NITRIC OXIDE LEVEL OF AQUEOUS HUMOUR AFTER PHOTOREFRACTIVE KERATECTOMY IN GUINEA PIGS

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ABSTRACT

Purpose: *To investigate the level of nitric oxide (NO) in aqueous humour after excimer laser keratectomy in guinea pigs.* ***Methods:*** *NO levels of the aqueous humour were measured by nitric oxide analyser with chemiluminescence assay in three Groups. Group I served as control, 10 mμ excimer laser corneal photoablation was performed in Group II and 20 mμ corneal photoablation in Group III.* ***Results:*** *NO levels of all groups showed no difference when compared to the control ($p>0.05$).* ***Conclusion:*** *Excimer laser keratectomy did not change the NO level of aqueous humour in guinea pigs 24 hours following photoablation and this finding could be explained by increased transforming growth factor-β1 (TGF-β1) level of aqueous humour after excimer laser therapy.*

Key Words: *Excimer Laser Keratectomy, Aqueous Humour, Nitric Oxide.*

INTRODUCTION

Excimer laser emits ultraviolet radiation at 193 nm (UV-C) and causes a specific photochemical reaction that results in an ablation of corneal tissue (1). The presence of free oxygen radicals after excimer laser photoablation has been demonstrated in many studies (2-8). Free oxygen radicals cause tissue damage by reacting with lipid components of the cell membranes, nucleic acids and sulphur containing enzymes (2).

Nitric oxide (NO) is a diffusible chemical mediator which is thought to be involved in regulating several physiological and pathological processes (9). NO is a free radical gas with the essential property of producing unstable toxic chemicals (10). The formation of the powerful oxidant peroxinitrite from the reaction of superoxide anion with NO has been shown to be a kinetically favored reaction contributing to cellular injury and death at sites of tissue inflammation (11). Many inflammatory stimuli induce the production of NO (12). The high influx of polymorphonuclear cells into rabbit corneal stroma after excimer laser therapy was reported previously (5,13,14).

In the present study NO levels of the aqueous humour were measured after deep and superficial excimer laser photoablations in guinea pigs.

MATERIALS AND METHODS

Eighteen albino guinea pigs were used in the study. Institutional guidelines regarding animal experimentation were followed in the study.. Anaesthesia was induced by an intramuscular

injection of 25 mg/kg ketamin HCl injection, and topical proparacaine hydrochloride.

NO levels of aqueous humour were measured in 3 Groups. Group I consist of unwounded 8 eyes of 4 animals and served as control. The corneal epithelium was removed by a blunt spatula (Visitec, Sarasota, USA) in Group II and III. We performed 10 μm corneal photoablation in Group II (7 eyes) and 20 μm corneal photoablation in Group III (7 eyes) after mechanical deepithelization. The eyes were irradiated with 193 nm excimer laser (MEL 60, Aesculap - Meditec, Heroldsberg, Germany) under in-vivo conditions. The fluence at the cornea was 220 mJ / cm^2, firing rate of 20 Hz, and diameter of ablation zone was 4 mm. Animals were euthanized 24 hours following photoablation. We performed limbal corneal microperforation with MVR blade (Visitec, Sarasota, USA) and then aqueous samples were taken with a scaled 25μl fire-polished microcapillary tube and immediately frozen at -70°C.

NO level was measured by nitric oxide analyser (Sievers 280 NOA: Sievers, Boulder, CO) with chemiluminescence assay, and detection of NO was based on the observation that ozone interacted with NO to generate light. Luminescence, measured by a sensitive photomultiplier tube, was directly proportional to NO levels (15).

The Mann-Whitney U test was used to evaluate the differences between groups.

RESULTS

The NO levels of all groups and statistical analysis are given in table I.

The NO level of the control group was found as 143.22±57.51μmol/ml. The NO level of aqueous humour did not change after superficial excimer laser photoablation (10 μm corneal ablation) ($p>0.05$).

In Group III the dose of the excimer laser energy was duplicated (20 μm corneal ablation), but NO levels of aqueous humour again did not change significantly ($p>0.05$).

DISCUSSION

The presence of free oxygen radicals after excimer laser photoablation has been demonstrated (2, 4-8). Excimer laser keratectomy changes the free radical balance of the eye. Previous studies demonstrated that excimer laser keratectomy increases the corneal temperature, decreases the superoxide dismutase activity of the aqueous humour, and induces lipid peroxidation in the superficial corneal stroma (2, 5). Excimer laser treatment also changes the corneal activities of aldehyde dehydrogenase and glutathione S-transferase. These two enzymes play an important role in detoxification of aldehydes, which are generated from free radical reactions (16). We have evaluated the effects of topical vitamin E therapy after PRK very recently and demonstrated that topical vitamin E treatment has beneficial effects in preventing excessive corneal wound healing after PRK (3).

In a recent study, excimer laser induced radical formation was demonstrated by electron spin-resonance by Shimmura (7). In another recent investigation, oxidative tissue damage in the form of lipid peroxidation was detected by fluorescent peroxidized carbonyl compounds using confocal microscope (5).

NO is a diffusible free radical gas and produces unstable toxic chemicals, such as superoxide anion (O_2^-) (10). Peroxinitrite, the reaction product of superoxide (O_2^-) and NO, may be a major cytotoxic agent produced during inflammation, peroxinitrite is highly reactive

Table - 1 : The mean aqueous NO levels and statistical analysis of all groups.

	n	NO(pg / ml) (mean± SD)	p value	Mann-Whitney U test
Group 1	8	143.22±57.51		
Group 2	7	157.42±35.04	p> 0.05*	U: 18.00
Group 3	7	179.5±43.26	p> 0.05*	U: 14.00

* Compared to control group.

molecule and induces lipid peroxidation (11).

There were several reports characterising inducible and constitutive NO synthase activities in the eye. Ciliary processes and trabecular meshwork contain a constitutive form of NO synthase activity, moreover trabecular meshwork may contain more than a single form of NO synthase activity (9).

The production of NO could be induced by an inflammatory stimulus, as activated polymorphonuclear cells produce large amounts of NO (12). Excimer laser keratectomy induces influx of polymorphonuclear cells into the corneal stroma after 24 hours (7, 14). As NO is a diffusible molecule we investigate the NO levels of aqueous humour after excimer laser therapy (9). NO levels of aqueous humour showed no difference when compared with the control after excimer laser photoablation ($p>0.05$).

We have previously showed that excimer laser treatment of high myopia increases the transforming growth factor-ß1 (TGF-ß1) levels of aqueous humour in the second postoperative day (17). TGF-ß1 has been reported to suppress NO production directly by reducing the expression of inducible nitric oxide synthase at the transcriptional, posttranscriptional, translational and posttranslational levels (18).

The influx of polymorphonuclear cells into the corneal stroma after excimer laser therapy may increase the NO level of the cornea and the aqueous humour .Our results showed that the NO level of aqueous humour slightly increased after superficial and deep excimer laser photoablation, but this was not statistically significant. This finding could be explained by the increased TGF-ß1 level of aqueous humour after excimer laser therapy, which plays a negative regulatory role by inhibiting the inducible nitric oxide synthase production and probably decreasing the NO level of aqueous humour (17, 18).

In conclusion, excimer laser keratectomy did not change the NO level of aqueous humour in guinea pigs 24 hours following photoablation and this finding could be explained by increased TGF-$ß_1$ level of aqueous humour after excimer laser therapy.

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