Trakia Journal of Sciences, Vol. 3, No. 3, pp 6-9, 2005

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

Original Contribution

COMPARING EFFECTS OF LOW-INTENSITY LASER IRRADIATION AND VITAMIN E ON OXIDATIVE DAMAGES IN EXPERIMENTAL MODEL OF DIQUAT-INDUCED CATARACT IN RABBITS

M. Mileva*, G. Zlateva

Department of Physics and Biophysics, Medical University, Sofia

ABSTRACT

The aim of this study is to compare the effects of low-intensity laser irradiation and those of the essential antioxidant vitamin E on oxidative stress markers during diquat-induced cataract in rabbits. Male rabbits Chinchila were used. The grade of the lens opacity was observed during the cataract development. After enucleating, the levels of the markers of the oxidative stress – malone dialdehyde and fluorescent lipofuscine-like products were determined in lens homogenate. Vitamin E supplementation reduces the oxidative stress significantly while the low intensity level irradiation did not influence significantly the levels of the products of lipid peroxidation.

Key Words: cataract, oxidative stress, low-intensity laser irradiation

INTRODUCTION

The pathogenesis of cataract is accompanied by oxidative stress manifested as an increase in generation of reactive oxygen species and lipid peroxidation (LP) (1).

Laser therapy is widely used in biomedical treatment of many diseases, but the possible molecular mechanisms of laser actions remain unclear and the damaging effects of laser irradiation are still controversial. The application of high-powered lasers in ophthalmology (surgery and treatment of eye diseases) is well known (2, 3). The side effects of laser therapy involve the generation of reactive oxygen and nitrogen species, which in turn initiate lipid peroxidation, protein damage or DNA modification (4).

It is well known that vitamin E is used as an essential antioxidant because it is oxidised in the presence of free radicals (5). It has been reported that He-Ne laser stimulation provides some favourable effect on bone repair (3), reduces inflammatory processes and restores corneal sensitivity after some corneal wound (6).

The same therapy leads to quicker normalisation of the content of LP products and of the activity of antioxidant enzymes (5, 7, 8).

The aim of this paper is to compare the effects of low-intensity laser (LIL) irradiation and those of the essential antioxidant vitamin E on the levels of the markers of oxidative stress during diquat-induced cataract in rabbits.

MATERIALS AND METHODS

Male rabbits Chinchilla were used. The rabbits, whose eyes were to be treated, were divided into six groups, as follows: Gr. I (n = 6) - control group (non-treated eyes); Gr. II (n = 6) – eyes treated with LIL (He-Ne laser, λ = 632 nm, 0.1 mW/cm^2) 3 min daily for 19 days; Gr. III (n = 10) – left eyes treated with vitamin E (100 mg/kg b.w. for 7 days as autocontrol Gr. IV (n = 10) – right eyes with Diquat-induced cataract as autocontrol; V (n = 10) – left eyes with Diquat-induced cataract and treated with LIL-irradiation 3 min daily for 5 days before and 14 days after Diquat-application; VI (n = 10) – right eyes with Diquat – induced cataract and treated with vitamin E (100 mg/kg b.w. for 7 days – 3 days before and 4 days after Diquat application).

Cataract development took 9 weeks (the time for a full darkening of the lens). After enucleating, the eyes were subjected to analysis of LPO-products and antioxidant enzymes.

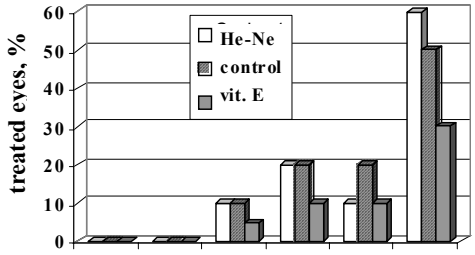
* ***Correspondence to***: *Milka Mileva, Department of Physics and Biophysics, Medical University, 2 Zdrave Str., 1431 Sofia, Bulgaria; E-mail: mm_mileva@hotmail.com*

Malondialdehyde (MDA) (9) and fluorescent and lipofuscine-like (10) products in lens homogenates were analysed. The contents of all analysed LPO products in the lens of treated eyes were expressed as % of control (control levels were considered to be 100 %).
All reagents of analytical or HPLC grade were obtained from Aldrich Chemical Co., Henkel Co., Merck, Sigma Chemical Co. Diquat was kindly provided by the Bulgarian National Institute of Drugs.

One-way analysis of variance (ANOVA) was used, followed by Bonferroni's test for significant differences. Statistical significance was defined at $p<0.05$. The statistical procedures were performed with GraphPad InStat software (USA).

RESULTS

The treatment of eyes by Diquat resulted in a marked increase of lens' opacity (**Figure 1**).



	0	I	II	III	IV	V
He-Ne			10	20	10	60
control	0	0	10	20	20	50
vit. E	0	0	5	10	10	30

Grading of the level of lense opacities

Figure 1. *Development of Diquat-induced cataract in normal, LIL-treated and vit. E-treated rabbit eyes on the 10-th week after Diquat-administration*

On the 10th week, the lens of Diquat-treated animals developed 4th-5th grade opacity. In this case, LIL-irradiation does not alter the level of opacity. Vitamin E decreases the level of lens opacity about two times, in comparison with Diquat-treated lens.

All analysed LP products increased significantly after Diquat-application (**Figures 2 and 3**): MDA and conjugated dienes (data are not shown) raised about 50 %, and fluorescent products – about 2 times, in comparison with control lens. Application of LIL on Diquat-treated eyes does not affect the levels of LPO products in lens. Vitamin E supplementation in animals with Diquat-induced cataract (Group VI) leads to significant decrease in the content of MDA – by 34 %, fluorescent products – with 41 % and conjugated dienes (data are not shown) – with 35 % versus the Diquat-treated eyes (Group IV).

DISCUSSION

The antioxidant balance in patients is disorganised during many diseases (11-14). The data obtained support the important role of oxidative stress in the development of Diquat-induced cataract. Such conclusion is in good agreement with the results reported previously by some authors using other models of cataract (15, 16).

Our previous data show that Vitamin E application has, without doubt, favourable effect on the free-radical pathogenesis of some disease, i.e. influenza virus infection (17). The results obtained confirm the protective role of Vitamin E on the oxidative damages as well as in Diquat-induced cataract (**Figures 2 and 3**).

In many diseases low-intensity laser treatment has a positive effect on the level of LPO (5, 7, 8). Traditionally low-intensity laser therapy is used as an anti-inflammatory factor. He-Ne laser stimulation reduces the inflammatory processes, and restores the corneal sensitivity after some corneal wounds of various origins (6) and promotes the tissue repair process of diabetic wound (18). Our findings exclude potentialities of low-intensity laser irradiation to influence significantly the lipid peroxidation in lens homogenate in this therapeutic scheme.

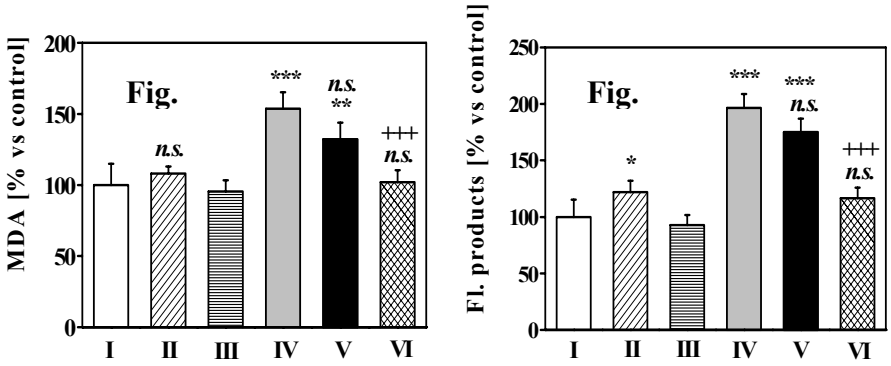


Figure 2 and Figure 3. *Effect of LIL and Vitamin E on the level of MDA and fluorescent lipofuscine-like products in rabbit eyes. The experimental group was as described in* ***Materials and Methods****.*
**** - p<0.05 vs group I; +++ - p< 0.05 vs group V; n.s. – non significant.*

CONCLUSION

The results demonstrate a non-essential effect of LIL-irradiation on the levels of lipid peroxidation products in normal lens as well as in Diquat-treated one. Probably, the effect of LIL-irradiation depends on the dose applied and prolongation of the treatment.
It is well demonstrated that the Vitamin E administration has a strong protective effect on the oxidative stress and lens opacity during the experimental model of Diquat-induced cataract while LIL application does not have such effect.

ACKNOWLEDGMENTS

The assistance of Dr. D. Mehandjiev from Department of Ophthalmology, Medical University, Sofia, is gratefully acknowledged.

REFERENCES

1. Bilgihan, K., Bilgihan, A., Akata, F., Hasanreisoglu, B., Turkozkan, N., Laser cornea surgery and free oxygen radicals. *Jpn J Ophthalmol*, 40:154-157, 1996.
2. Grossman, N., Schneid, N., Reuveni, H., Halevy, S., Lubart, R., 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. *Laser Surg Med*, 22:212-218, 1998.
3. Merli A., Santos M., Genovese W., Faloppa F., Effect of low-intensity laser irradiation on the process of bone repair. *Photomed Laser Surg,* 23:212-5, 2005.
4. Kujawa J., Zavodnik I.B., Lapshina A., Labieniec M,. Bryszewska M., Cell survival, DNA, and protein damage in B14 cells under low-intensity near-infrared (810 nm) laser irradiation. *Photomed Laser Surg*, 22:504-508, 2004.
5. Sies H. and Murphy M., Role of tocopherols in the protection of biological systems against oxidative damage. *J Photochem Photobiol,* 8:211-224, 1991.
6. Chentsova O., Prokof'eva G., Mozerenkov V., Kharchenko L., Balarev A., Sergushev S., Low-intensity helium-neon laser irradiation in multimodal treatment of corneal injuries. *Vest Oftalmol*, 107:23-26, 1991.
7. Rakitina D., Urias'ev O., Garmash V., Ivanova M., Krasnovid N., Lebedev V., Effects of laser therapy on lipids and antioxidants in blood of patients with bronchial asthma. *Ter Arkh,* 69:49-50, 1997.
8. Safafov R.M., Ianenko E.K., Nikitinskaia L.P., Golovanov S.A., Drozhzheva V.V., Kon'kova T.A., Danilkov A.P., Safavov R., Nikitinskaia E., The dynamic level of β 2-microglobulin, the basic lipid peroxidation indices and middle molecules in the blood and urine in patients with acute calculous pyelonephritis against a background of endovascular helium-neon laser therapy. *Urol Nefrol*, 1:11-13, 1997.
9. Bakalova, R., Mileva M., Kotsev Ch., Bardarov V., Ribarov St., Determination of malondialdehyde in biological samples by solid-phase extraction and HPLC. *Meth Find Exp Clin Pharmacol*, 22:267-269, 2002.

11. Dilliard, C., Tappel A., Fluorescent damage products of lipid peroxidation. *Meth Enzymol*, 105:337-341, 1984.
12. Alexandrova M., Bochev P., Markova V., Bechev B., Popova M., Danovska M., Simeonova V., Dynamics of free radical processes in acute ischemic stroke: influence on neurological status and outcome. *Journ Clin Neurosci* 11:501-506, 2004.
13. Kuchukova D., Gadjeva V., Vlaykova T., Ivanova V., Georgieva R., Influence of chemotherapy on the antioxidant status in patients with various hematological diseases. *IMAB*, 7:111-113, 2001.
14. Vasil'ev A., Strel'tsova N., Kiianiuk N., The stress-limiting action of low-intensity laser radiation in patients with ishemic heart disease. *Vopr Kulturol Fizioter Lech Fiz Kult*, 6:3-5, 1997.
15. Gadjeva V., Kuchukova D., Radostina Georgieva. Influence of polychemotherapy on the antioxidant levels and lipid peroxidation in patients with lymphoproliferative diseases. *Comp Clin Pathol,* 14:13-18, 2005.
16. Obara Y., The oxidative stress in cataract formation. *Nipp Ganka Gakkai Zasshi*, 99:1303-1341, 1995.
17. Spector A., Oxidative stress-induced cataract: mechanism of action. *FASEB J*, 9:1173 – 1182, 1995.
18. Mileva M., Bakalova R., Tantcheva L., Galabov A.S., Ribarov St., Effect of vitamin E supplementation on lipid peroxidation in blood and lung of influenza virus infected mice. *Comp Immunol Microbiol Inf Dis*, 25:1 – 11, 2002.
19. Maiya G.A., Kumar P., Rao L., Effect of low intensity helium-neon (He-Ne) laser irradiation on diabetic wound healing dynamics. *Photomed Las Surg*, 23:187-190, 2005.