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Ocular hypotensive effects of cholinergic and adrenergic drugs may be influenced by prostaglandins E2 in the human and rabbit eye

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PURPOSE. *Increased PGE2 production by the iris and ciliary body regulate intraocular pressure (IOP)* in vivo*. Various cholinergic and adrenergic compounds are traditionally used as antiglaucoma drugs, and their effect on IOP reduction is antagonised by cyclooxygenase inhibitors, indicating a role for eicosanoids in their hypotensive activity. One of the most potent antiglaucoma drugs, PG2 alpha (Latanoprost), reduces IOP by increasing uveoscleral outflow and also increases PGE2 production by the iris and ciliary body* in vivo*. We investigated whether cholinergic and adrenergic antiglaucoma drugs induce the production of prostaglandin E2 (PGE2)* in vitro *by: 1) the iris-ciliary body (ICB) of rabbits and, 2) irises of glaucoma patients.*

METHODS. *Pilocarpine 2%, epinephrine 1% and echothiophate iodide 0.125% were applied topically to both eyes of Albino rabbits. Control groups were treated with the corresponding vehicles, or untreated completely. Human iris specimens were obtained from nine untreated cataract eyes, and five eyes under antiglaucoma medication undergoing surgery. PGE2 were determined by a radioimmunoassay.*

RESULTS. *PGE2 production by the ICB of treated rabbits* in vitro *was twice that of vehicle-treated or untreated rabbit eyes (p< 0.001, for either group).* In vitro *PGE2 production by treated glaucoma patients' irises was three times higher (p<0.001) than in cataract control patients.*

CONCLUSIONS. *The study found an increase in* in vitro *production of PGE2 by the irises of eyes treated with cholinergic and adrenergic antiglaucoma medications. This suggests a role for endogenous PG production in the hypotensive effect of both classes of drug. (Eur J Ophthalmol 2003; 13: 18-23)*

KEY WORDS. *Prostaglandin E2, Iris-ciliary body, Rabbit, Cholinergic, Adrenergic*

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INTRODUCTION

Most definitions of glaucoma include elevated intra-ocular pressure (IOP) as a fundamental component. However the one characteristic common to all forms of glaucoma is optic neuropathy, resulting in a specific pattern of visual field loss. Since no direct neuroprotective treatment is yet available, the main goal of glaucomatous visual field loss prevention still resides in reducing IOP, and the first-line treatment resides in topical antiglaucoma medication. New drugs have been added to the traditional pharmacologic arsenal, but we still know too little about the mechanisms of action of the various compounds.

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Exogenously applied prostaglandins (PG) reduce IOP (1), and a recently developed PGF2α derivative is effective in patients with ocular hypertension and glaucoma (2).

PGE2 and PGF2α are the major prostanoids produced by the rabbit iris-ciliary body (ICB) (3) and prostanoid receptors have been found in the iris, ciliary body (EP1), ciliary processes, and ciliary muscle (4). Additionally, cyclooxygenase (COX) expression was documented in the inflamed anterior uvea, indicating endogenous PG synthesis (5).

The fact that the ocular hypotensive response to norepinephrine (6), or epinephrine – both long used as antiglaucoma drugs – in rabbits (7) and humans (8) was inhibited by the addition of indomethacin (a COX-1 inhibiting non-steroidal antiinflammatory drug), suggests that endogenously synthesized PGs play a role in the IOP-lowering effect of these drugs but no data is yet available on whether cholinergic antiglaucoma agents alter the PG metabolism.

This study investigated whether routinely used cholinergic and adrenergic antiglaucoma drugs achieve their ocular hypotensive effect through regulation of PG production. We studied the effect of these topical medications on PGE2 production by the iris of glaucomatous patients, and by the ICB in normotensive rabbits.

MATERIALS AND METHODS

Human iris specimens

Human irises were obtained by peripheral iridectomy on nine eyes operated for cataract removal, and another five glaucomatous eyes undergoing trabeculectomy, the filtering surgery for IOP reduction (Tab. I). An iris specimen was obtained from one eye of each patient. Cataract patients had no other known ocular pathology. Excluded from either group were patients with prior ocular surgery or patients receiving any topical ocular medication other than antiglaucoma agents in the three weeks preceding surgery.

The surgical procedures were approved by the institutional human experimentation committee and are in agreement with the Declaration of Helsinki. All subjects gave their informed consent prior to the operation, after being given a full explanation of the nature and possible consequences of the study.

The distribution of antiglaucoma topical treatment is set out in Table I. All glaucoma patients were taking systemic acetazolamide 1 g/day. Surgery was done under local anesthesia for all cataract and glaucoma patients except one in each group (no. 3 and 14). Local anesthesia was induced with meperidine hydrochloride (Demerol 50 mg), and promethazine hydrochloride (Phenergan 25 mg). General anesthesia included induction

TABLE I - AGE, RACE, COLOR OF IRIS AND TOPICAL TREATMENT OF GLAUCOMA AND CATARACT PATIENTS

Patient no.	Age	Race	Iris color	Diagnosis	Treatment
1	61	white	brown	cataract	none
2	57	white	brown	cataract	none
3	74	white	brown	cataract	none
4	60	white	brown	cataract	none
5	55	white	blue	cataract	none
6	65	white	blue	cataract	none
7	81	white	brown	cataract	none
8	57	white	brown	cataract	none
9	60	black	brown	cataract	none
10	61	white	brown	POAG	Pilocarpine 2%
11	44	black	brown	POAG	Pilocarpine 2% and epinephrine 1%
12	64	black	brown	POAG	Echothiophate iodide 0.125%
13	64	white	brown	POAG	Echothiophate iodide 0.125% and epinephrine 1%
14	56	white	brown	CACG	Pilocarpine 2%

POAG= Primary open-angle glaucoma
CACG= Chronic angle-closure glaucoma

with thiopental (4 mg/kg), scolopamine (1 mg/kg body weight) and maintenance with N20/O2 50%, and halothane 1.5%. Topical and systemic antiglaucoma medications were stopped 12 hours before surgery. Pupils were not dilated until the iridectomy was performed. After iridectomy, iris specimens were frozen in a Tris buffer solution.

Rabbit ICB specimens

Adult albino New Zealand rabbits of either sex, weighing 3-4 kg, were divided into ten groups. All the animals were treated in adherence to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. The three antiglaucoma medications studied were levo-epinephrine hydrochloride 1% (Epifrin 1%, Allergan Pharmaceuticals), pilocarpine hydrochloride 2% (Fischer Company), or echothiophate iodide 0.125% (Ayerst Laboratory). Benzalkonium chloride (for pilocarpine and Epifrine preparations), and Chloral vehicle (echothiophate iodide's vehicle), was prepared as stated by the manufacturer. In groups I to III, the appropriate drug was applied bilaterally, in groups IV to VI the drug was applied unilaterally and the vehicle was applied in the fellow eye. In group VII saline was applied to each eye, and in group VIII no medication was applied. The two vehicles were studied, each bilaterally, in groups IX and X. The drug, vehicle, or saline, were applied three times daily for three days. On the fourth day the last dose was applied three to four hours before the rabbits were euthanased by an overdose of pentobarbital (Nembutal) injected intravenously. The iris and cillary bodies were removed, cut into several specimens, and frozen in a Tris buffer solution (pH 7.4).

Determination of prostaglandin E2

Human and rabbit iris specimens were thawed, weighed and homogenized as described (9). Arachidonic acid (25 microgram/ml) was added to the iris homogenate which was incubated in a shaking bath at 37°C for two minutes. The homogenate was then processed for PGE2 determination using a radioimmunoassay with a specific antibody for PGE2 (Yeda Co., Rehovot, Israel) (9). PGE2 levels in the iris homogenate were considered indicative of PGE2 *in vitro* production by the iris. Statistical analysis was done using Student's t-test, and Spearman correlation coefficients (rs) when required. Rabbit data were analyzed either by one-way analysis of variance (ANOVA), or a two-sample t-test.

RESULTS

The main human iris details for cataract and glaucoma patients are given in Table I. The mean ages (mean ± SD) were respectively 63.3 ± 8.8 years and 57.8 ± 8.4 years for the cataract and glaucoma patients.

PGE2 *in vitro* production by the iris of glaucoma patients was approximately three times that of cataract patients (t=8.64, p<0.001) (Tab. II). There was no significant difference in PG metabolism between the three topical antiglaucoma treatments. Similarly, no correlation was found between PGE2 *in vitro* production and other variables, such as age, race, or iris color.

In the rabbit ICB, bilateral application the three drugs caused a two-fold elevation in the mean *in vitro* PGE2 production, compared with saline treated or control rabbits (Tab. III). ANOVA indicated no significant difference between the three bilaterally treated groups, while analysis of the same treated groups in comparison with the saline-treated and control groups was significant (F= 51.47, df=4, p<0.0001) (Tab. III). Two-sample t-tests comparing each of the bilateral drug-treated groups with either the vehicle or saline-treated eyes, as well as controls, showed significant differences (p<0.0001).

In vitro PGE2 production by ICB of eyes treated unilaterally with the drugs (Tab. III) was similar to the bilaterally treated groups. Saline and both vehicles applied unilaterally had no effect on PGE2 levels.

TABLE II - PGE2 PRODUCTION BY SURGICAL IRIS SPECIMENS FROM CATARACT AND GLAUCOMA PATIENTS

Diagnosis	PGE2 (ng/mg wet tissue)	Specimen weight content (mg wet tissue)
Glaucoma	2.98 ± 0.42 (5)	1.32 ± 0.2
Cataract	1.02 ± 0.40 (9)	1.75 ± 0.35

DISCUSSION

This study found a significant increase of PGE2 production by the ICB of rabbits exposed to epinephrine, pilocarpine, or echothiophate iodide. All three drugs enhanced PGE2 production to double the baseline level. Our assumption is that the increased production of PGE2 is related to a direct pharmacological effect of the drugs, although one must take into account that any reduction of IOP leads to an upregulation of PG.

Our findings with epinephrine are in agreement with reports that topical application of adrenergic agonists stimulated PG synthesis in the rabbit (10), or monkey (11), causing an elevation of these prostanoids in the aqueous humor. The interaction between epinephrine and PG has been substantiated by studies showing that COX inhibitors such as indomethacin antagonised the ocular hypotensive effect of epinephrine (8). Epinephrine increased outflow facility and raised cyclic AMP in the perfused human anterior segment (12), and boosted uveoscleral outflow in monkeys (13). In that study, the rise in cyclic AMP preceded the increase of outflow, suggesting that the ultimate effect of epinephrine was slower, involving synthesis and release of PGs or protein synthesis.

In our study the cholinergic agent pilocarpine caused a doubling of PGE2 production by ICB *in vitro*, similar to that induced by epinephrine. This finding is in accordance with previous studies of acetylcholine stimulating the *in vitro* release of PGs by the rabbit iris (14), and of a report that pilocarpine stimulated the *in vitro* release of PGs by bovine ciliary muscle, to 1.8 times baseline (15). In addition, PGs such as PGE2 and PGD2 increased cAMP formation and induced bovine ciliary muscle relaxation (15). It was suggested that in the ciliary muscle certain PGs, such as PGE2 and PGD2, may modulate the responses to muscarinic stimulation in the ciliary muscle via cAMP.

Our study suggesting a pilocarpine/PG interaction is substantiated by findings that pilocarpine's IOP-lowering effect (16) was blocked by various non-steroidal anti-inflammatory agents (NSAID). Concurrently, pilocarpine caused disruption of the blood/aqueous barrier in humans (17) and dogs (16), this being PG-related because protein leakage was inhibited by treatment with NSAID. However our theory is contradicted by the work of Kaufman (18) who showed that disinsertion of the iris prevents the effect of pilocarpine on IOP in monkeys. Moreover the effect of pilocarpine on aqueous humor dynamics is not related to an increase in uveoscleral outflow. The IOP-lowering effect of pilocarpine and epinephrine is reached within 2 hours where prostaglandins reduce IOP more slowly.

TABLE III - PGE2 PRODUCTION BY RABBIT IRIS CILIARY BODY IN EYES TREATED WITH ANTIGLAUCOMA MEDICATION, THEIR VEHICLES, OR SALINE, AND UNTREATED EYES

	Prostaglandin E2 production by iris ciliary body [ng/mg of wet tissue (mean ± SD)]				
Drug tested	**Epinephrine 1%**	**Pilocarpine 2%**	**Echothiophate iodide 0.125%**	**Saline**	**Control untreated**
Both eyes drug-treated	2.47 ± 0.41 (7)*	2.75 ± 0.29 (7)	2.88 ± 0.29 (7)	1.39 ± 0.29 (13)	1.38 ± 0.31 (13)
One eye drug-treated	2.53 ± 0.40 (5)	2.48 ± 0.37 (5)	2.72 ± 0.48 (8)	–	–
Both eyes vehicle-treated	1.24 ± 0.51** (8)	1.24 ± 0.51** (8)	1.25 ± 0.89** (8)	–	–
One eye vehicle-treated	1.45 ± 0.15 (4)	1.25 ± 0.20 (5)	1.28 ± 0.16 (5)	–	–

*=Number of animals in each group

**=$p < 0.01$ for two-sample t-test comparing drug-treated eyes with the appropriate vehicle-treated group

Thus, further research is required to confirm that the hypotensive effect of epinephrine, and to a lesser degree pilocarpine, is partly mediated by endogenous PG production.

Echothiophate iodide on the eye is associated with excessive endogenous levels of acetylcholine. Therefore our findings of enhanced PGE2 synthesis *in vitro* in eyes treated with this hypotensive agent follow the same reasoning as for pilocarpine. However, so far there is no data on a relationship between echothiophate iodide and alterations in PG metabolism.

In our study, *in vitro* PGE2 production by the ICB of all our glaucoma patients was three times higher than in the cataract group which served as control. Three of our glaucoma patients were treated with either pilocarpine or echothiophate iodide alone, while two were on a combined therapy consisting of epinephrine with one of the latter. In patients on pilocarpine 2% or echothiophate iodide alone, the altered PG metabolism was similar to that in the patients on combined therapy including epinephrine. These findings are in accordance with our rabbit study, which showed the PG metabolism was enhanced by either the cholinergic or adrenergic agonist medications. Nevertheless, the difference between PGE2 production by the iris in the glaucoma patients and the control group of cataract patients may reflect the difference between these two eye conditions, with less relation to the drugs. The small number of patients prevents us being conclusive, though the statistical significance raises interest in this possibility.

Exogenously applied PGF2 alpha and its analogs increased the production of PGE2 by ICB and ciliary muscles in different mammalian species by stimulating phospholipase A2 (19). We suggest that, similarly to latanoprost, the antiglaucoma drugs we tested might also exercise their hypotensive effect through an effect on endogenous PG production. PGs exert their ocular hypotensive effect by increasing uveoscleral outflow (20). The role of increased uveoscleral outflow in the induction of ocular hypotension by PGF2α and other PG derivatives is confirmed (21), on the basis of the ciliary muscle relaxation and modification of its extracellular matrix by PGs. In the former report (20) aqueous humor flow rate was not altered by PGF2α and there was only a minimal or no increase in trabecular outflow facility. However the other report (21) showed in the monkey that application of 8-iso PGE2, a PG with an unusual stereochimal configuration, caused a reduction of IOP with an increase of tonographic outflow facility.

In summary we have shown that cholinergic or adrenergic antiglaucoma drugs, such as pilocarpine, epinephrine and echothiophate iodide, endogenously increase the formation of PG, which acts on IOP. We suggest that this PG formation is involved in part of the hypotensive action of these drugs. Endogenously formed prostanoids may exert their hypotensive effect by acting on different hemodynamic mechanisms through stimulation of the muscarinic and cholinergic systems, with the involvement of cAMP.

These findings also raise the question of the efficacy of combined treatment with these drugs, and the causes of their side effects. Further controlled trials are necessary to clarify the mechanisms of action of topical antiglaucoma drugs.

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