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26th June, 1989

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Re: Final Report for DAMD17-87-G-5013

Dear Ms. Madigan,

Enclosed please find our final report covering the period of Feburary 12, 1985 through January 31, 1987, of our old Grant No. DAMD17-85-G-5013. This report was delayed due to a misunderstanding, which has been clarified by a recent letter from Ms. R. McHenry, LAIR.

Sincerely yours,

Nava Naveh, M.D.
The Maurice and Gabriela Goldschleger
Eye Research Institute

Encl.

cc. Becky McHenry/LAIR
Jeannie Shinbur/USA MRAA
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TREATMENT OF LASER-INDUCED RETINAL INJURIES

FINAL REPORT

February 12, 1985 - January 31, 1987

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Supported by US Medical and Development Command,
Fort Detrick, Frederick, MA 21701-5012
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FOREWORD

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals: prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life and Sciences, National Research Council (NIH Publication, No. 86-23, revised 1985).

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This study investigated the effect of steroids know for their inflammatory effect on the laser-induced retinal injury. In an attempt to minimize this we studied prostaglandin E_2 (PGE_2), known for their mediatory role in any inflammatory reaction, as well as changes in protein leakage. The latter is indicative of blood retinal barrier disruption.

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Our study revealed an enhanced PGE_2 response as manifested by: 1) excessive production in vitro of PGE_2 by the retina/choroid of laser-exposed eyes; 2) accumulation of both PGE_2 and protein in the vitreous body to above prelaser values. Corticosteroid treatment abolished the increase in the vitreal PGE_2 response, but it only partially reduced the excessive PGE_2 production in vitro by retina/choroid. Treatment was effective during the first week, but later failed. The finding of the transient nature of the anti-PGE_2 effect of the steroids does not necessarily point to the steroids inefficacy as anti-inflammatory agents, but rather may point to the cytoprotective nature of PGs themselves.

However, in further studies the effect of other anti-inflammatory agents in minimizing the damaging effect of irradiation might be considered, as well as other mediatory substances.

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INTRODUCTION

Laser instruments are used in the battlefield, as well as in medicine, industry, and communication, and it is certain that future armed conflicts will result in many laser-induced eye injuries. Such injuries may cause temporary or permanent visual *incapacitation.* Their severity depends on the extent of the burn to the retina, the part of the eye most vulnerable to laser irradiation. The area surrounding the retinal laser site, which is a few times larger in diameter than the laser lesion itself, is also subject to inflammatory reaction and subsequent scarring (12). Any measure that will reduce this inflammation will accelerate healing, diminish the extent of scarring, and improve the visual prognosis of the patient.

Our investigation on the ocular reaction to laser irradiation was based on two hypothesis: (1) prostanoids are involved in laser-induced retinal burns; and (2) anti-inflammatory drugs that inhibit arachidonic acid metabolism - and thus decrease prostanoid production - will reduce retinal inflammation, and accelerate healing. We chose steroid treatment known anti-inflammatory agents, which are also anti-PGs, to reduce the inflammatory response elicited by laser irradiation of the eye.

The histopathological changes of laser-induced retinal lesions in steroid-treated as well as in untreated eyes were investigated in this study. We also used the following parameters as indicators of the inflammatory response: Prostaglandin E_2 (PGE_2) production

by the retina/choroid, vitreal PGE_2 levels, and vitreal protein levels.

We decided to look for changes in PGE_2 based on information that the inflammatory changes following a suprathreshold-induced laser lesion is characterized by necrosis of the retina and the underlying choroid, surrounded by an area with severe inflammatory reaction with edema and cellular infiltration (1, 2). In addition, a suprathreshold laser-induced retinal damage is associated with rod outer segment lipid peroxidation (3, 4), and increased production by the retina of leukotreines (LTCs), the lypooxygenase products of arachidonic acid metabolism (5). However, prostaglandin production by noncoherent light-exposed retina via the cyclooxygenase pathway of arachidonic acid was not elevated (20).

However, no data are as yet available on arachidonic acid metabolism either by the retina or choroid of eyes subjected to retinal laser irradiation.

It is likely that retinal laser irradiation may be associated with an augmentation in PGE_2 production by the laser damaged retina and choroid, for PGE_2 production is induced by various stimuli including trauma, ischemia, and laser irradiation (6). PGE_2 produced in excess by the retina are immediately released into the vitreous body, an avascular extracellular space occupying 7/8 of the eye, and might play a major role in

regulating the ocular response to laser-induced trauma.

The inhibitory effect of corticosteroids on prostaglandin (PGs) production is well-established (7, 8), and is partially responsible for their anti-inflammatory activity. This suppression of PGE_2 production is mediated through reduction of the availability of arachidonic acid (8), a substrate for PGs formation.

In the present study we investigated the effect of corticosteroid treatment of argon laser-induced retinal injury on vitreal accumulation of both PGE_2 and protein, and their relationship with PGE_2 production in vitro by the retina/choroid.

MATERIAL AND METHODS

The study consisted of seven groups of age- and sex-matched rabbits, raised in alternating 12-hour periods of light and dark, using regular fluorescent light source. The provisions of the Arvo Resolution on the Use of Animals in Research were adhered to.

1) Control Group - Twenty-eight rabbits that were not exposed either to laser irradiation or noncoherent light illumination, and had no ocular pathology.
2) Noncoherent Light Exposure (sham exposure): Noncoherent light exposure (30 rabbits) involved pupil dilatation, contact lens fitting, and exposure to the noncoherent light

of the slit lamp attached to the argon laser, and used during laser procedure for a length of time similar to that used in the argon group, but without laser irradiation.

3) Subthreshold Laser Exposure - Irradiation in these cases (45 rabbits) involved low-energy levels with power setting of 20 milliwatts, spot size 60-70 microns, and duration of 0.2 seconds, and resulted in lesions that were invisible ophthalmoscopically.

4) Untreated Single Argon Laser Lesion - Seventy rabbits with a single argon laser-induced retinal burn divided into four subgroups: 20, 18, 12, and 20 rabbits in which PGE_2 and protein were determined one, three, seven, and 14 days after exposure, respectively.

5) Untreated Argon Laser-Exposed Group with Multiple Lesions - Seventy-three rabbits with multiple argon laser-induced retinal burns divided into four subgroups in which PGE_2 were determined one, three, seven, and 14 days after the exposure, respectively.

6) Corticosteroid Treated Argon Laser-Exposed Group - Fifty-seven rabbits with a single argon laser-induced retinal burn, that were treated with corticosteroids according to the procedure described below. This group was also divided into four subgroups: 12, 22, 11, and 12 rabbits in which PGE_2 and protein were determined one, three, seven, and 14 days after exposure, respectively.

Thirty minutes before the laser procedure all rabbits were

anesthetized by 35 mg/kg Ketamine and 5 mg/kg Xylazine injected intramuscularly; this was followed by pupil dilatation with tropicamide 0.5% and local anesthesia with benoxinate 0.1%.

Argon Laser Exposure

A continuous wave green-blue argon laser was used (Lasertek, 265 Excitor). The laser beam was focused on the retina of the right eye only of each rabbit, through a Goldmann-coated lens. Laser exposure consisted of a single burn at a power setting of 200 milliwatt, a spot size of 500 microns, and a duration of 0.5 seconds aimed 2 disc diameters below the optic disc, resulting in an ophthalmoscopically visible burn. During the procedure the animals were exposed both to the laser irradiation and to the noncoherent light of the slit lamp used for illumination required for a precise laser burn application.

Sample Preparation

Following enucleation the cornea was cut at the limbus, the lens and iris were removed and discarded, while the vitreous was separated as described, and put in another vial. Likewise, a retina/choroid preparation consisting of the whole retina attached to the choroid was separated as described, and put in another vial.

Prostaglandin E_2 Determination

The retina/choroid preparation was incubated in 0.6 ml Krebs Ringer Bicarbonate Hepes buffer, pH 7.4, in a slow-shaking bath

at 37°C for 15 minutes. At the end of the incubation period the retina/choroid underwent centrifugation at 3000 RPM for five minutes, the tissue was removed, and samples form the incubation media were withdrawn for PGE_2 determination, and extracted with two volumes of ether at pH 3.8. The aqueous phase was then assayed for PGE_2 using radioimmunoassay as described (4).

The vitreous body of each eye was similarly incubated in 1.0 ml of the same buffer, and on completion of the incubation period a sample was withdrawn from the media for PGE_2 determination, extracted with ether as described, and then assayed.

To determine PGE_2 recovery rate the appropriate tritiated prostaglandin was added to the medium, which was then cooled to 4°C, and acidified to pH 4 with sodium acetate buffer (1M pH 3.8), and extracted as described. The dissolved aqueous phase was used partly to assess procedural loss, and the remainder was used for the radioimmunoassay.

PGE_2 was measured using the radioimmunoassay technique (4) with a specific antibody for PGE_2 (Miles-Yeda, Rehovot, Israel) with a 3% cross reactivity with PGE_2. The cross reaction of this antiserum with other prostaglandins was less than 3%. Day-to-day reproducibility and within-run precision of the analytical procedure was examined by processing spiked samples of vitreous and buffer.

Protein Determination

Protein was measured in the vitreous body using the modified Roseborough Lowery method (5).

Steroid Treatment Procedure

Steroid treatment with a daily dose of Dexamethasone (0.5 mg/kg weight, intramuscularly) was started during the first hour after laser exposure, and repeated daily.

RESULTS

The results will be divided into three sections: 1) changes following multiple suprathreshold laser lesions; 2) changes following subthreshold exposure; 3) effect of steroid treatment on laser-induced injury.

1. Suprathreshold Multiple Laser Lesions

Vitreal PGE_2 Levels (Table 1): The vitreal PGE_2 levels in the control group were 7.47 ± 3.77 ng/mg weight, and were considered as baseline levels. In the laser-exposed eyes the vitreal PGE_2 levels showed a biphasic elevation above baseline levels over a two-week period, a transitory initial peak on day three, followed by a second peak on day 14 (17.87 ± 4.80 and 15.99 ± 4.22 ng/kg weight, respectively), in the noncoherent light exposed eyes the PGE_2 levels were found to be elevated only once, on day 14 (16.68 ± 4.94 ng/mg weight) (Table 1).

PGE_2 Production Retina/Choroid (Table 2): In the control group

PGE_2 production by retina/choroid was 0.36±0.13 ng/mg weight, and was considered as baseline values. PGE_2 production in the laser-exposed group was elevated at the various timing throughout the two-week period, with highest level at days three and 14 (2.55±0.98 and 1.11±0.46 ng/mg weight, respectively). In the noncoherent light-exposed eyes it remained unchanged during the first week compared with baseline levels, but showed a progressive elevation on days seven and 14 (0.82±0.43 and 1.21±0.40 ng/mg weight, respectively) (Tables 2, 3).

Vitreal Protein Levels (Table 3): In the control group vitreal protein levels were 0.57±0.17 ng/mg weight, and considered as baseline values. In the laser-exposed eyes vitreal protein levels exceeded baseline values during the first week after exposure (1.33±0.32, 1.03±0.24, and 0.95±0.17 ng/mg weight on days one, three, and seven, respectively), ($p<0.0000$, $p<0.002$, and $p<0.0008$, respectively). However, during the second week the levels returned to baseline. In the noncoherent light-exposed group vitreal protein content peaked only once, on day three (0.83±25 ng/mg weight, and was significantly higher than baseline, $p<0.0001$).

PGE_2 production by the retina/choroid was closely associated with changes in vitreal PGE_2 levels both in the laser and noncoherent light-exposed groups, as depicted in Figures 1 and 2. However, similarly in both groups the vitreal protein levels did not coincide with changes in vitreal PGE_2 content, as in the laser-

treated group protein level elevation above baseline preceded vitreal PGE_2 peak levels (Fig. 2), whereas in the other group protein levels peaked on day three, when PGE_2 was its lowest (Figs. 1, 2).

2. Subthreshold Argon Laser-Induced Retinal Injury

PGE_2 production by the retina/choroid following multiple subthreshold laser retinal burns as well as PGE_2 and protein vitreal levels are depicted in Fig. 3. PGE_2 production by the retina/choroid in this group was significantly elevated at one hour following exposure, and remained at this higher level at the one day followup point. It returned to control level at two days, but on the third day and seven-day time intervals retina/choroid PGE_2 production was again significantly augmented compared to control levels.

PGE_2 vitreal levels of eyes exposed to multiple subthreshold laser retinal applications were significantly augmented at the time intervals coinciding with the peaks in PGE_2 production by the retina/choroid. It is noteworthy that at time intervals later than one hour, for both retinal and vitreal PGE_2 levels, the sample number was smaller than usual, and did not exceed 11; thus, further studies are required to achieve conclusive data.

Vitreal protein levels following multiple subthreshold laser exposure were not elevated above control levels at all time intervals. An earlier evaluation using 11 samples of vitreal

protein levels at the one-hour interval showed a significant increase; however, when a larger number of samples (19) was evaluated, protein levels did not exceed control limits. Further study to clarify this difference is required.

3. Effect of Steroid Treatment on Laser-Induced Injury

Steroid Treatment of Laser-Treated Eyes (Table 4): In the steroid-treated group PGE_2 production measured as the amounts released into incubation media peaked only on day seven, and levels were lower than in the corresponding untreated laser group (21.0 ± 8.6 ng/mg protein vs 32.9 ± 4.9 ng/mg protein, $p<0.000$). However, on day 14 production was further enhanced in the treated eyes whereas in the corresponding untreated group levels were already declining at this time (32.2 ± 12.4 ng/mg protein, and 10.6 ± 4.5 ng/mg protein, $p<0.000$, respectively).

Vitreal PGE_2 Levels (Table 5): In the control group vitreal PGE_2 levels were 5.8 ± 1.7 ng/ml, and were considered as baseline. In the untreated laser-exposed group vitreal PGE_2 content peaked only once (day seven) above baseline values (10.7 ± 3.6 ng/ml, $p<0.0002$), as compared with the steroid-treated group in which vitreal PGE_2 content did not exceed baseline levels throughout the observation period. Thus, steroid treatment prevented the accumulation of vitreal PGE_2 above baseline values.

Vitreal Protein Levels (Table 6): In the control group vitreal protein levels were 0.57 ± 0.17 mg/ml, and were considered as

baseline. Following laser exposure vitreal protein content in the untreated groups was elevated twice during a two-week period; on day three and 14 reached values of 0.68 ± 0.16 mg/ml and 0.79 ± 0.13 mg/ml, respectively, which were significantly higher than baseline levels ($p<0.03$ and $p<0.004$, respectively).

In the steroid treated laser exposed eyes vitreal protein levels during the two-week followup peaked only once above baseline values (day seven), but reached values higher than those in the corresponding untreated group (1.00 ± 0.31 mg/ml vs 0.48 ± 0.13 mg/ml, $p<0.001$, respectively). Thus, steroid treatment only partially prevented the accumulation of protein in the vitreous body.

In the laser-exposed group a close association between changes in vitreal PGE_2 content and PGE_2 production by the retina/choroid was noted only during the second week after exposure. However, in the steroid-treated group (Table 6) no such correlation was observed during the observation period, as excessive PGE_2 production was not reflected in the vitreal levels, which remained unchanged from baseline.

Changes in protein vitreal levels in both the untreated and the steroid-treated groups (Table 3) did not coincide with changes of either vitreal PGE_2 content or PGE_2 production.

DISCUSSION AND CONCLUSIVE REMARKS

It is well-established that light-induced retinal changes are associated with membrane and lipid peroxidation of rod outer segment (3, 4); retinal lipid hydroperoxides were reported to be increased following light exposure of frogs (3) and albino rats (3), whereas increased water-soluble peroxides were released by light-exposed rats' retina (10).

Prostaglandin production by the noncoherent light-exposed retina might also be affected by the light-induced release of excessive amounts of lipid hydroperoxides (11), as their synthesis is highly sensitive to hydroperoxide levels (9).

No data are available thus far on arachidonic acid metabolism by the retina or the choroid of eyes subjected to laser irradiation, although laser irradiation of the rabbit iris by various laser modalities (12, 13) was shown to be associated with elevated PGE_2 production in the anterior segment of the eye.

Our demonstration of an increased PGE_2 production in our noncoherent light-exposed group has not been reported so far. The fact that PGE_2 production in the noncoherent light-exposed group was elevated above baseline levels on day 14 after exposure, but not earlier, is in agreement with histologic studies on photic light injury in rats' retina, in which, following a 24-hour exposure, alteration of the outer retinal layer became more severe with time (14).

In the laser-irradiated eyes the augmented PGE_2 production followed a different pattern, and was of higher magnitude compared with the light-exposed eyes. Higher PGE_2 production levels, that persisted for a longer time in the laser group, might be due to the additive effect of the noncoherent light photochemical changes and the laser-induced trauma. A laser-induced retinal trauma might be expected to elicit a response similar to that of the nervous system, whose reaction to trauma is characterized by an enhancement in prostaglandin production (15, 16). Another source of PGE_2 production at the laser lesion site might be the polymorphonuclear cells infiltration (17) of the surrounding area lesion.

Vitreal PGE_2 Content

A transitory accumulation of PGE_2 in the vitreous to above baseline levels was shown in both our laser and noncoherent light-exposed groups (a biphasic elevation and a single peak of the two, respectively). Vitreal PGE_2 content is determined partly by: 1) the amounts produced by the retina as PGE_2 immediately released into the vitreous body, which is an extracellular space, and 2) the rate of their removal from the vitreous by an active transport mechanism located at the ciliary processes and the blood retinal barrier (BRB).

In both our groups the accumulation of PGE_2 in the vitreous to above baseline levels occurred only when production was enhanced to at least three times that of baseline.

Vitreal Protein Content

Both the laser and noncoherent light exposure were associated with an elevation vitreal protein content indicative of a "break" in the BRB. In the former group elevated levels were maintained on days three and 14 following exposure, as compared with a transient peak on day three in the noncoherent light-exposed group. Our demonstration in the laser group that vitreal protein content resumed control levels during the second week after exposure is in agreement with vitreous fluorophotometric studies showing that BRB permeability recovered to near normal levels 14 days after xenon (18) or argon (19) retinal laser photocoagulation.

Changes in vitreal protein levels in both groups did not coincide with PGE_2 accumulation in the vitreous body. This might indicate that vitreal PGE_2 levels in our exposed groups were too low to affect the BRB, as it was demonstrated (20) that BRB remained intact when the amounts of PGE_2 injected intravitreally did not exceed 200 g, a quantity higher by four orders of magnitude than the highest vitreal PGE_2 levels observed in our study.

The Effect of Steroid Treatment on Laser-Induced Retinal Injury

The inhibitory effect of corticosteroids on prostaglandins production is well-established, and is partly responsible for their anti-inflammatory activity (6, 7).

Steroid treatment of our laser-irradiated eyes prevented the

initial elevation in PGs production on day one, but not the development of a later peak on day seven, when production was only 32% lower than in the corresponding untreated group. By day 14 a further enhancement of production resulted in maximal peak values at a time when levels were already declining in the untreated group.

The steroidal inhibitory effect on PGE_2 production in the eye and elsewhere in the body is well-established (21) so that our observation of such an inhibitory effect in the retina/choroid is not unusual. However, the demonstration of the absence of this inhibitory effect during the second week is less common, and might be related only with reports on the lack of an inhibitory effect of maternally administered dexamethasone on $PGE_{1\alpha}$ synthesis by fetal rat lung (22). Thus we demonstrated that the steroidal inhibitory effect of PG formation was transitory and was evident during the early phase following treatment, but was lost during the later phase. This effect has not yet been reported.

PGE_2 levels in the vitreous are regulated, however, both by the rate of its production and release by the retina, and its removal by active transport mechanisms located at the BRB (23). As PGE_2 production was measured in vitro, whereas its vitreal levels were determined in vivo, the applicability of the production values might be relative. Our finding of normal PGE_2 vitreal levels despite excessive production might indicate that corticosteroid

treatment enhanced the efficacy of the BRB absorptive mechanisms.

Corticosteroid treatment only partially reduced the above baseline elevation of vitreal protein levels; peak levels were evidence once, as compared with a biphasic elevation in the untreated group. This finding is in accordance with a study on endotoxin-induced uveitis (24), in which steroids had little effect on excessive vitreal protein levels, while it had a significant effect in suppressing the clinical signs, and reducing the polymorphonuclear cellular infiltration. Elevation of protein levels in the vitreous to above baseline in our steroid-treated group might be indicative of persistent leakage and/or inability of the BRB absorptive mechanisms to remove the excessive vitreal accumulation.

Our finding of the transient nature of the steroidal inhibitory effect on PGE_2 production must be considered in view of three facts: 1) steroid treatment of various inflammatory conditions maintains its effectiveness over a long period; 2) the anti-inflammatory effect of corticosteroids might be mediated through various mechanisms besides prostaglandins, by stabilizing lysosomal and cell membrane, by inhibition of complement induced granulocyte aggregation, or by prevention of the polymorphonuclear infiltration into the inflamed tissue or exudate (25); 3) various prostaglandins suppress the inflammatory response when administered exogenously (19-21). In like manner, intravitreal administration of PGs in rabbits with bacterial

endotoxin uveitis resulted in decreased vascular permeability (26), whereas application of PGE_1 and $F_{2\alpha}$ prior to the induction of corneal trauma reduced the resultant inflammatory response and the formation of endogenous PGs (27). Therefore the loss of the steroidal inhibitory effect on PGE_2 synthesis as observed by us during the later phase of the inflammatory response does not necessarily reflect a loss or reduction of their anti-inflammatory activity. It has been suggested that excessive levels of PGE_2 generated during the inflammatory reaction might act as suppressors of the inflammatory response due to secondary elevation in cyclic AMP (28).

Table 1: The effect of suprathreshold argon laser retina exposure on prostaglandin E_2 levels in the vitreous body - comparison with noncoherent light exposure.

Prostaglandin E_2 Vitreal levels (ng/mg weight) (mean±SD)			
Time after exposure (days)	noncoherent light exposed group	laser exposed group	p value
1	5.84±2.29 n=41	8.69±3.47 n=26	N.S.
3	5.35±1.92 n=30	17.87±4.80 n=42	< 0.0000
7	8.67±3.49 n=18	6.09±2.49 n=20	N.S.
14	16.68±4.94 n=8	15.99±4.22 n=7	N.S.
Baseline 7.47±3.77 n=30			

* p value ( ) p value for t-student test comparing each of the noncoherent light exposed eyes at each time interval with corresponding laser exposed group.

**n n indicates number of eyes involved in each group.

***N.S. statistically not significant.

Table 2: Prostaglandin E_2 production by retina/choroid of suprathreshol argon laser exposed eyes - comparison with the effect of noncoherent light exposure.

Prostaglandin E_2 Production by retina/choroid (ng/mg wet weight) (mean±SD)

Time after exposure (days)	noncoherent light exposed group	laser exposed group	p value
1	0.45±0.22 n=36	0.52±0.21 n=31	N.S.
3	0.35±0.13 n=30	2.55±0.98 n=33	0.0000
7	0.82±0.43 n=24	0.55±0.20 n=24	0.0076
14	1.21±0.40 n=12	1.11±0.46 n=9	N.S.

Baseline 0.36±0.13 n=30

*p value ( ) p value for t-student test comparing each of the noncoherent light exposed eyes at each time interval with corresponding laser exposed group

**n n indicates number of eyes involved in each group.

***N.S. statistically not significant.

Table 3: The effect of suprathreshold argon laser retinal exposure on protein levels in the vitreous body - comparison with noncoherent light exposure.

	Protein vitreal levels (μg/mg weight) (mean±SD)		
Time after exposure (days)	noncoherent light exposed group	laser exposed group	p value
1	0.63±0.21 n=40	1.33±0.32 n=34	<0.0000
3	0.83±0.25 n=30	1.03±0.24 n=56	<0.002
7	0.49±0.19 n=24	0.95±0.17 n=23	<0.0008
14	0.54±0.17 n=12	0.43±0.13 n=9	N.S.
Baseline	0.59±0.17 n=30		

*p value ( ) p value for t-student test comparing each of the noncoherent light exposed eyes at each time interval with the corresponding laser exposed group.

**n n indicates number of eyes involved in each group.

***N.S. statistically not significant.

Table 4:

Prostaglandin E_2 relase by the choriod-retina of argon laser exposed eyes – the effect of steroid treatment.

Time after exposure (days)	Prostaglandin E_2 levels (ng/mg protein) (mean±SD) untreated laser group	steroid treated laser group	*p Values (s)
1	13.0±3.9	4.4±2.1	<0.0000
	+n=19	n=12	
3	7.5±3.9	6.6±2.7	‡N.S.
	n=22	n=20	
7	32.9±4.9	21.0±8.6	<0.000
	n=10	n=11	
14	10.6±4.5	32.2±12.4	<0.000
	n=19	n=11	
baseline	6.0±2.3		
	n=30		

*p (S) p-value for t-student test comparing the untreated laser exposed eyes at each time interval with the corresponding steroid treated group.

\+ n n indicates number of eyes involved in each group

‡ N.S. statistically not significant

Table 5

Vitreal prostaglandin E_2 levels following argon laser induced retinal lesion - The effect of steroid treatment

Time after exposure (days)	Prostaglandin E_2 levels (ng/ml) (mean±SD) untreated laser group	steroid treated laser group	* p Value(s)
1	3.64±1.7 n=20	3.4±1.2 +n=12	‡ N.S.
3	3.0±4.5 n=20	5.9±2.2 n=22	<0.0014
7	10.7±3.6 n=10	5.9±2.2 n=11	<0.002
14	3.6±1.9 n=23	4.0±1.5 n=12	N.S.
baseline	5.8±1.7 n=30		

* p p value for t-student test comparing each of the laser untreated eyes at each time interval with the corresponding steroid treated group.

\+ n n indicates number of eyes involved in each group.

‡ N.S. statistically not significant.

Table 6

Vitreal protein levels in argon laser exposed lesion - the effect of steroid treatment

Time after exposure (days)	Vitreal protein levels (mg/ml) (mean±SD) untreated laser group	steroid treated laser group	* P Value(s)
1	0.52±0.15	0.41±0.13	‡N.S.
	+ n=20	n=18	
3	0.68±0.16	0.41±0.10	N.S.
	n=22	n=22	
7	0.48±0.13	1.00±0.31	<0.0000
	n=10	n=10	
14	0.79±0.13	0.47±0.10	<0.0001
	n=23	n=12	
baseline	0.43±0.12		
	n=32		

*p (s) p value for t-student test comparing each of the laser untreated eyes at each of the time intervals with the corresponding steroid treated group.

+ n n indicates number of eyes involved in the involved group.

‡ N.S. statistically not significant.

Fig. 1 Changes in prostaglandin E_2 production its accumulation in the vitreous and protein vitreal levels in the noncoherent light exposed eyes.
Each point represents the mean±SD for the number of eyes (given as n) involved in this group. The vertical lines represent the standard deviation.

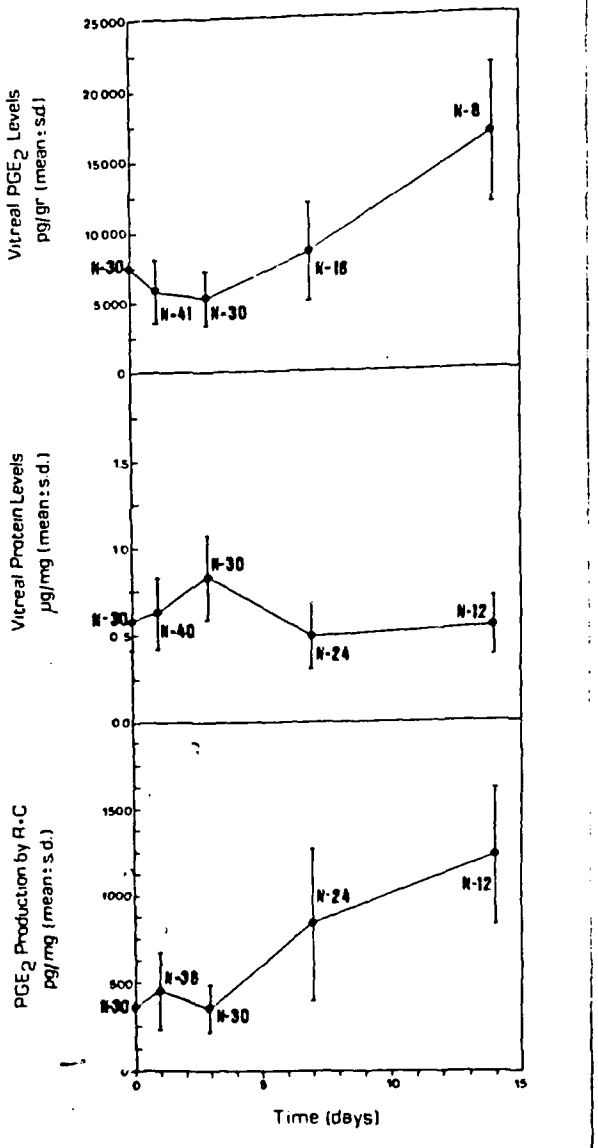


Fig. 2 Changes in prostaglandin E_2 production, its accumulation in the vitreous and protein vitreal levels in the laser irradiated eyes.
Each point represents the mean±SD for the number of eyes (given as n) involved in each group. The vertical lines represent the standard deviation.

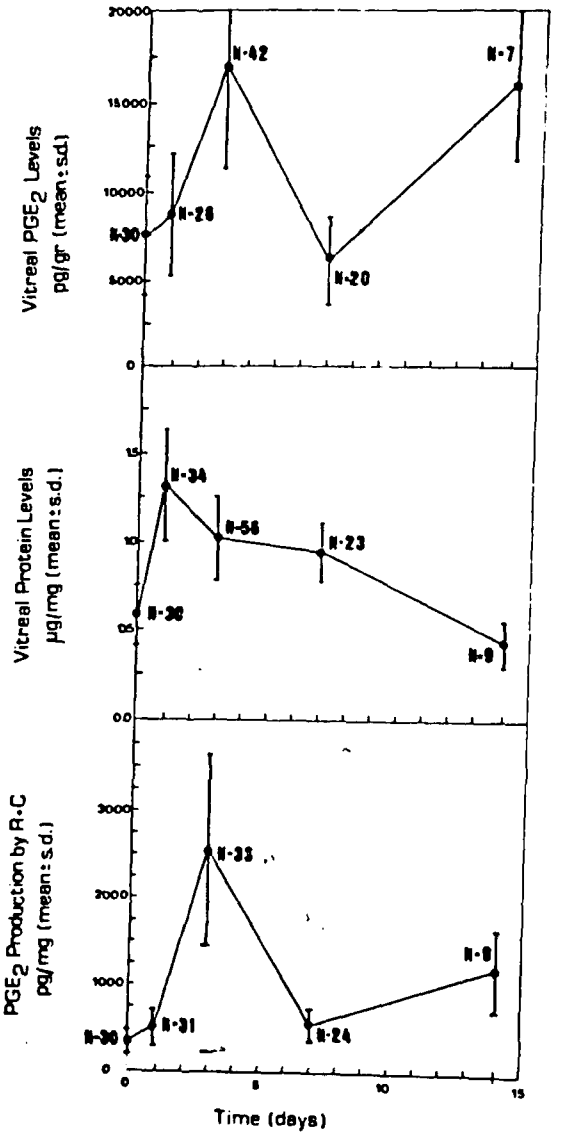
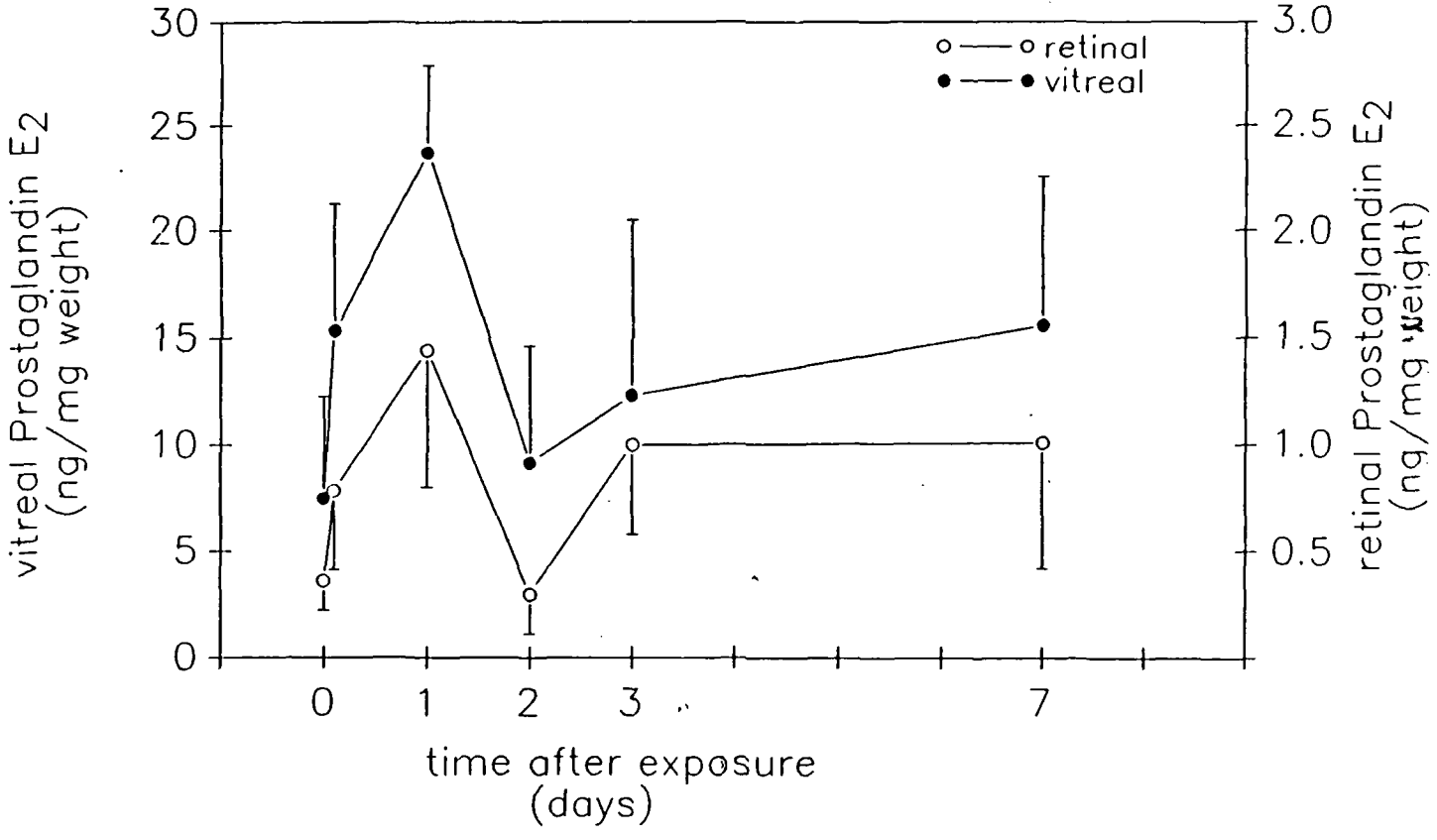


Figure 3

Subthreshold Laser Retinal Damage–
effect on Prostaglandin E_2 response



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