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Effect of photodynamic doses of laser irradiation on endogenous intoxication indices

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Background: *One of the promising areas in ophthalmology is photodynamic therapy (PDT).*

Purpose: *To assess the effect of laser irradiation of the rat eye at the doses applied in PDT (without photosensitizer) on endogenous intoxication indices.*

Materials and Methods: *Fifty rats were used to investigate the effect of different modes of photodynamic irradiation of the rat eye on changes in endogenous intoxication indices over time.*

Results: *Exposure to 630-nm laser irradiation of 300-mJ pulsed mode (3 min) and 60-J photodynamic dose (PDD) mode (15 s) does not result in the development of endogenous intoxication. According to the literature, serum level of middle-weight molecules (MWM) is an integral marker of metabolic disorders of the body and can be used as evidence of the intensity of endogenous intoxication.*

Conclusion: *Exposure to 630-nm laser irradiation of 300-mJ pulsed mode (3 min) and 60-J PDD mode (15 s) does not result in the development of endogenous intoxication, making possible the application of these modes in practical ophthalmology. Because exposure to 890-nm laser irradiation of 300-mJ pulsed mode (3 min), and, especially, to 630-nm laser irradiation of 120-J PDD mode (30 s) results in the increased accumulation of MWM in circulating blood of animals, the eye should not be exposed to such irradiation doses.*

Key words: photodynamic therapy, endogenous intoxication, middle-weight molecules (MWM)

Introduction

Advances in photobiology have laid the groundwork for the development and implementation of a variety of laser applications, including ophthalmic ones. Stimulating, microcirculating and anti-inflammatory effects of therapeutic doses of laser irradiation are well known. One of the most promising areas in ophthalmology is photodynamic therapy (PDT) which is based on the selective destruction of proliferating cells resulted from their photochemical exposure and the response of photosensitizer accumulated in them to a specific wavelength of light [1, 2].

In PDT, low-energy lasers are used, and the irradiation dose should be selected properly to avoid the thermal effect above a thermal sensitivity threshold of about 400 mW/ cm2. The patient feels burning pain should the dose result in the effect above the threshold. The method requires neither local nor general anesthesia, and is particularly helpful when anesthesia is either contraindicated or dangerous [3, 4].

Until recently, the introduction of PDT into common clinical practice in Uzbekistan was

substantially hampered by the high price of foreign photosensitizing agents and laser equipment. A 640 ± 20 nm and 890 nm therapeutic laser apparatus Vostok was proposed by Uzbek scientists R.Sh. Mavlian-Hodzhaev and R.A. Sadykov in 2009 and has been authorized for use in PDT applications (for septic, infected and persistent wounds) in surgery [5, 6] by the Uzbek government agency.

However, there have been no works related to ophthalmic PDT applications using national laser equipment in Uzbekistan.

The purpose of the study was to assess the effect of laser irradiation of the rat eye at the doses applied in PDT (without photosensitizer) on endogenous intoxication indices.

Materials and Methods

A total of 50 non-pedigree mature rats weighing about 150 g and maintained under standard vivarium conditions were used in the study. Irradiation from the Uzbek laser apparatus Vostok was applied at a therapeutic dose (300 mJ; 890 and 630 nm; pulsed mode) or a photodynamic dose (PDD) (60 and 120 J; 630 nm; continuous wave mode) into the plane of the right rat eye (aperture, 5 mm), on 7 consecutive days.

Depending on the specific irradiation dose used, the animals were divided into the control group comprising 10 intact animals and four experimental groups comprising 10 animals each: (1) 300-mJ pulsed mode (control group, 890 nm, 3 min); (2) 300-mJ pulsed mode (630 nm, 3 min); (3) 60-J PDD mode (630 nm, 15 s), and (4) 120-J PDD (630 nm, 30 s).

Animals were sacrificed at day 3, day 7 and day 14 after the initial irradiation session. All the animal experiments were performed in compliance with the WHO guidelines on animal studies, and with all the precautions taken. Animals were euthanized in compliance with approved protocols, when they were assessed as dying.

The serum levels of middle-weight molecules (MWM) were determined by the Korochkin et al. method [7] at the Tashkent Medical Academy Central research laboratory directed by prof. B.U. Iriskulov. 0.2 mL of trichloroacetic acid (TCA) (16%) was added to 0.2 mL of the bioassay, and the proteins were precipitated by centrifugation. 2.7 mL of distilled water was added to 0.3 mL of the supernatant. Quartz cuvettes with 10-mm path length were used for spectrophotometric measurements of the UV absorbance at 254 nm against the blank sample containing 0.1 mL of original TCA solution and 2.9 mL of distilled water. MWM levels were expressed in arbitrary units.

Statistical data analysis was performed using Microsoft Office Excel-2003 (including built-in statistical processing functions) and Biostatistics v.4.03 for Windows.

Results and Discussion

By day 14, the MWM serum levels in rats of group 1 (300-mJ pulsed mode; 890 nm; 3 min) were (1.81 times) statistically significantly higher than those in the intact animals (Table 1). Although the levels in experimental group 2 (300-mJ pulsed mode; 690 nm; 3 min) were not statistically significantly different from those of the controls at any of the time points of the study, they demonstrated a decreasing trend with time. Additionally, although by day 7, the MWM serum levels in experimental group 3 (60-J PDD mode (630 nm, 15 s)) were within the normal range, they were gradually rising within the following period to become (1.27 times) statistically significantly higher than those in the intact animals by day 14. The use or a higher energy mode in experimental group 4 resulted in the

Table 1. Serum levels of middle-weight molecules (expressed in arbitrary units) in animals of the study depending on the mode of photodynamic therapy (n=10 animals in each group; M ± m)

Groups	Time points, days after PDT		
	3	7	14
Controls (intact animals)	0.091±0.007		
Group 1, 300-mJ pulsed mode (890 nm, 3 min)	0.210±0.012	0.187±0.013	0.165±0.009*
Group 2, 300-mJ pulsed mode (630 nm, 3 min)	0.110±0.008	0.102±0.008	0.083±0.007
Group 3, 60-J PDD mode (630 nm, 15 s)	0.092±0.007	0.094±0.008	0.116±0.011*
Group 4, 120-J PDD mode (630 nm, 30 s)	0.122±0.009	0.137±0.010*	0.187±0.012*

Note. *, statistically significant difference ($P < 0.5$) in MWM levels between intact and experimental groups of animals

increased accumulation of MWM in circulating blood, with the MWM serum levels being 1.51 and 2.05 higher than those in control animals by day 7 and day 14, respectively.

Therefore, exposure to 630-nm laser irradiation of 300-mJ pulsed mode (3 min) and 60-J PDD mode (15 s) does not result in the development of endogenous intoxication. According to the literature, serum level of middle-weight molecules is an integral marker of metabolic disorders of the body and can be used as evidence of the intensity of endogenous intoxication [7, 8]. The results of this study have something in common with those produced by R.A. Sadykov et al. (2012), which showed a decrease in the leukocytic intoxication index (LII) from 6-8 arbitrary units to 1.95 arbitrary units in patients with septic wounds following a course of photodynamic therapy with methylene blue for the lesion focus by day 3, and normalization of that index by day 10 of complex treatment [6]. The authors believe that that was associated with the increase in non-specific resistance of the patient's body and the decrease in intoxication events.

Conclusion

1. Exposure to 630-nm laser irradiation of 300 mJ pulsed mode (3 min) and 60-J PDD mode (15 s) does not result in the development of endogenous intoxication, making possible the application of these modes in practical ophthalmology.

2. Because exposure to 890-nm laser irradiation of 300-mJ pulsed mode (3 min), and, especially, to 630-nm laser irradiation of 120-J PDD mode (30 s) results in the increased accumulation of MWM in circulating blood of animals, the eye should not be exposed to such irradiation doses.

The results of this experimental study will be submitted to the Pharmacological Committee of the Republic of Uzbekistan to take them into consideration when making a decision on clinical studies in patients with inflammatory ocular disorders.

We are going to conduct clinical studies in patients with ophthalmic pathology, and to present the results of such studies in our following publications.

References

1. Volodin PL. [Photodynamic therapy with chlorin photosensitizer in ophthalmology (experimental and clinical study)]. [Dr. Sc. (Med.) thesis]. Obninsk (Russia): Medical Radiological Research Center of RAMS; 2009. 265 p. Russian.
2. Gelfond ML, Arseniev AI, Levchenko EV et al. [Photodynamic therapy in complex treatment of malignant tumors: current state and future prospects]. Laser Med. 2012;16(2):25-30. Russian.
3. Samlin RM, Sten'ko AA, Zhuk IG, Bragov MIu. [Main areas of photodynamic therapy in medicine]. Nov Khir. 2008;3:155-162.
4. Takhchidi HP, Belyĭ IuA, Tereshchenko AV et al. [Photodynamic therapy in ophthalmology (review)]. Oftalmokh. 2005; 1:45-51. Russian.
5. Nazyrov FG, Sadykov RA, Mirzakulov A, Sadykov RR. [Potential and prospects of photodynamic therapy for tumors]. Uzb Med J. 2010;2:55-8. Russian.
6. Sadykov RA, Kasymov KR, Sadykov RR. [Technical and scientific aspects of photodynamic therapy]. Tashkent;2012. 167 p.
7. Korochkin IM, Chukaeva Sh.I. [Determination of serum levels of middle molecule peptides in patients with acute myocardial infarction]. Lab Delo. 1988;9:15-8. Russian.
8. Kariakina EV, Belova SV. [Middle-weight molecules as an integral marker of metabolic disorders (literature review)]. Clin Lab Diagn. 2004;3:3-8. Russian.

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