10. G. Rudnick, Annu. Rev. Physiol., 48, 403 (1986).
11. R. D. Schwartz, P. D. Suzdak, and S. M. Paul, Mol. Pharmacol., 30, 419 (1986).
12. M. Willow and G. A. R. Johnston, Int. Rev. Neurobiol., 24, 15 (1983).

EFFECT OF LOW-INTENSITY LASER IRRADIATION ON STATE OF BLOOD PROTEINS

V. M. Genkin, V. F. Novikov,
L. V. Paramonov, and B. I. Él'kina

UDC 615.849.19.015.4:616.153.96].09

KEY WORDS: proteins; blood; fluorescent probes; laser

Low-intensity laser radiation is widely used at the present ime in medicine for the treatment of various diseases [1,6-9,11,13,14]. However, the mechanism of its action is not yet clear. This is largely due to the fact that most investigators have studied the response of the whole body to irradiation. There have been few studies in vitro. For instance, the effect of irradiation has been studied on platelet and leukocyte function, and on the state of the blood clotting system and enzyme activity [3-7, 10, 12]. Even these parameters, however, are essentially a reflection of a series of complex processes taking place in the blood and its components under the influence of irradiation. Simpler parameters reflecting the response to irradiation in vitro are required. In the investigation described below the degree of binding of a fluorescent probe by blood proteins and cells was chosen as the parameter.

EXPERIMENTAL METHOD

Whole blood, plasma, and platelet mass. The effect of irradiation was assessed with the aid of the fluorescent probes 1,8-anilinonaphthalene sulfonate (ANS) and 4-(p-dimethylaminostyryl)-1-methylpyridinium (DSM). Fluorescence of ANS was excited at a wavelength of 270 nm and recorded within a wide range of wavelengths in excess of 360 nm. The ranges for DSM were 470 and 530 nm respectively. All investigations were carried out on the "Specord M40" spectrophotometer.

Whole bood was irradiated in a Petri dish in a thin layer (1-2 mm) by the unfocused beam of a helium-neon laser (wavelength 632.8 nm), with power density of 0.9 mW/cm^2 for 5-60 min. The irradiated blood was then incubated under room conditions in darkness for between 10 min and 24 h, after which the blood cells were separated by centrifugation at 1500 rpm for 15 min. The supernatant was diluted with 0.9% NaCl solution in a ratio of 1:50 by volume. To 2 ml of the resulting solution 0.01 ml of 10^{-4} M ANS in water was added. The optical density of the plasma and probe were verified photometrically at 270 nm. Platelet mass or plasma was irradiated in a test tube by a laser beam with power density of 45 mW/cm^2. The difference in power density for irradiation of blood compared with irradiation of platelet mass and plasma is due to the necessity of irradiating the blood with an unfocused laser beam in a thin layer.

All the materials studied (blood, plasma, platelet mass) were divided into two series: control and experimental; the difference between them was limited to the irradiation procedure.

The "effective binding constant" K_{ef} was chosen as the quantitative characteristic reflecting the results:

$$K_{ef} = \frac{I}{D_1 (D_2 - D_1)},$$

where I denotes the intensity of fluorescence in the selected spectral range in arbitrary units, D_1 the optical density (in relative units) of the test sample at 270 nm without the probe, and D_2 the optical density of the same sample with the probe. The value of $D_2 - D_1$ is evidently proportional to the concentration of the probe, whereas D_1, even allowing for the contribution of scatter to the optical density, is basically proportional to the protein concentration for this particular

Institute of Applied Physics, Academy of Sciences of the USSR. S. M. Kirov Gor'kii Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR B. A. Korolev.). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 188-190, August, 1989. Original article submitted November 25, 1988.

0007-4888/89/0008-1127$12.50

TABLE 1. Results of an Experiment with Irradiation of Blood by Helium-Neon Laser (power density 0.9 mW/cm^2, duration of irradiation 10 min, incubation time 60 min)

Series of experiments	No. of probe	D_1, rel. units	D_2, rel. units	I	K_{ef}
Control	1	1,2639	1,7709	65,61	102,38
	2	1,2662	1,7769	65,75	101,70
	3	1,2604	1,7624	65,66	103,70
					$\Delta = 0,83$
Experimental	1	1,2220	1,7320	69,86	112,09
	2	1,2258	1,7398	69,93	110,98
	3	1,2278	1,7408	70,20	111,45
					$\Delta = 0,45$

experimental situation. This follows from the fact that we were operating close to the protein absorption line, strong frequency dispersion of D_1 being observed near 270 nm. Thus K_{ef} incidates the degree of binding of the probe with proteins. To avoid any misunderstandings, we may point out that K_{ef} is not the binding constant as usually defined (see, for example [2]), but for our purposes it is sufficient to use K_{ef}.

EXPERIMENTAL RESULTS

The fluorescent probes which we used carry opposite charges under normal conditions: ANS a negative charge, DSM a positive. The quantum yield of both probes during binding with proteins, membranes, and intracellular organelles is significantly higher than in an aqueous medium. The intensity of fluorescence is thus an indicator of the degree of binding with the test object. The change in K_{ef} as a result of laser irradiation is independent of the method of measurement, for it is determined by comparison with K_{ef} of the control specimen. Natural luminescence of proteins was not observed in the dilution which we used.

The quantum yield after irradiation increases as a result of an increase in binding of ANS with plasma proteins.

Table 1 gives the results of an experiment involving irradiation of blood. Three measurements were made for both control and experimental series in order to exclude possible errors. In some experiments, as Table 1 shows, the optical density of the protein was changed by irradiation. However, the conversion of K_{ef} cannot be attributed entirely to a change in D_1, for the intensity of fluorescence also changed, and a 3% change in D_1 cannot lead to a 10% change in K_{ef}. Nevertheless, the effect of a change in optical density as a result of irradiation calls for further study.

Experiments with blood, plasma, and platelet mass showed that the magnitude of the effect and also its sign were subject to individual variation for different specimens. A change in K_{ef} under 3% is not significant, as shown by analysis of the results of the measurements in each series.

With a dose of 0.5 J (irradiation for 10 min) K_{ef} in different specimens changed relative to K_{ef} in the control by between 7 and 17%. With this dose, incidentally, we observed no change in K_{ef} within the 3-7% range. This was probably due to the small number of specimens studied.

The magnitude of the effect of irradiation was shown to depend on the time elapsing after irradiation for several blood specimens. After incubation for 24 h the effect of irradiation fell appreciably, down to 0 in some cases.

To explain the dependence of the change in K_{ef} of the irradiated specimen on the charge of the probe a series of experiments was carried out with DSM, whose charge is opposite to that of ANS. It was found that whereas K_{ef} in the experiment with ANS was increased by irradiation, in the experiment with DMS it was reduced. Thus in one investigation, conducted on platelet mass, K_{ef} of ANS was increased by 10% after irradiation whereas K_{ef} of DSM was reduced by 14%. For another sample of platelet mass, reduction of K_{ef} for ANS under the influence of irradiation led to a parallel increase in K_{ef} of DSM. This type of change of fluorescence of oppositely charged probes suggests that irradiation with a helium-neon laser affects the charge of the blood proteins to some degree.

It was shown that irradiation with light with a wavelength of 632.8 nm induces processes which evidently lead to a change in the charge of the blood proteins. The magnitude of the effect depends on the individual properties of the material and the dose and time of incubation after irradiation. It must be pointed out that the results obtained in this study do not allow the cause of the change in the charge on the proteins to be identified, for it may be produced by several different factors.

LITERATURE CITED

1. U. Ya. Bogdanovich, Kazan. Med. Zh., No. 5, 59 (1981).
2. Yu. A. Vladimirov and G. E. Dobretsov, Fluorescent Probes in the Investigation of Biological Membranes [in Russian], Moscow (1989).
3. V. N. Dreval', S. A. Bazhanova, V. M. Klyuchnikov, and O. N. Manueva, Comparative Biochemistry of Metabolism in Animals [in Russian], Kuibyshev (1982), pp. 48-52.
4. R. M. Lapteva, S. A. Bashieva, and O. I. Makarova, Zdravookhr. Kazakhstana, No. 11, 51 (1984).
5. V. I. Popova, Gig. Truda, No. 11, 44 (1985).
6. Application of Methods and Materials of Laser Technology in Biology and Medicine [in Russian], Kiev (1980).
7. E. N. Meshalkin (ed.), Application of Direct Laser Irradiation in Experimental and Clinical Heart Surgery [in Russian], Novosibirsk (1981).
8. I. M. Suvorov, V. V. Dobrynina, I. N. Ushakova, and L. N. Lychakova, Vrach. Delo, No. 9, 10 (1981).
9. Abstracts of Proceedings of an All-Union Conference on the Use of Lasers in Medicine [in Russian], Krasnoyarsk (1984).
10. N. N. Tapeznikov, V. I. Kupin, A. V. Ivanov, et al., Vest. Akad. Med. Nauk SSSR, No. 5, 40 (1984).
11. J. A. Carruh, J. Med. Eng. Technol., 8, No. 4, 161 (1984).
12. Y. J. Joyce, A. D. Meyers, and J. J. Cohen, Laser Surg. Med., 6, No. 2, 171 (1986).

EFFECT OF ANTAGONISTS OF EXCITATORY AMINO ACIDS ON NEURODEGENERATIVE ACTION OF QUINOLINIC ACID IN VITRO COMPARED WITH THEIR ANTICONVULSANT ACTION IN SITU

L. G. Khaspekov, I. P. Lapin,
I. V. Rhyzov, and I. V. Viktorov

UDC 615.213:547.831/.015.4.07

KEY WORDS: nerve cell culture; quinolinic acid; seizures; antagonists of excitatory amino acids

The results of recent experimental investigations show that quinolinic acid (QA), one of the strongest neuroactive products of the kynurenin metabolism of tryptophan, has a marked excitatory, convulsant, and neurodegenerative action [11, 14]. This action of QA is similar in many respects with the effects of excitatory amino acids (glutamate, aspartate) and of their exogenous analogs (N-methyl-D-aspartate, ibothenate, quisqualate, cainate, etc.). As it has been shown [4, 12, 14], the QA concentration in various brain structures rises during aging and also in epilepsy, senile dementias, Alzheimer's disease, Huntington's chorea, and hepatic coma, and this may be one of the causes of destruction of neurons. Identification and study of the mechanisms of action of QA antagonists is thus an urgent problem.

Soem of the investigations to be described below were conducted on dissociated cultures of cells from various brain structures, by means of which the destructive action of QA and its analogs on living neurons could be studied at the cellular level and the protective effect of putative antagonists of this action revealed [8, 13]. Experiments to study the anticonvulsant action of these compounds in situ by their systemic administration and injection into the cerebral ventricles, preceding injection of cytotoxins, are another traditional and effective method of identifying antagonists [1, 6].

The aim of the investigation was to compare the effects of various antagonists on the neurodegenerative action of QA in dissociated cultures of hippocampal cells and on its convulsant action in situ.

Laboratory of Experimental Neurocytology, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR. Laboratory of Psychopharmacology, V. M. Bekhterev Leningrad Psychoneurologic Research Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Andrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 190-193, August, 1989. Original article submitted July 18, 1988.

0007-4888/89/0008-1129$12.50