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TREATMENT OF LASER INDUCED RETINAL INJURIES

ANNUAL/FINAL REPORT

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Prostanoids are mediators of the inflammatory process in many organs including the eye, and their production from arachidonic acid is triggered by a variety of stimuli including laser burns.

We have suggested that prostanoids might be involved in laser induced retinal burns and that it is likely that anti-inflammatory drugs which inhibit arachidonic acid metabolism can help accelerate retinal healing. In addition,

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we have suggested that an increase in prostanoid levels in the retina/choroid or vitreous cavity might cause a break in the blood retinal barriers and protein leakage into the vitreous.

Our preliminary study indicates that both a single retinal laser burn and 10 laser burns cause an increase in PGE_2 and prostacyclin levels of the choroid at 18 hours after lasing. However, results were inconsistent and required the use of 3 samples of each tissue for a single determination.

Modifications done in order to overcome these problems involved the use of a tissue preparation of unseparated retina/choroid, and exposed the retina to 30 laser burns. Thus, a single tissue sample was adequate for measurement. Indeed, PGE_2 levels in retina/choroid and vitreous of eyes subjected to 30 moderate laser applications demonstrated an increase in prostaglandin E_2 (PGE_2) and prostacyclin levels throughout a 3 day period after lasing.

This mode of lasing was chosen as our working model and confirmed our hypothesis of PGE_2 and prostacyclin involvement in laser induced retinal damage.

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Background

With the increase in the number and uses of laser instruments in the battlefield it is certain that future armed conflicts will result in laser induced eye injuries, even if laser weapons will not be used.

Eye casualties are also expected in peace time as a result of training maintenance and research activities associated with the ever increasing laser uses. Laser eye injuries will cause temporary or permanent visual incapacitation.

The damage caused in biological systems subjected to laser irradiation depends mainly on wavelength, energy concentration, the duration of laser pulse, and the absorption characteristics of the tissue (1), The eye is the part of the body most susceptible to laser irradiation, and in the eye, the part most vulnerable to most lasers is the retina. The retinal laser lesion includes destruction of the neuroretinal layers, retinal pigment epithelium, and the underlying choroid. The lesion site is surrounded by an area a few times larger in diameter, which consists of inflammatory edema and damaged cells (2-3), change that are partially potentially reversible. These areas surrounding the lesion site appear normal or almost normal ophthalmoscopically but even light microscopy reveals the associated edema and cellular damage (4,5).

The extent and rate of visual recovery following laser retinal burn will depend on the amount of scarring that will occur in the inflamed area surrounding the lesion. Any measure that will reduce the inflammation and accelerate the absorption of edema will diminish the amount of scarring and improve visual prognosis of the patients. There is no proven method to reduce inflammation and edema of laser induced retinal burns, as very little is known on their pathogenesis.

Prostanoids are mediators of the inflammatory process in many organs including the eye (6,7). Prostanoids # (a term referring to prostaglandin type). D,E,F, and prostacyclin) are unsaturated fatty acid derivatives formed from arachidonic acid (A.A.) (6,8). ## Prostanoids production from A.A. is triggered by a variety of stimuli (9) including laser burns (10,11). Prostaglandin E_2 (PGE_2) is one of the prostanoids produced by most cells while prostacyclin is produced mainly by the endothelium of the blood vessels and is a potent inhibitor of platelet aggregation involved in regulation of blood coagulation.

Prostanoids are known to be involved in the regulation of the inflammatory process in many organs (9) including the eye (10-13), causing hyperalgesia, either vasodilatation or vasoconstriction, local hyperemia, increased blood vessel permeability and leukocyte migration to inflammatory sites (8,9).

Both corticosteroid & non-steroidal anti-inflammatory (NSAI) drugs, like aspirin, decrease prostanoids production, the NSAI group by inhibiting a cyclooxygenase pathway (9) and corticosteroids by decreasing the availability of A.A. (14). It has already been shown that laser irradiation of pigmented rabbit iris is accompanied by augmented production of PGE_2. No data was previously available on prostanoid metabolism in the retina and choroids of the eyes subjected to retinal laser irradiation.

We have suggested that prostanoids might be involved in laser induced retinal burns, and that it is likely that anti-inflammatory drugs which inhibit arachidonic acid metabolism can help accelerate retinal healing. It is well established that prostaglandin E_2 (PGE_2) and prostacyclin increase the permeability of blood vessels in many organs with subsequent protein leakage to extracellular space. In the eye, prostanoids cause a break in blood aqueous barrier associated with increased levels of proteins in the anterior chamber. Exogenously applied PGE_2 cause an elevation of PGE_2 levels in the aqueous humor, likewise an increase in PGE_2 formation by iris and ciliary body as seen in uveitis, is associated by elevation of aqueous humor protein levels. Therefore, protein was used by some investigators as an additional indicator for demonstration of augmentation in prostanoid levels. We suggest that an increase in prostanoid levels in the retina, choroid or vitreous might cause a break in blood retinal barriers and protein leakage into the vitreous cavity. Changes in the active absorption mechanism of blood retinal barrier might have an additional effect on protein and prostanoid levels of the vitreous body.

This report will be divided into two parts:

I. Preliminary Study - a summary of the various regimens of retinal laser application used during the first few months and their results which led to development of the best suitable experimental models. An extensive preliminary study was required because on initiation of our study no previous data on Prostaglandin E_2 (PGE_2) formation following retinal lasing was available, and we were obliged during the first few months of our study to examine several different methods of retinal laser application. Similarly we had to try various methods of handling the retina, choroid and vitreous as they will be the least traumatized. At the end of this period, a satisfactory experimental model was obtained.

II. Main Study - involves data on prostanoid formation and protein levels following two modes of retinal laser application.

I. PRELIMINARY STUDY

Material and Methods

A-I. Animals

Pigmented dutch rabbits of either sex weighing 1.5-2.0 kg were used. They were anesthetized by 35 mg/kg Ketamine and 5 mg/kg Xylazine injected intramuscularly 30 minutes before laser application. Both eyes of each rabbit were similarly lased 1 hour after the pupils were dilated using 0.5% tropicamide. The eyes of age and sex-matched non-irradiated rabbits served as controls.

B-I. Laser Lesion Production

During our preliminary attempts at finding a suitable experimental model, the effects of various methods of retinal lesions on PGE_2 formation by the retina, choroid and vitreous were studied. Retinal laser lesions were produced using a continuous wave argon laser beam (Argon-Krypton Lasertek 265 Excitor) through a coated Goldman fundus contact lens. Four methods of retinal laser applications were used during the study.

1) A Single Intense Burn - A single burn at the posterior pole 2D from the optic disc (Power setting - 1.0 watt; spot size - 1000 microns; pulse duration - 0.5 sec).

2) Ten Laser Burns - Ten intense laser burns (power setting 1.0 watt; spot size - 1000 microns; pulse duration 0.3 sec, each) were applied at the posterior pole as far apart as possible.

3) Thirty Moderate Laser Burns - Thirty laser burns applied as far apart as possible. The initial power setting of 0.5 Watt was changed so as to get slight whitening at the application site (initial power setting - 0.5 Watt; Spot size - 300 microns; duration - 0.3 sec. each).

4) Multiple Small Laser Burns - 250 to 300 small laser burns (power setting - 0.3 Watt; spot size - 200 microns; pulse duration - 0.3 sec each) applied close together, so as to form a large confluent lased area.

C-I. Tissue Preparation

Rabbits were sacrificed using an overdosage of pentobarbitone

1) Vitreous Separation - eyes were enucleated, the cornea was excised at the limbus, iris and ciliary body removed by pulling at the iris base. Following lens removal, the vitreous was excised using blunt scissors at its anterior attachment and carefully removed into a vial containing 0.5 ml of Krebs Ringer Bicarbonate Heppse (Buffer) pH 7.4. Each vial contained vitreous from 3 eyes.

2) Retina and Choroid Separation - Two different preparations of retina and choroid were used:

a) Retina and choroid as isolated preparations. Removal of retina from choroid was obtained by careful dissection mostly using a spatula, and blunt scissors at the retinal adhesion to the optic nerve. Careful separation of choroid from the sclera followed, and was facilitated by frequent cutting at sites of vascular attachments (9). Retinas of 3 eyes were put together in a vial containing 0.6 ml Buffer pH 7.4. Similarly, choroids of 3 eyes were pooled together in a vial containing 1.0 ml of the same buffer.

b) Retina and choroid as a combined preparation. The retina and choroid were not separated from each other but careful dissection of the retina-choroid together from the sclera took place.

The dissected retina-choroid of each eye was put separately into a vial containing 1.0 ml of the buffer.

D-I. Prostaglandin Determination

1) Prostaglandin E_2 Determination - Retina choroid and vitreous from different animals were incubated in buffer pH 7.4 in a slowly shaking bath at 37°C for 15 minutes. At the end of the incubation period, the tissues were removed and the contents of each vial were put in 0.6 ml of the same buffer, homogenized separately and samples were taken for protein determination. PGE_2 and Prostacyclin levels in the incubation media were determined separately using a radioimmunoassay with a specific antibody for PGE_2 and 6-keto PGF_{1a} the stable metabolite of prostacyclin.

E-I. Protein Determination

Protein levels of vitreous retina and choroid were determined separately, following homogenization of each tissue, in a vial containing 0.6 ml Buffer, and using the Lowry Rosenbrough Methods.

Results and Comments

a. Prostaglandin E_2

In our earlier studies we started with multiple laser burns (see methods A-3,4) as we believed that the resulting extensive damage following multiple laser burns might result in excessive prostanoid production. However, early measurements showed that prostaglandins levels shortly after multiple laser applications were not much higher than after few burns (Table 1). This method was soon abandoned as it was time consuming and was felt to be of no military significance and was replaced by two other methods of lasing used concomitantly; a single laser burn and 10 laser burns (see under methods B, 1-2).

A single laser burn caused an increase in PGE_2 production by the choroid observed 45 minutes and 18 hours following laser treatment (Table 1 and Table 2). Retinal and vitreal PGE_2 levels in these eyes subjected to a single laser burn were not significantly different from control, at 45 minutes (Table 1), but were elevated at 18 hours after lasing (Table 2).

It is noteworthy that PGE_2 choroidal and vitreal measurements following a single laser application showed a wide range of variability at various time intervals (Table 2 and Table 3). In eyes subjected to 10 laser burns, an increase in choroidal levels of PGE_2 were noted 18 hours following laser (Table 3); however, retinal and vitreal levels of PGE_2 were not affected by this mode of lasing at 18 hours after irradiation.

In eyes subjected to 10 laser application, #PGE_2 levels of the choroid were significantly higher than those observed following a single laser burn and therefore it was suggested that an increased number of laser burns up to 30, applied widely apart might be associated with a larger area of retinal and choroidal damage resulting in enhanced amounts of PGE_2. Enhancement of PGE_2 was highly desired as it might have enabled us to use a single sample of either retina, choroid or vitreous for each determination instead of three samples used so far.

This argument coupled with the observation of an inconsistency of measurements with the use of a single laser burn made us focus our efforts on the study of a 30 laser burns.

A modification of tissue handling was done at that time, separation of retina from choroid as practiced during the first 4 months appeared to be a long traumatizing procedure. Trauma is a well known stimulus enhancing prostanoid production, and in order to avoid and minimize the effect of the surgical trauma we started using tissue preparation of combined choroid-retina, while the vitreous was still removed separately. Ceasing to separate the retina from choroid shortened significantly the time required for surgery and decreased the trauma associated with handling the tissues.

PGE_2 levels of retina-choroid and vitreous in eyes subjected to 30 laser applications to the retina at various time intervals are given in Table 4. PGE_2 levels in the retina-choroid 1 hour, 24 hours and 48 hours following retinal lasing were significantly increased. However, following a similar augmentation in PGE_2 levels of the vitreous at 1 hour and 24 hours leads to a decrease at 48 hours.

b. Proteins

Changes in protein levels of the vitreous and retina-choroid at various time intervals following 30 retinal laser applications are given in Table 5.

Increased protein levels of the vitreous and retina-choroid were observed 48 hours and 72 hours following retinal irradiation.

c. Prostacyclin

Prostacyclin levels were studied during the first and second quarters. A single laser burn caused elevation of choroidal, vitreal and retinal prostacyclin observed 18 hours after lasing (Table 6). In eyes subjected to 30 retinal laser applications, a significant increase in prostacyclin levels of combined retina-choroid was noted, but vitreal levels were unaffected (Table 7). As mentioned, PGE_2 and prostacyclin were determined each by separate

radioimmunoassay which is a time consuming technique. We decided to focus our efforts on the study of PGE_2 alone, so that larger samples might be studied. The study of prostacyclin was postponed until the completion of the study of the PGE_2 and protein.

Interim Summary and Conclusinn of Preliminary Study

Following both single intense retinal laser burn and 10 laser burns an increase in PGE_2 and prostacyclin levels of the choroid at 18 hours following lasing was measured. This observation indicated that following retinal lasing prostanoid formation was augmented. However, tissue handling was length and traumatic and results were inconsistent and required the use of 3 samples of each tissue for a single determination.

Modifications done in order to overcome these problems involved:

1. Use of combined retina-choroid instead of separated retina and choroid to facilitate handling and lessen trauma.
2. Use of 30 retinal laser burns to achieve consistent results and larger quantitites of PGE_2 so as a single tissue sample will be adequate for measurement.

Indeed PGE_2 levels in retina-choroid and vitreous of eyes subjected to 30 moderate laser applications demonstrated an increase in PGE_2 and prostacyclin levels at various time intervals following lasing. This mode of lasing was chosen as our working model.

Table 1 - PGE_2 levels in retina, vitreous and choroid measured 15 and 45 minutes after laser application to retina.

Group	Time following laser (min)	PGE_2 levels* pg PGE_2 / mg protein Vitreous	Retina	Choroid
Control	-----	2.56	2.16	9.5
		5.89	1.79	8.7
		3.46	2.39	
One laser burn**	45	3.05	2.52	
		2.67	2.73	11.52
		6.07	4.5	13.5
300 laser burns***	15	2.56	1.53	---
		2.2	2.27	---
	45	2.39	2.39	6.3
		5.90	3.12	7.5
		3.18	3.9	

* Numbers are the mean of a radioimmunoassay triplicate determined for each vial, each vial containing either retinas of 3 eyes, choroids of 3 eyes or vitreous bodies of 3 eyes.

** Spot size: 1,000 microns; power setting: 1.0Watt; duration 0.3 sec.

*** Spot sizes: 200 microns; power setting: 0.2 Watt; duration 0.3 sec.

Note: In this table PGs levels were given per mg protein, but later levels were calculated per mg tissue.

Table 2 - PGE_2 levels in retina, vitreous and choroid measured 18 hours after a single laser application* to the retina.

Group	PGE_2 levels pg/mg tissue weight (mean±SD) Vitreous	Retina	Choroid
Control	2.2 ± 0.6 (12)**	15.4 ± 2.2 (12)	160 ± 45 (12)
One laser burn*	2.69 ± 0.7 (10)	23.4 ± 6.8 (10)	200 (10)

* Laser setting - spot size 1 mm; power setting 1.0 Watt; duration 0.5 sec.

** Figure in brackets indicates number of eyes involved.

Table 3 - PGE_2 production by retina and choroid measured 18 hours after laser application to retina.

Group	PGE_2 levels ng PGE_2 / mg tissue weight* Vitreous	Retina	Choroid
Control	1.23	10.45	105.56
	1.3	13.45	69.13
One laser burn**	3.5	1.54	54.96
	1.24	1.85	
	0.955	1.75	76.33
Ten laser burns***	1.58	14.5	194.5
	1.59	21.6	138.14

* Numbers are the mean of radioimmunoassay triplicates determined for each vial, each vial containing either retinas of 3 eyes, choroids of 3 eyes or vitreous bodies of 3 eyes.

** Spot size: 1,000 microns; power setting: 1.0 Watt; pulse duration: 0.3 sec.

*** Spot size: 1,000 microns; power setting: 1.0 Watt; pulse duration: 0.3 sec. each.

Table 4 - PGE_2 levels in retina + choroid, and vitreous measured at different time intervals following multiple laser application to retina*

Group	Time following laser (hours)	PGE_2 levels pg/mg weight (mean ± S.D.) Vitreous	Retina + Choroid
Control	---	3.08±0.28 (17)**	197.3 ± 72.7 (17)
30 laser burns	1	10.3±247.0 (12)	323.0 ± 75.9 (12)
30 laser burns	24	13.67± 3.15 (12)	427.8 ± 104.0 (12)
30 laser burns	48	7.18±2.83 (12)	490.0 ± 180.0 (12)

* Laser setting - spot size 0.5 mm, power setting - 0.3 Watt, duration 0.3 sec.

** Figure in brackets indicates number of eyes involved.

Table 5 - Protein levels in retina choroid and vitreous measured at different time intervals following multiple laser application to the retina*

Group	Time following laser (hours)	Protein levels (mg)(mean ± SD) vitreous	retina + choroid
Control	----	2.0±0.4 **(10)	7.15±0.52 (10)
30 laser burns	1	2.2±0.52 (10)	8.0±0.95 (10)
30 laser burns	24	2.4±0.3 (6)	9.0±1.1 (6)
30 laser burns	48	3.6±0.4 (6)	10.0±1.2 (6)
30 laser burns	72	3.8±0.5 (6)	7.5±0.8 (6)

* laser setting - spot size 0.5 mm, power setting - 0.3 Watt, duration 0.3 sec.

** Figure in brackets indicates number of eyes involved.

Table 6 - Prostacycline (PGX) production by retina- vitreous and choroid measure 18 hours after laser application to retina.

Group	PGX levels ng PGX / mg weight * Vitreous	Retina	Choroid
Control	0.263	2.28	4.63
	0.61	1.80	2.58
One laser burn**	4.09	55.98	117.68
	4.54	60.84	133.3
Ten laser burns***	1.39	7.05	20.4
	0.98	5.46	16.46

*Numbers are the mean of a radioimmunoassay triplicate determined for each vial, each vial containing either three retinas of three eyes; choroids of 3 eyes or vitreous bodies of 3 eyes.

**Spot size: 1,000 microns; power setting: 1.0 Watt; pulse duration: 0.3 sec.

***Spot size: 1,000 microns; power setting: 1.0 Watt; pulse deuration: 0.3 sec.

Table 7 - Prostacyclin (PGX) levels in retina-choroid, and vitreous measured at different time intervals following multiple laser application to retina*.

Group	Time following laser (hours)	PGX levels pg/mg weight (mean ± S.D.)** Vitreous	Retina-Choroid
Control		0.59 ± 0.24	7.45 ± 1.8
30 laser burns	1	0.57 ± 0.09	21.9 ± 5.73
30 laser burns	24	0.50 ± 0.1	34.2 ± 11.9
30 laser burns	48	0.45 ± 0.07	25.5 ± 3.88

* Laser setting - spot size 0.5 mm, power setting 0.3 Watt, duration 0.3 sec.

** Six animals were involved in each experiment.

Table 8 - Prostaglandin E_2 levels in retina-choroid and vitreous at different time intervals following multiple laser applications to the retina*

Group	Time following laser (hours)	PGE_2 levels pg/mg weight (mean ± S.D.) Retina-Choroid	Vitreous
Control	----	359 ± 141 (n=32)	7873 ± 3327 (n=28)
30 laser burns	1	1031 ± 360 (n=21) *** p=0.0000	10395 ± 3636 (n=31) p=0.007
30 laser burns	24	558± 210 (n=24) p=0.0003	9301 ± 4222 (n=30) p=0.16 N.S.
30 laser burns	48	437 ± 260 (n=26) p=0.15 N.S.	5215 ± 2548 (n=30) p=0.0011
30 laser burns	72	3098 ± 1423 (n=35) p=0.0000	16138 ± 8153 (n=44) p=0.0000

* Laser setting - spot size 0.5 mm, power setting 0.3 Watt, duration 0.3 sec.

** Figures in brackets indicate number of eyes involved.

*** p value for t-student test comparing PGE_2 levels between each of the groups and corresponding control group.

N.S. No statistically significant difference

Table 9 - Protein levels in retina-choroid and vitreous at different time intervals following multiple laser applications to the retina*.

Group	Time following laser (hours)	Protein levels pg/mg weight (mean ± S.D.) Retina-choroid	Vitreous
Control		59.9 ± 17.4 (n=39)	1.707 ± 0.45 (n=41)
30 laser burns	1	64.6 ± 12.5 (n=41) N.S.	2.08 ± 0.47 (n=39) ***p=0.0005
30 laser burns	24	51.4 ± 10.2 (n=56) p=0.013	2.70 ±0.56 (n=33) p=0.0000
30 laser burns	48	69.3 ± 23 (n=19) N.S.	2.22 ± 0.78 (n=18) P=0.0022
30 laser burns	72	58.3 ± 19.5 (n=40) N.S.	2.09 ± 0.56 (n=46) p=0.0007

* Laser setting - Spot size - 0.5 mm, power setting 0.3 Watt, duration 0.3 sec.

** Figure in brackets indicates number of eyes involved.

*** p value for t-student test comparing protein levels in each group with the corresponding control group.

N.S. - No statistically significant difference.

Table 10 - Prostaglandin E_2 and protein levels in retina-choroid and vitreous - effect of sham lasing and a single intense laser application to the retina*, at 24 hours

		Control	Sham exposure (24 hours)	A single laser burn (24 hours)
Prostaglandin E_2 levels pg/mg weight mean ± S.D.	Retina-choroid	359±141 (n=32)	557±334 (n=36) ***p=0.001	964±305 (n=24) p=0.0000
	Vitreous	7873±3327 (n=38)	6637±2783 (n=37) N.S.	10864±720 (n=29) p=0.05
Protein levels mg/mg weight (mean ± S.D.)	Retina-choroid	59.9±17.4 (n=39)	56.1±15.5 (n=46) N.S.	55.6±17.1 (n=36) N.S.
	Vitreous	1.707±0.45 (n=41)	1.74±0.59 (n=46) N.S.	1.78±0.4 (n=32) N.S.

* Laser setting - spot size 0.5 mm, power setting - 0.3 Watt; duration 0.3 sec.

** Figure in brackets indicates number of eyes involved.

*** p value for t-student test comparing each group with the corresponding control group.

N.S. - No statistically significant difference.

II. MAIN STUDY

Materials and Methods

A-II. Animals same as in Preliminary Study (A-I. "Animals").

B-II. Laser Lesion Production

Retinal laser lesions were produced using the Lasertek and a coated Goldmann lens as described in Preliminary Study (Part I, B-I 1,2) "A single intense burn").

Two methods of retinal laser applications were used:

1. a single intense burn - as described in the preliminary study (Part I, B-1 "A single intense burn").
2. Thirty moderate laser burns - described in the preliminary study (Part I, B-3. "thirty moderate laser burns").

C-II. Tissue preparation

Vitreous - removed separately, as described in preliminary study (Part I, C-1 "Vitreous separation").

Retina and choroid - as a combined preparation - removed unseparated from each other as described in preliminary study (Part I, C-2 "Retina and choroid separation").

Experimental design - the study involved 4 groups of animals, age and sex matched.

1. Control group - normal rabbits, unexposed to laser irradiation.
2. Sham control - rabbits anesthetized, pupils dilated and sham exposed, meaning that these animals were set-up on the laser instrument and viewed with the illuminating light from the slit lamp through the Goldman contact lens for the same period of time as though it were to be laser exposed, but with no laser irradiation. Animals were sacrificed 24 hours after sham laser exposure.
3. Argon single exposure - animals were exposed to a single intense laser application as described, sacrificed 24 hours later, and prostanoid and protein levels were determined in the retina-choroid and vitreous.
4. Thirty argon laser burns - animals were exposed to 30 retinal laser applications as described and were sacrificed at various time intervals following lasing: 1) 1 hour following irradiation
   2) 24 hours following irradiation
   3) 48 hours following irradiation

4) 72 hours following irradiation.

Results

PGE_2 determined in the incubation media of retina-choroid represent the amounts of PGE_2 produced by this tissue during incubation period, as prostaglandins are not stored by the tissue but released. # PGE_2 determined in each of the vitreal samples represent the amount present, as the vitreous lacks cells capable of producing prostaglandins.

The effect of 30 laser applications to the retina on PGE_2 levels of the combined retina-choroid and vitreous at different time intervals are given in Table 8. It show that PGE_2 formation by the retina-choroid 1 hour following lasing increased 3-fold when compared to normal (p=0.0000). At 24 hours PGE_2 formation decreased by 50% but levels were still significantly higher than control (p=0.0003). PGE_2 formation by the retina-choroid were further reduced to control levels at 48 hours following retinal lasing; however, at 72 hours a 10-fold increase in PGE_2 levels were noted when compared to control levels. The increase in PGE_2 production by retina-choroid 72 hours following retinal laser application was significantly higher than that observed 1 hour after lasing (p= 0.001).

The increase in PGE_2 levels of the vitreous was already noted one hour following retinal lasing however, at 24 hours PGE_2 levels were reduced to normal. PGE_2 levels were further reduced at 48 hours and reached levels significantly lower than control levels (p=0.001). A secondary increase in PGE_2 levels of the vitreous was noted 72 hours following retinal irradiation and reached levels two fold higher than normal (p=0.0000) and also significantly higher than augmented PGE_2 levels observed at 1 hour (p= 0.0001).

Protein levels in the combined retina-choroid and vitreous at different time intervals following 30 laser applications to the retina are given in Table 9. Protein levels of the retina-choroid 1 hour , 48 hours and 72 hours following retinal irradiation remained unchanged when compared to control. However, a slight decrease in protein levels of the retina-choroid were observed 24 hours following retinal lasing (p=0.013).

An increase in protein vitreal levels was already noted 1 hour following irradiation, when compared to control (p=0.0005) with a further increase observed at 24 hours (2.08 ± 0.47 mg/mg weight (mean $\pm$ SD) and 2.70 ± 0.56 mg/mg weight respectively). Vitreous levels of protein were gradually reduced at 48 hours

and 72 hours, but remained statistically significantly higher than control ($p=0.0022$, and $p=0.0007$ respectively).

PGE_2 production by the retinal-choroid, as well as PGE_2 vitreal levels 24 hours following a single intense laser application to the retina are given in Table 10. PGE_2 production by retina-choroid of eyes subjected to a single intense retinal lasing was significantly increased when compared to normal ($p=0.0000$). PGE_2 levels observed 24 hours following a single irradiation though increased when compared to control (10864 ± 7252 pg/mg tissue weight mean$\pm$ SD and 7873 ± 3327 pg/mg tissue weight respectively) was not statistically significant ($p=0.05$). Protein levels of the vitreous and retina-choroid 24 hours following a single retinal irradiation were unchanged when compared to normal (Table 10). The effect of sham lasing on protein and PGE_2 levels of the retina-choroid and vitreous, are given in Table 10 . It shows that 24 hours following sham lasing PGE_2 production by retina-choroid was significantly higher than control levels ($p=0.001$), and did not differ significantly from PGE_2 levels observed 24 hours following 30 moderate laser burns ($p=0.06$). However, sham lasing did not affect either vitreal PGE_2 levels or protein levels of the vitreous.

Discussion

In this study protein and PGE_2 levels of the retina-choroid and vitreous of rabbits after argon laser irradiation at exposure levels to produce either a 30 moderate burns or a single intense retinal burn were investigated. Included in the study were control untreated rabbits as well as a control group of anesthetized rabbits, with dilated pupils which were sham exposed. Changes in protein and PGE_2 levels of eyes irradiated by multiple laser burns were studied at 1 hour, 24 hours, 2 days and 3 days after lasing. In eyes subjected to either a single intense burn or sham exposed, protein and PGE_2 levels were studied 24 hours later.

It is well established that PGs produced by any tissue are not stored but released. Therefore, PGE_2 present in the incubation medium of retina-choroid represent the amounts produced by this tissue during incubation period. However, PGE_2 vitreal levels represent the amounts of PGE_2 present at that time interval, as the vitreous lacks cells capable of PGE_2 production.

Our study shows that during a period of 3 days following 30 moderate laser applications to the retina PGE_2 production by retina-choroid is changed in a biphasic mode.

An initial increase in PGE_2 production by retina-choroid is evident 1 hour after multiple retinal irradiation followed by a 50% decrease of production at 24 hours, and a further decrease to control levels at 48 hours. A second increase in PGE_2 formation by retina-choroid of multiply irradiated eye was to levels 3 times higher than the initial increase and 10 times higher when compared to control. PGE_2 vitreal levels following the same irradiation showed biphasic changes similar to those involving PGE_2 production by retina-choroid, with a larger second peak, but with a slightly different time course. The initial increase in vitreal PGE_2 levels was noted 1 hour following retinal lasing, but levels were reduced to normal at 24 hours and were significantly lower than control at 48 hours. The second increase in PGE_2 vitreal levels occurred 3 days after lasing and levels were significantly greater than those observed during the initial rise in PGE_2 level and coincided with the increase in PGE_2 production by retina-choroid. Reduction in vitreal PGE_2 levels following multiple irradiation was noted earlier than reduction in PGE_2 production by retina-choroid. It is noteworthy that at 2 days following lasing vitreal PGE_2 levels reached levels lower than control. Protein levels of

retina-choroid following multiple irradiation were unchanged when compared to control except for a significant decrease observed 24 hours after lasing. Protein levels of the vitreous 1 hour following multiple retinal lasing were increased when compared to normal and were further augmented at 24 hours. A gradual decrease in vitreal protein levels was noted 2 days and 3 days following multiple retinal irradiation, but remained significantly higher than control levels.

PGE_2 production by retina-choroid following a single intense laser burn was augmented; however, PGE_2 vitreal levels, though increased, showed a borderline p value of 0.05, with a large variability. Protein levels of the retina-choroid and vitreous 24 hours following a single intense burn were unchanged when compared to normal. This is in contrast with protein elevated levels observed 24 hours following multiple irradiation.

PGE_2 production by retina-choroid of sham exposed eyes at 24 hours was significantly higher than normal but was lower than levels observed at that time interval following either a multiple or a single laser application. However, vitreal PGE_2 levels 24 hours following sham exposure were unchanged when compared to normal in contrast with the observation of elevated protein levels at 24 hours following multiple irradiations.

Conclusions

I) In eyes subjected to multiple retinal laser irradiation, we demonstrated during 3 days follow-up:
   a) a biphasic increase in PGE_2 production by retina-choroid.
   b) A biphasic increase in PGE_2 vitreal levels.
   c) An increase in vitreal protein levels while protein levels of the retina-choroid remained unchanged.

II) In eyes subjected to a single intense laser burned, we demonstrated at 24 hour following irradiation:
   a) a significant increase of PGE_2 production by retina-choroid, and increased PGE_2 vitreal levels with borderline statistical significance.
   b) protein levels of retina-choroid and vitreous remained unchanged when compared to normal.

III) Sham exposure - 24 hours following sham exposure an elevation in PGE_2 production by retina-choroid was noted. However, vitreal protein levels were unchanged as opposed to increased levels observed at that time interval following multiple laser application.

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