change into a neurochemical signal. This study sought to determine whether levels of ATP were elevated in the aqueous humor of patients with acutely elevated IOP.

Methods: ATP levels were determined with the luciferin-luciferase assay using aqueous humor samples collected from patients following the principals outlined by the Declaration of Helsinki. Samples were obtained from 14 patients undergoing an emergency anterior chamber paracentesis, a routine procedure following an acute attack of primary acute angle closure glaucoma at the Zhongshan Ophthalmic Center. Control samples were obtained from 18 cataract patients with normal IOP. ATP was measured using Goldmann tonometry. All samples were processed with respect for patient privacy following an accepted protocol. Rank Sum Test was used to analyze between two groups of ATP level and IOP level, respectively. Spearman Rank Correlation Analysis was used to evaluate the relationship between IOP and ATP levels.

Results: The mean IOP of patients with primary acute angle closure glaucoma was 58.9±3.6 mm Hg, while that from controls was 13.2±0.4 mm Hg (p<0.001). The mean level of ATP in samples of aqueous humor were ten-fold higher in patients with primary acute angle closure glaucoma compared to control, being 224±36 pm in glaucoma eyes and only 25±4 pm in control (p<0.001). The positive correlation between the magnitude of IOP elevation and ATP concentration was highly significant (r=0.83, p<0.001).

Conclusions: ATP levels are increased in the aqueous humor of patients with acute glaucoma in proportion to the rise in IOP. The cellular source of this ATP is unknown, although several cell types in the anterior eye are known to release ATP in response to swelling. The tight correlation between ATP and pressure suggests the rise results from controlled release, although cell lysis cannot be ruled out. It remains to be determined whether this excess ATP can initiate changes by stimulating local receptors.

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4822 - B1015
Fixed Combination Timolol/Dorzolamide versus Timolol/Brimonidine: A Randomized Clinical Trial
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Purpose: A prospective randomized controlled trial comparing fixed combination timolol 0.5%/dorzolamide 2% with timolol 0.5%/brimonidine 0.2% to determine which combination preparation provides superior intraocular pressure (IOP) control and a better side-effect profile.

Methods: Patients with any type of glaucoma using timolol/dorzolamide were identified from the Moorfields Eye Hospital pharmacy database and review of clinical notes. Those with stable IOP of ≤22mmHg using timolol/dorzolamide alone or with other medications were invited to take part in a prospective, randomized trial and allocated timolol/dorzolamide or timolol/brimonidine in a double masked fashion. Randomization was by patient according to a computer generated list held by the dispensing pharmacist. Where both eyes were eligible the right eye was selected for analysis. Primary variable was IOP measured at baseline, four weeks and 12 weeks. Secondary variables included side-effects and patient preference.

Results: 1440 patients were screened of whom 825 were eligible for enrolment. 54 patients have completed the trial; 42 were male, mean [range] age was 69 [58-78]. Of the 26 eyes randomized to timolol/dorzolamide mean IOP was 15.2mmHg [9-20] at baseline, 15.5 mmHg [12-23] at four weeks and 17.8mmHg [14-22] at twelve weeks. Of 28 eyes randomized to timolol/brimonidine mean IOP was 15.5mmHg [11-22] at baseline, 15.0mmHg [10-19] at four weeks and 15.7mmHg [12-23] at twelve weeks. Three patients developed a "red eye", the trial and were unmasked. Two were using timolol/dorzolamide and one timolol/brimonidine.

Conclusions: Both fixed combinations had a comparable effect on IOP and a low incidence of side-effects.

CR: A. Spratt, Allergan, F. L. Ogusbawale, Allergan, F. W. Franks, Allergan, F.
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4823 - B1016
Latanoprost versus 0.5% Timolol After Phacoemulsification and Intraocular Lens Implantation in Patients With Primary Open-Angle Glaucoma


Purpose: To evaluate the efficacy of Latanoprost and Timolol in early post-operative period after phacoemulsification and posterior chamber intraocular lens (IOL) implantation in patients with primary open-angle glaucoma (POAG).

Methods: This prospective randomized double-masked clinical study comprised 80 patients with unoperated POAG, who had uneventful phacoemulsification and IOL implantation surgery. Patients were randomly assigned to 1 of 2 groups: pre- and post-operative application of 0.5% Timolol (Group II) or Latanoprost 0.005% (Group II). Intraocular pressure (IOP) was measured (pneumotonometry) before and on first, seventh, and fourteenth days after surgery. The anterior chamber was examined for the levels of cells and flare using slitlamp biomicroscopy.

Results: Pre-operatively, Mean IOP (± Standard Deviation) was 20.0±0.63 mmHg in Group I and 19.0±0.74 mmHg in Group II. Two patients in group I had transient hypotension on the first day after surgery. In both groups, Mean IOP at all three post-operative measurements was below 22.0 mmHg. However, on the seventh day, Mean IOP (± Standard Deviation) in Group II (17.68±0.33 mmHg) was lower than in Group I (21.81±0.53 mmHg). This difference appeared statistically significant (p<0.05).

Conclusions: In patient with unoperated POAG in early postoperative period after phacoemulsification and IOL implantation, Latanoprost is as safe as Timolol. Latanoprost exerts more pronounced hypotensive effect than Timolol regardless surgery-associated inflammatory events in the anterior chamber.

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4824 - B1017
A Multicenter Evaluation of the Effect of Patient Education on Acceptance of Hyperemia Associated With Bimatoprost Therapy for Glaucoma or Ocular Hypertension

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Purpose: The purpose of this study was to evaluate the incidence of hyperemia in patients using bimatoprost and to determine if simple interventions result in increased understanding of glaucoma and hyperemia.

Methods: Multicenter, open-label, evaluator masked trial. Patients (n=64) were randomized to intervention (Group 1) or no intervention (Group 2). For Group 1, office staff were asked to review a fact sheet explaining the importance of IOP lowering and the efficacy of bimatoprost. Patients were given this sheet to take home. Group 2 was instructed only to instill bimatoprost daily and was given no additional instructions.

Visits were at baseline, days 1 and 7, month 1, and week 6. At each study visit, patients completed a questionnaire about any hyperemia and how it affected their willingness to continue bimatoprost.

Results: As graded by investigators, conjunctival hyperemia peaked 1 day after commencing bimatoprost, with a mean of 1.1 (0.5-trace, 1-mild, 2-moderate, 3-severe). By Day 7, hyperemia levels were approximately trace (0.69) and continued to decrease throughout the study. At each visit, patients in Group 2 were slightly more bothered than patients with intervention than were patients in Group 1. At each visit, Group 1 was more likely than Group 2 to report that lowering IOP was very important to preserving vision (for example, at Day 7, 90% and 65%, respectively, P<0.01). Group 1 was more likely than Group 2 to be willing to continue to use bimatoprost, despite hyperemia (97% vs. 88% at Day 1, P<0.05).

Conclusions: Overall, hyperemia peaked at day 1 (to mild levels) and quickly returned to baseline. Patients in Group 1 were more aware of the importance of IOP lowering and were more willing to tolerate hyperemia. Most patients were not bothered by hyperemia. Patient education can improve patient acceptance of a prescribed regimen and potentially increase compliance.

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4825 - B1018
Efficacy and Safety of a Systematic Switch From Latanoprost to Travoprost in Patients With Glaucoma

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Purpose: After a tender for prostaglandin analogs, glaucoma patients previously treated with latanoprost were systematically switched to travoprost. The switch was a systematic switch and not based on intolerance or poor response. The aim of this study was to assess the efficacy and safety of systematically switching a large number of hospital-based glaucoma patients from latanoprost to travoprost therapy.

Methods: In this prospective non-randomized, observational case-series, patients on latanoprost were systematically switched to travoprost without washout and followed up for 12 weeks. The main outcome measures were intraocular pressure (IOP), rate of switching back and tolerability. IOP was measured at baseline (while on latanoprost), and at weeks 6 and 12 after switching to travoprost. Adverse effects were assessed and conjunctival hyperemia was graded using a standardized scale as 0 (none to trace), 1 (mild), 2 (moderate) and 3 (severe). At the final visit, all patients were subjected to a questionnaire that enquired about comfort of use and their assessment of degree of hyperemia.

Results: Ninety-three consecutive patients were enrolled; data was analyzed for 82 subjects. The mean age was 62.9 ± 11.9 years (25-87 years). Majority of the patients were Chinese (89%); there were 47 males (57%). The mean IOP at baseline (16.92 ± 3.81 mm Hg) was not statistically different from that at week 6 (15.86 ± 4.21 mm Hg) (p=0.10) or week 12 (16.34 ± 5.69 mm Hg) (p=0.25). Forty-six patients (58.2%) showed no change in the hyperemia scores at week 12, while 21 (26.5%) had an increase in hyperemia by 1 grade. There were 11 patients (13.2%) who showed improvement of hyperemia by 1 grade. Sixty-six patients (83.5%) felt that subjectively, both drugs were the same in terms of comfort and degree of redness. Ten patients (12.6%) felt that latanoprost was better tolerated and 3 patients (3.8%) felt travoprost was more comfortable. None of the patients exited the study due to hyperemia. Three patients were switched back to latanoprost after 6 weeks due to travoprost intolerance; eight were lost to follow-up.

Conclusions: When glaucoma patients were systematically switched from latanoprost to travoprost, the efficacy and safety of the 2 medications were found to be comparable with few of them switching back.

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