European Journal of Ophthalmology / Vol. 11 no. 1, 2001 / pp. 47-52

Increase of free oxygen radicals in aqueous humour induced by selective Nd:YAG laser trabeculoplasty in the rabbit

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PURPOSE. *To investigate the impact of selective Nd:YAG laser trabeculoplasty on free oxygen radicals and antioxidant enzymes of the aqueous humour in the rabbit.*
METHODS. *One eye of 18 rabbits was subjected to 360° selective laser trabeculoplasty (LT) with a frequency-doubled Nd:YAG laser (532 nm). The anterior chamber aqueous humour was aspirated 3, 12 hours and 1, 3, 7, 10 days after treatment. Lipid peroxide (LPO) and glutathione S transferase (GST) levels and superoxide dismutase (SOD) activities of aqueous humour were measured.*
RESULTS. *Concentrations of LPO in the aqueous humour of the treated eyes were significantly higher than the untreated eyes until the 7th day. Aqueous SOD activity significantly decreased 3 hours after LT and remained low until day 7. Aqueous GST levels were significantly decreased between 12 hours and 7 days after the LT.*
CONCLUSIONS. *Selective LT was followed by an immediate increase in the aqueous humour LPO concentration and decreases of SOD and GST in the rabbit, probably due to photovaporization and photodisruption caused by the frequency- doubled Nd:YAG laser. The increased aqueous LPO levels suggest that free oxygen radicals are formed in the pigmented trabecular meshwork during LT, and may be responsible for the inflammatory complications of this procedure. (Eur J Ophthalmol 2001; 11: 47-52)*

KEY WORDS. *Selective laser trabeculoplasty, Frequency-doubled Nd:YAG laser, Free oxygen radicals, lipid peroxidation, superoxide dismutase, glutathione S transferase*

Accepted: April 10, 2000

INTRODUCTION

The clinical application of a Nd:YAG laser to selectively target the pigmented trabecular meshwork was proposed as a new treatment for primary open angle glaucoma (1). Unlike in argon laser trabeculoplasty, thermal diffusion from targeted cells to the surrounding nonpigmented trabecular meshwork cells is minimal; therefore the technique has been named selective laser trabeculoplasty (2-4).

Visible wavelength lasers are employed to create laser energy that is absorbed by pigmented intraocular tissue, transforming the light energy into heat. A temperature increase sufficient to denature protein occurs and thermal degradation liberates free oxygen radicals in tissues (5). Free oxygen radicals generated by the infiltrating polymorphonuclear cells are toxic and create tissue damage and inflammation. They can also cause peroxidation of fatty acids or lipids of cell membranes, which is known as lipid peroxidation (6). The iris and corneal endothelium is a potential site of free radical damage as it is rich in polyunsat-

 1120-6721/047-06$03.00/0

urated fatty acids that are especially susceptible to peroxidation (7,8). Reactive oxygen metabolites appear to play an important role in the amplification of the inflammatory process and have direct cytotoxic effects as well.

The balance between production and catabolism of oxygen metabolites by cells and tissues is critical for the maintenance of biochemical processes, and may have a direct impact on the extent of free radical-induced tissue damage (9). The eye has effective defenses against toxic oxygen products. It contains a variety of antioxidants, including SOD, catalase, glutathione peroxidase, P-phenyl diamine peroxidase, GST, ascorbate, and vitamin E (10). These antioxidants protect the eye either by trapping the radicals or by interfering with oxidative chain reactions. Because of conflicting biochemical and histochemical data, the distribution of these antioxidants in the eye is not clearly defined. It is hoped that immunohistochemical studies will enable us to locate them more precisely and, so far, the ocular distribution of SOD has been reported (7). Specific immunohistochemical localization of copper-zinc SOD in ocular tissues has revealed its presence predominantly in the cornea, sclera and iris. This enzyme was seen primarily in ocular structures that may be frequently exposed to superoxide or its metabolites under physiological conditions and in pathological processes such as intraocular inflammation (11). It is reported that toxic aldehyde products of lipid peroxidation are natural substrates for GST. GST is the first enzyme in the mercapturic acid pathway, which detoxifies xenobiotic substrates including aldehydes, as by-products of membrane lipid peroxidation. Consequently, a major biological function of GST may be to protect the cell from these toxic compounds originating from the oxygen metabolism (12). GST activities were increased in some corneal disorders (13). In the present study we measured LPO levels, SOD and GST activities in aqueous humour after selective Nd:YAG laser trabeculoplasty in the rabbit.

MATERIAL AND METHODS

A total of 18 pigmented rabbits without ocular abnormalities, weighing between 3 and 4 kg, were used. The animals were cared for in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. One eye per animal was treated, the other eye remaining intact. Laser treatments and aspiration of aqueous humour were done under general anesthesia with intramuscular ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (5 mg/kg).

One eye of each rabbit was subjected to a 360° selective LT with a 532 nm frequency-doubled green Nd:YAG laser (Oculight GL-IRIS Medical Instruments) through a Trokel F/3 Gonio laser lens (Ocular Instruments), spot size 75 µm (68 µm in target tissue with Trokel lens), duration 50 ms, power 800 mw, number of laser spots 100 in 360°. Adjacent but not overlapping spots were made. The desired response is a blanching of the pigmented trabecular meshwork with or without minimal bubble formation.

The anterior chamber aqueous humour was aspirated using a 26-gauge needle 3, 12 hours and 1, 3, 7, 10 days after the treatment. The central cornea was cannulated, then the samples were immediately frozen and stored at -70°C until assay.

Lipid peroxidation levels in the aqueous humour was determined using the thiobarbituric acid (TBA) method (14). Malondialdehyde (MDA), an end-product of fatty acid peroxidation, reacts with TBA to form a colored complex that has maximum absorbance at 532 nm. MDA values were calculated from the absorbance coefficient of the MDA-TBA complex at 532 nm, 1.56×10^5 cm^{-1} mol^{-1}. The values were expressed as nanomoles MDA formed per ml aqueous.

SOD activities of the samples were determined by inhibition of nitroblue tetrazolium reduction, with xanthine-xanthine oxidase used as a superoxide generator (15). The results were expressed as U/mg protein aqueous. One unit of SOD is defined as the amount of protein that inhibits the rate of nitroblue tetrazolium reduction by 50%. Aqueous protein levels were determined with folin phenol reagent (16).

GST activity was determined according to the method of Habig et al (17). The assay mixture (1 ml) contained 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH in 0.1 M potassium phosphate buffer, pH 6.5. After adding 10 µl aqueous humour, the increase in absorption was measured at 340 nm for 3 minutes at 25°C. The enzyme activity was expressed in U/mg of protein. The results were analyzed by using the paired samples t-test.

RESULTS

Three and 12 hours, 1 and 3 days after the laser treatment LPO concentrations in the aqueous humour were significantly higher than in the untreated-control eyes of the same animals. Seven and ten days after the treatment, no differences were seen between the treated and untreated eyes (Tab. I, Fig.1).

By 3, 12 hours, 1 and 3 days after the laser treatment SOD concentrations in the aqueous humour were significantly lower than in the untreated eyes of the same animals and by 7 and 10 days no differences were seen between the treated and untreated eyes (Tab. II, Fig. 2).

At 12 hours, 1 and 3 days after the treatment GST concentrations in the aqueous humour were significantly lower than in the untreated eyes, and by 3 hours, 7 and 10 days after the treatment no differences were seen between the treated and untreated eyes (Tab. III, Fig. 3).

DISCUSSION

Laser effects in biological tissue can be divided into three general categories: photochemical, thermal, and ionizing. Although it is conceptually useful to differentiate between these actions, it is important to recognize that more than one may occur together and be responsible for clinical effects at intermediate values of irradiance and exposure time (18). Laser light entering biological tissue is either transmitted, absorbed or scattered. Absorption of a photon may alter the energy states of electrons of atoms in a molecule at the target site. The heat generated by the absorption of laser energy is dissipated in the surrounding tissue. If intense laser energy is focused onto a very small area for a short time, a reaction occurs that is independent of pigment absorption and is referred to as photodisruption. An instantaneous electric field is generated, which literally strips electrons from target atoms, producing a gaseous state, called “plasma”. As ionized atoms of a plasma recombine with a wide range of energies are emitted, producing a spark of incoherent white light. Associated shock and pressure waves create additional mechanical damage to target tissues, resulting in a reaction that can disrupt both pigmented and nonpigmented structures.

Thermal effects are also involved in the mechanism of photodisruption (19). The high irradiance ionizes in a small volume of the tissue at the focal point of the laser beam and degradation of organic materials may also produce homolytic fission and liberate toxic oxygen radicals. Reactive oxygen radicals create tissue damage by reactions with lipid components of the cell membranes, nucleic acids and sulphur-containing enzymes (5,20).

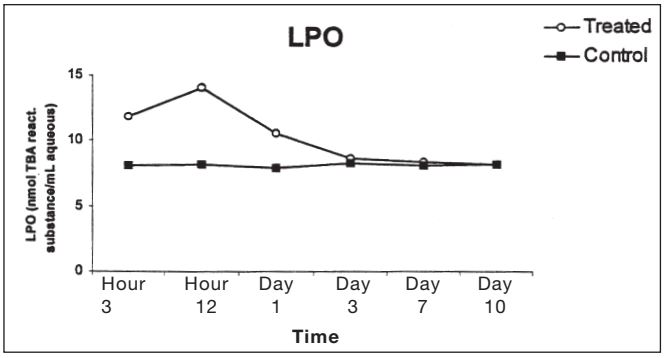

Fig. 1 - *LPO concentrations in aqueous humour after laser treatment for treated and control eyes.*

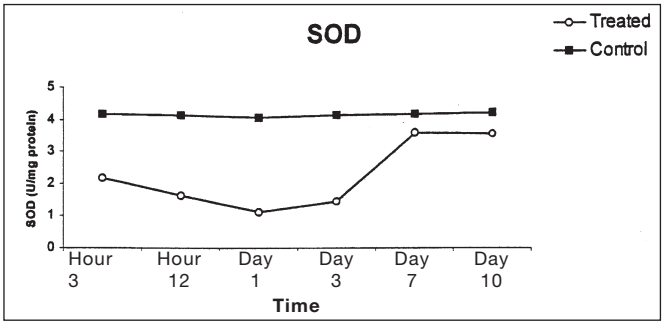

Fig. 2 - *SOD concentrations in aqueous humour after laser treatment for treated and control eyes.*

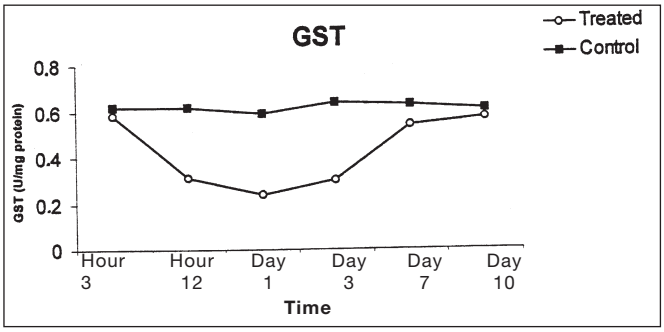

Fig. 3 - *GST concentrations in aqueous humour after laser treatment for treated and control eyes.*

The eye protects itself from toxic radical injury by two major protective mechanisms. There are antioxidant enzyme systems, e.g. SOD, GST, catalase, and free radical scavengers such as vitamins E, C and A (7). GST and copper-zinc SOD have been located within the cellular components of the iris; anterior pigmented myoepithelium, fibrocytes, and melanocytes in both the iris stroma and anterior border layer. Glu-

TABLE I - LPO CONCENTRATIONS IN AQUEOUS HUMOUR (nmol OF TBA REACTIVE SUBSTANCE/ml AQUEOUS) AFTER LASER TREATMENT FOR TREATED AND UNTREATED CONTROL EYES

	LPO (mean±SD)		
Time	**Treated eyes**	**Control eyes**	**Paired samples t-test**
Hour 3	11.89 ± 2.42	8.12 ± 0.99	4.55***
Hour 12	14.11 ± 1.90	8.22 ± 0.97	8.11****
Day 1	10.56 ± 2.43	7.91 ± 0.75	3.23**
Day 3	8.61 ± 1.00	8.24 ± 1.04	3.02**
Day 7	8.36 ± 0.99	8.08 ± 1.10	1.03*
Day 10	8.23 ± 0.92	8.16 ± 0.86	1.02*

* p>0.05 ** p<0.05 *** p<0.01 **** p<0.001

TABLE II - SOD CONCENTRATIONS IN AQUEOUS HUMOUR (U/mg PROTEIN) AFTER LASER TREATMENT FOR TREATED AND UNTREATED CONTROL EYES

	SOD (mean ± SD)		
Time	**Treated eyes**	**Control eyes**	**Paired samples t-test**
Hour 3	2.16 ± 0.95	4.17 ± 1.32	-3.85**
Hour 12	1.63 ± 1.02	4.13 ± 1.19	-4.94***
Day 1	1.14 ± 0.82	4.06 ± 1.31	-4.91***
Day 3	1.44 ± 0.89	4.12 ± 1.58	-4.30**
Day 7	3.57 ± 1.15	4.17 ± 1.09	-2.01*
Day 10	3.56 ± 1.24	4.20 ± 1.18	-2.02*

* p>0.05 ** p<0.01 *** p=0.001

TABLE III - GST CONCENTRATIONS IN AQUEOUS HUMOUR (U/mg PROTEIN) AFTER LASER TREATMENT FOR TREATED AND UNTREATED-CONTROL EYES

	GST (mean ± SD)		
Time	**Treated eyes**	**Control eyes**	**Paired samples t-test**
Hour 3	0.58 ± 0.16	0.62 ± 0.20	-1.94*
Hour 12	0.31 ± 0.15	0.62 ± 0.19	-3.94**
Day 1	0.24 ± 0.13	0.59 ± 0.19	-4.07**
Day 3	0.30 ± 0.12	0.64 ± 0.20	-6.20***
Day 7	0.54 ± 0.23	0.63 ± 0.19	-0.84*
Day 10	0.57 ± 0.19	0.61 ± 0.18	-1.49*

* p>0.05 ** p<0.01 *** p<0.001

tathione peroxidase has been found only in erythrocytes within the vasculature of the human iris (8).

Varma et al demonstrated that intraocular photocatalytic generation of oxygen radicals was harmful, as indicated by the formation of excessive lipid peroxides (21). Antioxidant enzyme systems appear to play a role in preventing damage from this photocatalytic generation of oxygen radicals through their distribution in the endothelium of the cornea, iris, trabecular meshwork and ciliary epithelium, and in the lens, as these structures are constantly exposed to the aqueous humour and its photocatalytic products.

In pathological conditions such as anterior uveitis, the inflammatory cells could produce superoxide and its metabolites. The enzyme SOD in the lining cells of ocular cavities may minimize the destructive effects of these metabolites on the endothelium, iris and ciliary processes. Similar beneficial effects may be operative in the vitreous by preventing superoxide-mediated depolymerization of hyaluronic acid (22,23).

The inflammatory reaction is an important source of oxygen free radicals in the anterior chamber. Large amounts of superoxide radicals are secreted by activated phagocytic leukocytes, and the radical is also formed as a byproduct during prostaglandin and leukotriene biosynthesis. The ocular tissues and fluids, except the retina, have little protection against superoxide radicals formed during an inflammatory reaction. The eye, however, is an immunologically privileged organ, apparently because of the widespread occurrence of fas-ligand resulting in the apoptosis of invading inflammatory leukocytes. The eye, therefore, is almost completely spared from inflammatory reactions, and this may explain the low basal content of the SOD isoenzymes in most of its compartments. However, should the barriers of immunological privilege be crossed, an inflammatory reaction would put many ocular tissues at great risk of damage (24-26).

The specific activity of SOD shows an age-dependent decline in the normal human trabecular meshwork (27). The potential role of SOD in primary open angle glaucoma, a disorder of the aging trabecular meshwork, requires further investigation. Oxidative damage after LT and antioxidant protection in the trabecular meshwork and aqueous humour have not been studied previously. Recent anterior segment studies related to oxidative damage have increasingly focused on excimer laser corneal surgery and uveitis (6, 28-32).

Bilgihan et al found the aqueous LPO levels were not changed after excimer laser photoablation, but low aqueous SOD activities suggest that free radicals were formed in the cornea during excimer laser keratectomy (33). Hayashi et al reported lipid peroxidation in the corneal stroma in excimer laser treated corneas. This might come from free oxygen radicals generated by the infiltrating polymorphonuclear cells at the site of tissue damage (34).

In our model selective LT resulted in raised aqueous humour LPO levels and low aqueous SOD and GST activities. These changes suggest that free oxygen radicals are formed in the pigmented trabecular meshwork during selective LT, and that they may be responsible for some of the complications of LT. The use of a topical or systemic antioxidant such as ascorbic acid to reduce lipid peroxidation might limit postoperative oxygen free radical tissue damage and the acute inflammatory anterior chamber reaction.

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