BIOPHYSICS AND BIOCHEMISTRY

Laser Modification of the Blood *in Vitro* and *in Vivo* in Patients with Parkinson's Disease

T. V. Vitreshchak, V. V. Mikhailov, M. A. Piradov, V. V. Poleshchuk, S. L. Stvolinskii, and A. A. Boldyrev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 5, pp. 508-511, May, 2003
Original article submitted December 23, 2002

The effect of He-Ne laser radiation on activity of MAO B, Cu/Zn-SOD, Mn-SOD, and catalase in blood cells from patients with Parkinson's disease was studied *in vivo* and *in vitro.* The effects of intravenous *in vivo* irradiation (intravenous laser therapy) were more pronounced than those observed in similar *in vitro* experiments. It is concluded that generalized effect of laser therapy involves interaction between blood cells.

Key Words: *Parkinson's disease; monoamine oxidase B; Cu/Zn-dependent superoxide dismutase; He-Ne laser*

Parkinson's disease (PD) is characterized by death of dopaminergic neurons accompanied by exhaustion of antiradical defense system in the brain [7]. Replacement therapy with long-term administration of DOPA is not efficient, since it provokes undesirable side effects [6]. Therefore, the development of nondrug therapy of PD allowing reduction of DOPA doses is an actual problem. A perspective approach is helium-neon laser (HNL) therapy, which is widely used in modern medicine [2]. Laser radiation produces both local (on irradiated organs and tissues) and general (on the whole organism) effects. Therefore, laser therapy affects not only irradiated region, but also involves distant organs and systems [4].

The study of monoamine oxidase B (MAO B) in blood platelets and Cu/Zn-SOD in erythrocytes of PD patients showed that these patients can be divided into two groups: group 1 with high and group 2 with low activity of these enzymes compared to the control group [1]. It was also shown that group 1 patients have more severe PD and longer course of DOPA-treatment than group 2 patients.

Institute of Neurology of the Russian Academy of Medical Sciences, Moscow

The present paper reports the effects of HNL on these enzymes and on Mn-SOD and catalase in PD patients after *in vivo* or *in vitro* irradiation of their blood. Our aim was to evaluate the contribution of the direct effects of laser radiation into the total changes of enzyme activities and to reveal intimate regulatory mechanism in the whole organism triggered by HNL irradiation of the blood in PD patients.

MATERIALS AND METHODS

We used an ALOK-1 He-Ne generator (λ=632.8 nm, 1 mW beam power, power density 0.5 W/cm^2).

To study the effects of laser radiation *in vitro,* the blood was drawn from the cubital vein after overnight fast into a vial with heparin (1500 U/50 ml blood). This blood was divided into two equal portions. One portion (25 ml) was irradiated while pumping through a silicone tube (5 mm diameter). The second portion was not irradiated and served as the control.

Platelets and erythrocytes were isolated from the control and experimental blood to determine MAO B and Mn-SOD (platelets) and Cu/Zn-SOD and catalase (erythrocytes). These tests were made with the blood of PD patients (n=10) and healthy donors (n=5).

0007-4888/03/1355-0430$25.00

The course of laser therapy consisted of 5 daily 20-min sessions of blood irradiation. The blood was examined on day 3 after the last session. The patients received no selegilin or other MAO B inhibitors. The effect of *in vivo* laser therapy on neurological status and activity of the above enzymes was studied in 70 patients.

The methods of MAO B and Cu/Zn-SOD assay were describes elsewhere [1]. Activity of Mn-SOD and catalase were measured as described previously [5].

In a special series, for evaluation of the effect of laser on MAO B activity, mitochondrial fraction isolated from platelets of control blood samples from PD patients and healthy donors was irradiated. To this end, a disposable light guide was inserted into a vial containing 0.2-0.4 ml mitochondria in 0.2 M Na/K phosphate buffer (pH 7.5). Protein concentration in the sample was 1.5-3.5 mg/liter and laser exposure was 0.5 min. MAO B activity was examined both in control and irradiated mitochondria.

The results were analyzed statistically using Excel software and Student's *t* test.

RESULTS

Laser therapy significantly improved neurological status of PD patients assessed by Fan—Elton scale (UPDRS). This improvement was accompanied by normalization of MAO B and Cu/Zn-SOD activities: these parameters significantly decreased in group 1 (n=40) and increased in group 2 (n=30) patients (Figs. 1, *a*; 2, *a*). Mn-SOD activity in platelets in both groups surpassed the control. Laser therapy decreased this parameter (the decrease was significant only for group 2 patients with low initial activities of MAO B and Cu/Zn-SOD). Catalase activity did not differ from the control in both groups (this parameter was slightly decreased before therapy and tended to increase (normalization) after it, Table 1).

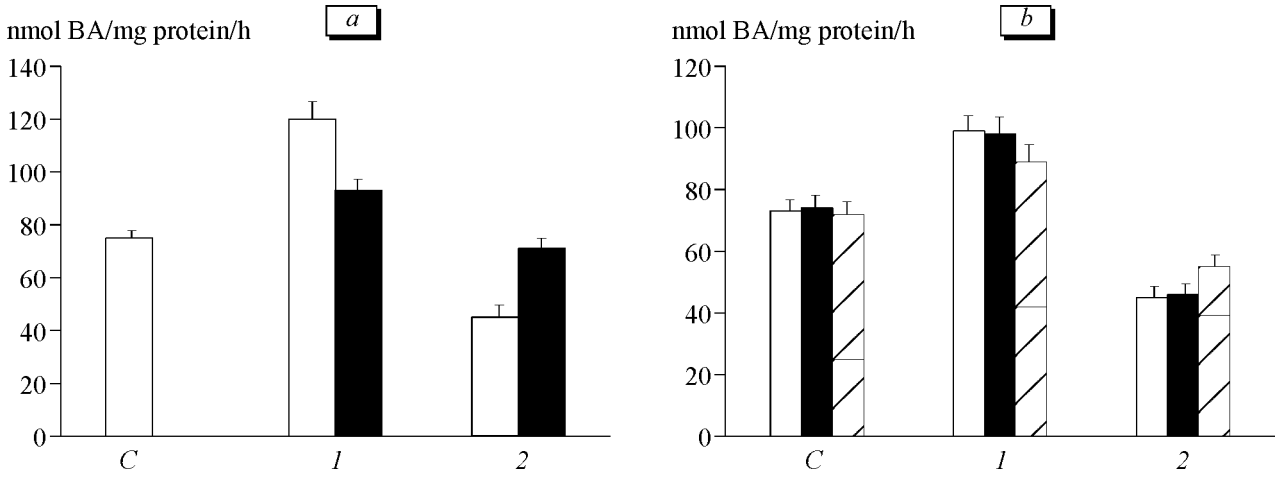

Fig. 1. Effect of laser irradiation on MAO B activity. *a*) MAO B activity before (light bars) and after *in vivo* laser therapy (dark bars); *b*) MAO B activity before (light bars) and after (dark and hatched bars) *in vitro* irradiation of mitochondrial fraction (dark bars) and the whole blood (hatched bars). Here and in Fig. 2: *C*) control (healthy donors), *1*) group 1, and *2*) group 2 patients with Parkinson's disease.

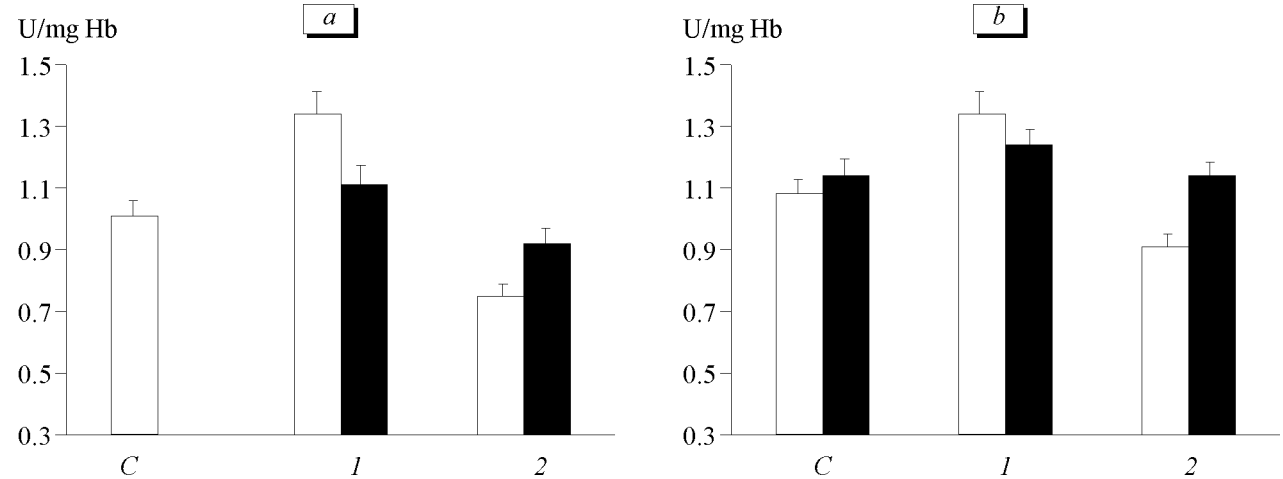

Fig. 2. Effect of *in vivo* (*a*) and *in vitro* (*b*) laser irradiation on Cu/Zn-SOD activity. Activity before and after irradiation is shown by light and dark bars, respectively.

TABLE 1. Effect of Laser Therapy on Activity of Mn-SOD, Catalase, and Neurological Deficit in PD Patients (*M*±*SEM*)

Group		Mn-SOD, U/mg protein	Catalase, μmol H_2O_2/mg Hb/min	PD severity, points
Control		19±3	85±4	—
1st	before treatment	27±4	83±5	72±5
	after treatment	25±4	89±5	58±3+
2nd	before treatment	36±5*	79±3	57±4
	after treatment	28±4+	82±3	47±3+

Note. $p<0.05$ *compared to the control and +to the corresponding values before treatment.

In *in vitro* study we used the blood of 10 PD patients with high (n=6, group 1) or low (n=4, group 2) MAO B and Cu/Zn-SOD activities. Similarly to *in vivo* laser therapy, irradiation of isolated blood partially normalized activity of MAO B: it decreased in group 1 ($p<0.05$) and increased in group 2 ($p<0.08$). Irradiation of blood samples changed Cu/Zn-SOD activity in a similar way ($p<0.07$ and $p<0.1$ in groups 1 and 2, respectively). Irradiation had no effect on MAO B activity of healthy donors. Activity of erythrocitic Cu/Zn-SOD in donors slightly increased after irradiation ($p<0.2$).

To examine, whether irradiation produces a direct effect on MAO B, a copper-containing enzyme, we irradiated mitochondrial fraction isolated from non-irradiated platelets of healthy donors and PD patients. This procedure produced no effect of MAO B activity in mitochondria (Fig. 1, *b*).

Mn-SOD activity in 10 examined blood samples from PD patients changed insignificantly. Its mean value demonstrated a decrease after *in vitro* irradiation, which was within the statistical error. Laser irradiation produced no effect on this activity in healthy donors (Table 2).

Activity of erythrocytic catalase increased after irradiation in all five donors ($p<0.05$). In PD patients this parameter tended to increase ($p<0.1$, Table 2).

Activation of catalase due to absorbtion of HNL red light by its chromophore group is well known [2]. This effect was clearly seen in the blood of healthy donors. Moderation of this effect in PD patients probably results from some pathological processes. It is possible that catalase structure is modified in PD patients in such a way that it became less sensitive to red light.

TABLE 2. Effect of *in Vitro* HNL Irradiation on Activity of Mn-SOD and Catalase (*M*±*SEM*)

Group	Mn-SOD, U/mg protein	Catalase, μmol H_2O_2/ mg Hb/min
Patients		
before treatment	25±3	78±4
after treatment	22±3	81±4
Donors		
before treatment	18±3	81±3
after treatment	18±3	90±4*

Note. $p<0.05$ *compared to the value before irradiation.

The described effects of HNL on blood enzymes were much stronger after *in vivo* application of laser therapy, than after *in vitro* blood irradiation (Figs. 1, 2). Moreover, irradiation of isolated mitochondria produced no effect on the enzymes. Simplification of the experimental system revealed only the direct unspecific effects of radiation. Irradiation of the whole blood modifies enzyme activity via two possible mechanisms: first, by the response of individual cells to low-intensity laser irradiation (LILI) and second, by the interaction between these cells. There are data that LILI triggers receptor-mediated processes of respiratory burst in phagocytes leading to the release of various bioactive agents into the blood, e.g. cytokines responsible for cell-cell interaction [3,4]. During *in vivo* laser therapy in PD patients, the response of some cells to LILI and modification of their interaction by laser irradiation can be accompanied by the effect of LILI on endothelial cells, which play the key role in the regulation of oxidative stress. These findings confirm the integral systemic effect of laser therapy on the whole organism.

This work was partially supported by the Russian Foundation for Basic Research (grant No. 00-04-48767).

REFERENCES

1. T. V. Vitreshchak, V. V. Poleshchuk, I. A. Ivanova-Smolenskya, *et al*., *Neirokhimiya*, **19**, No. 3, 213-218 (2002).
2. L. L. Goncharova, L. A. Pokrovskaya, I. N. Ushakova, and N. Yu. Mal'kova, *Mezhdunar. Med. Obz.*, **2**, No. 1, 15-19 (1994).
3. M. A. Pal'tsev and A. A. Ivanov, *Cell-Cell Interaction* [in Russian], Moscow (1995).
4. T. V. Chichuk, I. A. Strashkevich, and G. I. Klebanov, *Vestn. RAMN*, No. 2, 27-32 (1999).
5. A. D. Shalabodov and N. V. Guseva, *Principles of Membrane Transport* [in Russian], Tyumen' (2001).
6. Y. Agid, *Neurology*, **57**, Suppl. 3, 46-51 (2001).
7. C. W. Olanov, P. Jenner, N. A. Tatton, and W. G. Tatton, in: *Parkinson's Disease and Movement Disoders*, Eds. J. Jankovic and E. Tolosa, Baltimore (1998), pp. 67-103.