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Prostaglandin $F_{2\alpha}$-isopropylester eye drops: effects in normal human eyes*

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SUMMARY The effects of $PGF_{2\alpha}$-isopropylester eye drops on intraocular pressure (IOP) and aqueous humour dynamics were investigated in healthy male volunteers. The other eye was treated with vehicle and used as a control. Special attention was also paid to adverse effects. Single and repeated doses were tested. There was a dose related effect on IOP. Significant reductions were observed 4, 8, and 12 hours after application of 1·0, 2·5, or 10 μg $PGF_{2\alpha}$ equivalents of the drug. With 10 μg the effect lasted 24 hours. An initial tendency towards an increase in IOP was observed for these doses. Repeated doses of 1·0 μg daily or 0·5 μg twice daily produced a significant and lasting IOP reduction of about 2 mmHg for 1-2 weeks. Aqueous humour production was not altered, and outflow facility was not significantly changed. There was a dose dependent hyperaemia with a maximum within 2 hours after application. A foreign body sensation, some pain, and photophobia were noted with increasing doses. A slight miosis of 1 mm was seen in three of six eyes treated with 10 μg. No signs of intraocular inflammation were recorded, but a slight increase in penetration of fluorescein into the anterior chamber was observed after 16 days of treatment.

Prostaglandins have marked effects on the intraocular pressure (IOP). Intracameral injections increase the IOP in rabbits,[1] while low doses of topically applied prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) reduce the IOP in rabbits, cats, and monkeys.[2–7]

The topical application of prostaglandins may also increase the permeability of the blood-aqueous barrier, particularly in rabbits[1 2 5] but also in cats and monkeys.[4–6 8] No systemic side effects have been reported from these studies.

There are only a few studies of the effect of prostaglandins in the human eye. A study of intravenous or intrauterine injections of $PGF_{2\alpha}$ reported a slight IOP reduction following an initial minor increase.[9] The effects on the human eye of a single topical application of 200 μg of the tromethamine salt of $PGF_{2\alpha}$ have been reported.[10] A marked reduction of the IOP was found. Among the reported side effects were ocular pain, conjunctival hyperaemia, and headache, but no aqueous flare or cells in the anterior chamber were seen. The lipid solubility of the tromethamine salt is low, and lipid-soluble esters of $PGF_{2\alpha}$ penetrate the cornea better and can be used in lower doses.[11]

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We have evaluated the effects in the human eye of one such lipid soluble ester, $PGF_{2\alpha}$-isopropylester.[12] We found a dose dependent reduction in IOP without a concomitant reduction in aqueous flow or increase in outflow facility. The drug also produced a dose dependent conjunctival/episcleral hyperaemia and ocular external irritation, mainly in the form of a foreign body sensation.

Material and methods

STUDY DESIGN

$PGF_{2\alpha}$-isopropylester produces some foreign body sensation and conjunctival hyperaemia even in low doses, which prevented a reliable masking of the study. Thus all trials reported here were designed as unmasked, open studies but in which the IOP readings were made in a masked fashion. All participants were healthy male volunteers between 22 and 45 years of age. They did not receive any other topical or systemic drug during the studies. Some of the subjects participated in two of the trials. The study protocols were reviewed and approved by the National Board of Health and Welfare and by the Ethics Committee of the Medical Faculty, University of Umeå. A signed informed consent was obtained from each participant.

A. In a dose finding study IOP response and side effects were determined for 0·1, 0·5, 2·5, and 10 μg

$PGF_{2\alpha}$ administered as isopropylester in eye drops. Six subjects participated. The eye to be treated was randomly selected and a 25 µl eye drop was applied in the conjunctival sac at 8 am (T=0). Randomisation was carried out only once and the same eye was used for all four doses. The other eye was treated with the vehicle. The IOP was determined before treatment and at T=0·5, 1, 2, 4, 8, 12, and 24 hours. At each examination the eyes were evaluated for conjunctival hyperaemia and aqueous flare and cells. Corneal sensitivity was estimated with an aesthaesiometer (Luneau and Coffignon, Paris) 90 minutes after application of the eye drops, and the diameter of the pupils was determined on a Goldmann perimeter under standard light conditions (31·5 apostilbs) 30 minutes later. The treatment schedule started with the lowest dose, and then successively higher concentrations were tested with a washout period of at least 48 hours between doses.

B. Six subjects were treated with 1·0 µg $PGF_{2\alpha}$-isopropylester at 8 am (T=0) for six days in one randomly selected eye. The other eye was treated with vehicle. Corneal sensitivity and pupil diameters were not examined in this study, but the eyes were examined and IOP determined before treatment and at T=0·5, 2, 4, 8, 12, and 24 hours on days 1, 3, and 5. On the sixth day the blood-aqueous barrier permeability was estimated by fluorophotometry.

C. Ten subjects were treated according to the same protocol as in study B with the exception that the dose was divided into two doses of 0·5 µg $PGF_{2\alpha}$-isopropylester given at 8 am (T=0) and 8 pm (T=12), and given for 16 days. The IOP was determined and the eyes were examined before application of the morning dose and at T=4, 8, 12, and 24 hours on days 1, 8, and 15. On day 10 the aqueous humour production was determined in both eyes. Blood-aqueous barrier permeability was determined on day 16 and about two months later.

D. Sixteen subjects were given 1·0 µg $PGF_{2\alpha}$ in one randomly selected eye and vehicle in the other eye. Five hours later the IOP and outflow facility were determined.

AQUEOUS HUMOUR DYNAMICS

The IOP was determined with a Goldmann tonometer. Five consecutive readings were done in each eye, the right eye being taken first. The scale of the tonometer was masked to the examiner. The highest and lowest values were discarded, and the mean of the remaining three values was accepted as the true IOP. The tonometer prism was cleaned between readings in the right and left eye to avoid drug contamination.

Aqueous flow was determined by fluorophotometry (Fluorotron Master). Three drops of 2% sodium fluorescein was applied at 5-minute intervals. Measurements were done hourly 5 to 10 hours later, and from these data the aqueous flow was calculated.[13] The outflow facility was calculated from 4-minute tonograms performed with a pneumatonometer (Alcon, Fort Worth) with the 10 g weight.

SIDE EFFECTS

Hyperaemia was evaluated from photographs of the exterior of the two eyes. Pictures of the temporal conjunctiva were projected on to a screen with an overall magnification of approximately ×25. On the screen a square of 14×14 dots, 1·5 mm in diameter, was placed, with an interval of 1 cm between dots. All dots having some contact with a vessel were counted (Fig. 1). The evaluation of the photographs was masked. The difference between counts in the $PGF_{2\alpha}$ treated eye and the vehicle treated eye was used as an estimate of the drug induced hyperaemia. Fluorophotometry was used to estimate the blood-aqueous barrier permeability.

Corneal and aqueous fluorescein concentrations were determined in the two eyes 2, 4, 6, and 8 hours after an oral dose of 400–500 mg sodium-fluorescein diluted in orange juice. Subjective side effects were registered after each dose in A and after each day of IOP measurements in B and C. Visual disturbances, foreign body sensation, itching, pain, photophobia or any systemic symptom were asked for. The side effects were graded absent, slight, moderate, or severe and the duration was recorded.

STATISTICAL EVALUATION

The two-tailed Student's *t*-test of paired data was used to calculate observed differences. The results are presented as mean (with SEM). Drug effects were based on the difference in IOP between the two eyes. We assume that a possible, centrally mediated effect on IOP, induced by the experimental procedures, would affect the two eyes similarly. Furthermore, prostaglandins are rapidly inactivated in the lungs,[14] and an effect on the contralateral eye induced by systemically absorbed $PGF_{2\alpha}$ seems unlikely.

Results

AQUEOUS HUMOUR DYNAMICS

The effects on the IOP are presented in Tables 1–3. These tables include only the initial IOPs and values obtained at times where a significant effect was seen with at least one dose (study A) or on some day (studies B and C). Remaining non-significant differences are included in Fig. 2, which provides a summary of the observed differences between the two eyes for the three studies A-C. In the dose-response study (A) 0·1 µg and 0·5 µg $PGF_{2\alpha}$ had no

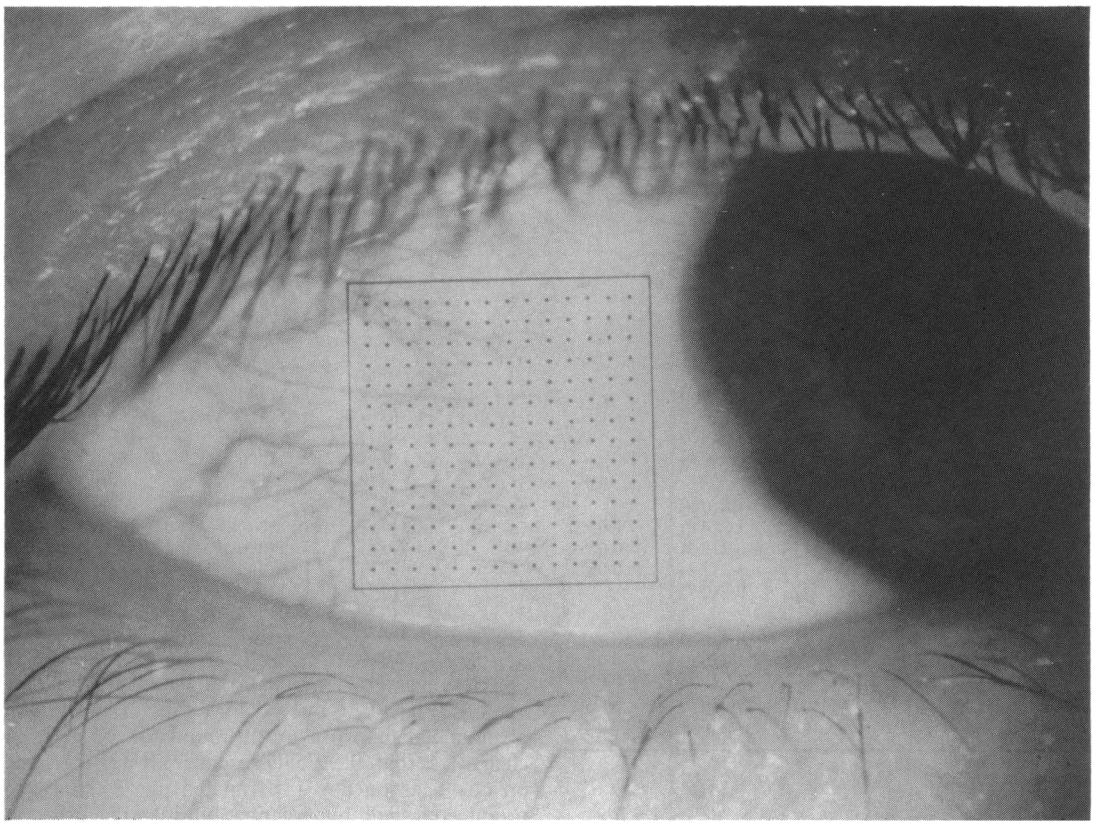

Fig. 1 *To quantitate conjunctival hyperaemia, photographs of the conjunctiva were projected on a screen with a dot pattern. The numbers of dots in contact with a conjunctival or episcleral vessel were counted in the two eyes. The difference in counts of dots between the treated eye and the control eye was used as an estimate of the hyperaemic response for each eye. The figure shows a medium responding eye 30 min after application of 2·5 μg $PGF_{2\alpha}$.*

significant effect on IOP, while the higher doses produced a dose dependent reduction with a similar pattern – a tendency towards an increase in IOP after 30 minutes, and then a slow decrease that reached a maximum 8 to 12 hours after application of the drug. The maximal mean difference between treated and non-treated eyes was 5·7 mmHg observed eight hours after application of 10 μg $PGF_{2\alpha}$. An effect that lasted 24 hours was observed only with the 10 μg dose.

With 1·0 μg given once daily for six days (study B) a significant IOP reduction of 2-3 mmHg was observed four to eight hours after application of the drug throughout the study. No 24-hour effect was obtained, and there was no tendency to an increase or decrease of the response. With repeated doses of 0·5 μg twice daily for 16 days (study C) a significant reduction in IOP of about 2 mmHg was obtained. The IOP was measured after conclusion of the study every twelfth hour, and 36 hours after the last drug application there was no longer any significant difference in IOP between the two eyes.

0·5 μg $PGF_{2\alpha}$ twice daily for ten days had no effect on aqueous flow (study C). Flow was 3·05 (SEM, 0·20) μl/min in the $PGF_{2\alpha}$ treated eyes and 2·97 (0·35) μl/min in the vehicle treated eyes. IOP and outflow facility (study D) are presented in Table 4. There was a significant reduction in IOP in the eyes treated with 1·0 μg $PGF_{2\alpha}$ when compared with the vehicle treated eyes, but no significant change in outflow facility.

SIDE EFFECTS

The hyperaemic response for the dose response study is presented in Fig. 3. A dose dependent hyperaemia was seen about 10 minutes after application of the drug. 0·1 and 0·5 μg $PGF_{2\alpha}$ produced a slight hyperaemia for about one hour. The hyperaemia after 2·5 or 10 μg was equally pronounced but more prolonged with 10 μg. The hyperaemia involved

Table 1 *Intraocular pressures in treated and untreated eyes at various times after application of 0·1, 0·5, 2·5, or 10 µg $PGF_{2\alpha}$-isopropylester in study A. The values are mean with SEM (n=6). Statistical significances were calculated for the paired differences*

Th	Vehicle	$PGF_{2\alpha}$	Difference	p<
0·1 µg				
0	15·7 (1·1)	15·4 (1·1)	0·3 (1·0)	
4	13·9 (0·7)	12·7 (1·1)	1·2 (0·9)	
8	15·4 (1·0)	13·5 (1·3)	1·9 (1·1)	
12	14·6 (1·0)	14·3 (1·4)	0·3 (1·0)	
24	13·0 (1·1)	12·2 (1·3)	0·7 (0·5)	
0·5 µg				
0	14·4 (1·1)	12·8 (1·2)	1·6 (0·9)	
4	14·8 (1·2)	12·9 (1·5)	1·9 (0·9)	
8	14·4 (1·2)	13·5 (1·4)	0·9 (0·8)	
12	13·6 (1·5)	11·9 (1·5)	1·7 (0·9)	
24	14·9 (1·5)	14·2 (1·4)	0·7 (0·5)	
2·5 µg				
0	14·1 (1·4)	12·3 (1·3)	1·8 (0·8)	
4	13·2 (1·3)	11·4 (1·0)	1·8 (0·6)	0·05
8	15·0 (1·8)	11·8 (1·1)	3·3 (0·9)	0·025
12	14·8 (1·5)	11·5 (1·1)	3·3 (1·2)	0·05
24	13·7 (1·4)	12·8 (1·5)	0·8 (0·7)	
10 µg				
0	14·0 (1·2)	13·2 (1·1)	0·8 (0·5)	
4	13·5 (1·7)	11·2 (1·3)	2·3 (0·8)	0·05
8	16·1 (1·1)	10·4 (1·4)	5·7 (1·4)	0·025
12	13·9 (1·3)	9·6 (0·9)	4·3 (0·9)	0·005
24	14·5 (1·3)	12·2 (1·3)	2·3 (0·3)	0·005

Table 2 *Intraocular pressures in treated and untreated eyes at various times after application of 1·0 µg $PGF_{2\alpha}$-isopropylester once daily for six days in study B. Mean with SEM (n=6). Statistical significances were calculated for the paired differences*

Th	Vehicle	$PGF_{2\alpha}$	Difference	p<
Day 1				
0	15·6 (1·7)	15·4 (1·6)	0·2 (0·8)	
2	15·1 (1·1)	13·6 (1·1)	1·5 (0·9)	
4	14·7 (0·8)	11·8 (1·2)	2·9 (0·6)	0·01
8	15·5 (1·4)	12·5 (1·6)	3·0 (0·8)	0·025
12	15·3 (1·3)	13·0 (1·3)	2·3 (1·2)	
24	14·3 (1·4)	13·1 (1·5)	1·2 (0·8)	
Day 3				
0	13·4 (1·3)	12·7 (1·6)	0·7 (0·7)	
2	14·9 (0·9)	13·3 (1·2)	1·6 (0·5)	0·025
4	15·2 (1·3)	12·7 (1·3)	2·4 (1·0)	
8	15·6 (1·4)	12·4 (1·3)	3·2 (1·2)	0·05
12	15·5 (1·5)	12·3 (0·9)	3·2 (1·0)	0·05
24	13·0 (1·3)	13·2 (1·5)	−0·2 (0·8)	
Day 5				
0	13·8 (1·2)	13·7 (1·3)	0·2 (0·4)	
2	14·5 (1·1)	13·2 (0·8)	1·3 (0·3)	0·025
4	14·9 (1·6)	12·5 (1·0)	2·4 (0·8)	0·05
8	13·9 (1·6)	11·0 (1·0)	2·9 (0·9)	0·025
12	13·2 (1·1)	12·2 (1·1)	1·0 (0·8)	
24	12·2 (1·4)	11·8 (1·4)	0·3 (0·5)	

Table 3 *Intraocular pressures in treated and untreated eyes at various times after application of 0·5 µg $PGF_{2\alpha}$-isopropylester twice daily for 16 days in study C. Mean with SEM (n=10). Statistical significances were calculated for the paired differences*

Th	Vehicle	$PGF_{2\alpha}$	Difference	p<
Day 1				
0	15·2 (1·0)	14·9 (0·7)	0·3 (0·4)	
4	15·3 (0·9)	13·1 (0·6)	2·2 (0·9)	0·05
8	14·8 (0·9)	13·3 (0·8)	1·6 (0·8)	
12	14·8 (0·8)	13·1 (0·8)	1·6 (0·7)	0·05
24	13·2 (0·9)	12·2 (0·7)	1·0 (0·5)	
Day 8				
0	13·7 (0·8)	12·0 (0·7)	1·7 (0·6)	0·025
2	14·3 (0·7)	11·8 (0·6)	2·5 (0·5)	0·001
4	14·0 (0·6)	11·5 (0·5)	2·5 (0·4)	0·001
8	13·6 (0·7)	11·8 (0·6)	1·8 (0·3)	0·001
12	13·5 (0·9)	11·4 (0·8)	2·1 (0·4)	0·001
24				
Day 15				
0	13·7 (0·8)	12·7 (0·6)	1·0 (0·4)	0·05
4	14·1 (0·8)	11·9 (0·5)	2·1 (0·5)	0·005
8	13·6 (0·8)	12·0 (0·6)	1·6 (0·4)	0·005
12	13·4 (0·7)	12·1 (0·7)	1·3 (0·3)	0·005
24	13·7 (0·8)	12·3 (0·8)	1·3 (0·5)	0·025

episcleral vessels as well as conjunctival vessels. The response to 1·0 µg once daily (study B) was more pronounced than previously found for 0·5 µg, but clearly less than the 2·5 µg response. It was the same on day 5 as on day 1. Even 0·5 µg×2 (study C) produced a slight hyperaemia, but it had vanished at the time of ocular examination four hours later.

No cells or flare were found in any of the studies. Fluorescein concentrations in the cornea and aqueous ranged between 42 and 328 ng/ml and 7 and 112 ng/ml respectively, two to eight hours after an oral dose of 400–500 g sodium fluorescein in studies B and C. The concentrations in the cornea were, as a rule two to three times that in the aqueous. The ratio of treated eye to untreated eye was calculated for both cornea and aqueous, and the results are presented in Table 5. Generally the fluorescein concentrations in the treated eyes tended to be lower than in the untreated eyes in study B while the opposite was true for study C. The participants in study C were re-examined two months later. At that time no difference was observed between the two eyes for either cornea or aqueous.

No effect on the pupil diameter could be shown for doses up to 2·5 µg $PGF_{2\alpha}$. 10 µg produced a miosis of 1 mm in three of six participants. No change in corneal sensitivity was observed. The subjective adverse effects showed a marked dose dependence. No discomfort was noted for 0·1 µg. 0·5 µg produced a minor foreign body sensation in the treated eye in three of the seven participants for about one hour.

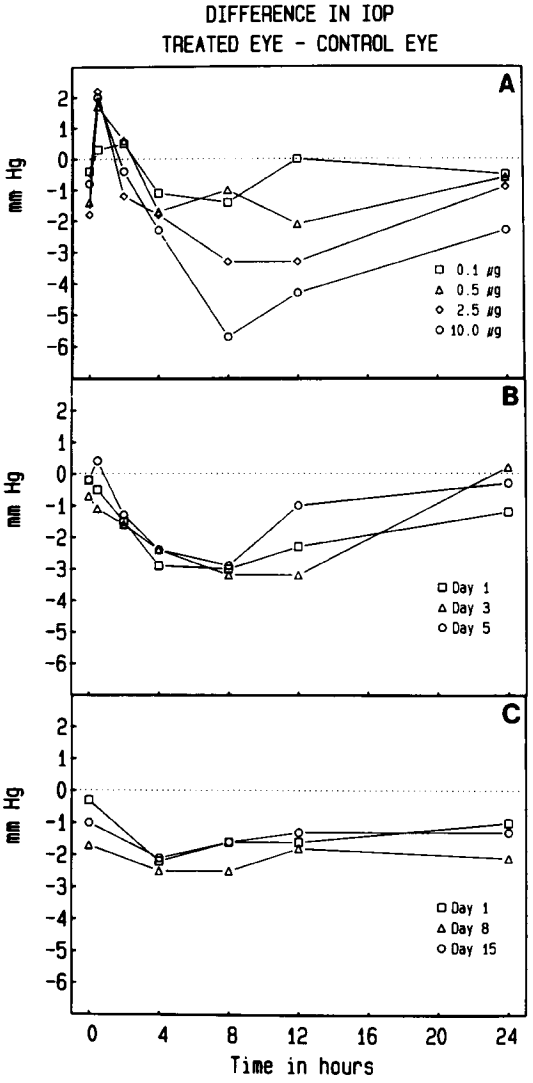

Fig. 2 *The mean difference (IOP in control eye minus IOP in treated eye) at various times after application of drugs.* Top: *Single doses of four different doses of $PGF_{2\alpha}$ (study A).* Middle: *Repeated doses of 1·0 µg $PGF_{2\alpha}$ once daily for 6 days (study B).* Bottom: *Repeated doses of 0·5 µg $PGF_{2\alpha}$ twice daily for 16 days (study C).*

After 2·5 µg discomfort was noted by all participants for two to three hours. In the treated eye a marked foreign body sensation was felt by three subjects, and the remaining three had an experience of pain for about the same time. 10 µg produced photophobia and pain in the treated eyes of all six participants, one having a foreign body sensation for 12 hours. The side effects noted by the participants receiving 1·0 µg daily for six days were regarded as mild by everyone and remained unchanged during the study. A foreign body sensation was noted about 15 minutes after application of the drug and lasted about two hours. With 0·5 µg twice daily a slight discomfort for about one hour was experienced by only two of 10 persons on the first day of treatment. On days 8 and 15, however, discomfort was felt for one to two hours in the $PGF_{2\alpha}$ treated eyes of nine of 10 participants. No discomfort was recorded from the vehicle treated eyes in any of the studies.

Table 4 *Intraocular pressures in mmHg and outflow facilities in µl/min/mmHg in treated and untreated eyes five hours after 1·0 µg $PGF_{2\alpha}$ (study D). Mean with SEM (n=16). Statistical significances were calculated for the paired differences*

	IOP	*Facility*
Control eye	17·0 (0·6)	0·311 (0·029)
Treated eye	15·3 (0·7)	0·334 (0·033)
Difference	1·7 (0·4)	0·024 (0·029)
p<	0·005	NS

Discussion

The purpose of study A was to find a suitable dose for further tests. Without previous knowledge of the effects on the eye we chose to use increasing concentrations of $PGF_{2\alpha}$ in the same eye. The study served its purpose, but an unexpected observation was the difference in IOP between the two eyes observed even before application of the three higher doses (Table 1). A similar difference in pretreatment IOP was not observed before 0·1 µg $PGF_{2\alpha}$ or before any of the other studies. 0·1 µg $PGF_{2\alpha}$ had no effect on IOP for the first 24 hours, and the likelihood that it caused a slow and almost significant ($p<0·1$) reduction 48 hours later seems remote. Thus we have to assume that this is due to random variation. The information obtained from study A enabled us to choose a dose for further studies, but the magnitude of the effect with higher doses of $PGF_{2\alpha}$-isopropylester may be exaggerated by the unexplained difference in pretreatment IOP between the two eyes. Moreover the IOP of the vehicle treated eyes tended to become lower during studies A–C. Obviously we cannot rule out a contralateral effect, but a likely alternative is that the participants become more relaxed as they become more familiar with the examination procedures. We could find no tendency to a reduction in IOP due to the five rapid measurements on each examination.

The IOP response in the human eye seems to follow the pattern seen in primate eyes, where a

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