APPLICATION OF A Cu-VAPOR LASER TO IDENTIFICATION OF A PRIMARY PHOTOACCEPTOR IN LASER THERAPY

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The setup, comprising a cytodiffractometer and a dye laser pumped by a Cu-*vapor laser, has been used to check the concept of photogeneration of singlet oxygen as a primary cell response to irradiation at the oxygen absorption bands. The results of laser therapy at the absorption band of singlet oxygen confirm this concept.*

1. INTRODUCTION

Use of low-power laser radiation for medical treatment, i.e., laser therapy (LT), in spite of 30-year world-wide experience, needs for a rigorous justification. To this end, three main requirements must be met. A new method must give reproducible results in independent medical institutions. In addition, a molecular or other agent, which reacts with photons and initiates the expected therapeutic effect, must be identified in experiments with cells. Finally, the therapeutic effect in a clinic must be shown to be initiated by one and the same agent.

As to the first requirement, it was fulfilled more than sufficiently.[1–3] In the research,[4–9] the second requirement was also met, at least for some spectral regions. In particular, for the most wide-spread version of LT with the use of a He–Ne laser (632.8 nm) it was shown that, under irradiation of red blood cells (erythrocytes), responsible for the process reminiscent of cell biostimulation[10] is the reaction of photogeneration of singlet oxygen (PhSO):

$$h\nu + {}^3O_2 \rightarrow {}^1O_2.$$

Oxygen dissolved in the out-of-cell space rather than bound to biomolecules (hemoglobin, for example) takes part in the reaction.

The results of blood cells investigation have been obtained using the method of recording of action spectra with the use of tunable lasers and the original diagnostic method of cytodiffractometry (erydiffravision).[11] Under exposure to radiation, the PhSO mechanism was discovered to dominate in at least four narrow spectral regions in the visible and near infrared. Among them are the visible 630–650 nm spectral band, within which the 633 nm lines of He–Ne laser lie, and the 580–590 nm band promising for application in medicine. In this paper we describe the results of the final stage of this research. This stage was aimed at demonstrating the fact that third requirement is also met, i.e., checking whether the PhSO mechanism forms the basis of the LT medical effect or not. We conducted clinical researches in the up-to-date regime of intravenous irradiation which has gained official recognition. (Two authors of the present paper, I.M. Korochkin and G.M. Kapustina were awarded with the USSR State Prize for the development of the method of intravenous irradiation.)

2. INSTRUMENTATION AND THE PROCEDURE

Patients were irradiated with the use of the setup for medical and biological researches designed by the group headed by A.N. Soldatov at Tomsk State University. Block diagram of the setup is shown in Fig. 1.

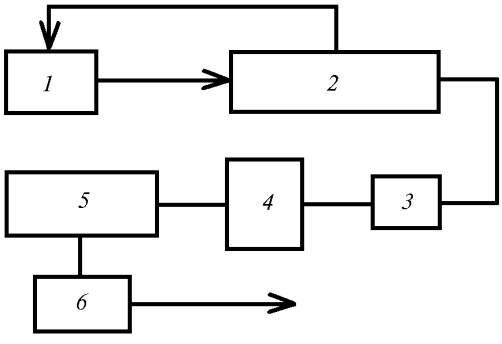

FIG. 1. Block diagram of the experimental setup: a power supply 1, a laser tube 2, dispersion elements 3, an MLK–02 dye laser converter 4, an attenuator 5, a waveguide for laser radiation 6.

The repetitively pulsed Cu-vapor laser of the MALAKHIT type[12] operated at 510.6 and 578.2 nm with the mean power up to 1000 mW, pulse duration 20–30 ns, and pulse repetition rate 12–18 kHz.

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The MALAKHIT laser was applied to pumping dyes. As a dye laser, we used a small-size MLK–02 converter[13] with a changeable autonomously circulated dye cell which allows an active medium to be changed without readjusting the optical scheme. We used the Rhodamine B for generation in the yellow spectral range and Rhodamine 6G for generation in the red range. Pulse duration was 10–15 ns, and lasing linewidth was 1–2 nm.

The radiation was directed into a changeable quartz waveguides 0.4 mm in diameter, whose distal ends were intended for irradiation of patients. Average power at the wavequide output can be varied within 0.3–50 mW under the control with an IMO–2 calorimeter.

For a more convenient procedure of wavelength measurement under clinical conditions, we have specially designed miniature attachment according to the scheme of a diffraction spectrograph with a *Z*-shaped optical tract. As a dispersion element in it, we used a diffraction grating with 1200 grooves/mm. The radiation was injected into waveguides in such a way that the wavelength can be monitored by eye at the wavequide output. The wavelength and the spectrum width were measured using the scale calibrated by the 510.6 and 578.2 nm lines accurate to 1 nm.

In a number of procedures as a source of radiation, we also used standard ALOK–1M apparatus with a cw He–Ne laser with up to 3 mW power, the radiation from which also was injected into analogous waveguide. The procedure of therapeutic irradiation with this laser and recommended doses are described in Ref. 14.

The measurement procedure with the use of cytodiffractometer is presented in Ref. 15. The value monitored is the dimensionless factor of erythrocyte elongation in the cytodiffractometer cell. In our research the value was measured 20 min after sample injection into the physiological solution circulating in the device.

Heart ischemic disease patients, to whom the laser therapy was prescribed, were selected voluntarily, grouped into uniform groups of 4–10 people (totally about 100 people). A standard treatment course usually consists of 5–6 laser procedures. In our experiments, in order to provide the patients' safety, they were undergone experimental irradiation one time in the course, during the first procedure.

The experiment was carried out in the following way. The required parameters of laser radiation were fitted at the waveguide output. Then the waveguide was injected into the patient's vein through a hollow syringe's needle. In so doing, a blood drop was sampled (sample volume needed was 0.125 ml) to measure the initial value of the parameter $\varepsilon(0)$. From this point onwards the irradiation started. After a period t preset, the waveguide was taken out of the needle for a moment; as this took place, a blood drop was squeezed out due to internal pressure and it was then used for measuring $\varepsilon(t)$, whereas the waveguide was inserted back to continue the irradiation.

In such a way we have obtained a curve $\varepsilon(t)$ for every patient. With this curve we determined time t when $\varepsilon(t)$ reaches maximum. As the previous researches had shown, this time corresponded the optimal dose rate for a given patient under specified conditions of a laser procedure.

The action spectrum was constructed as $t_m^{-1}(\lambda)$ reduced to one and the same value of the average power P at the waveguide output. To check the correctness of this reduction, we have checked the linearity of the function $t^{-1}(p)$ within the values of the power used.

3. RESULTS AND DISCUSSION

When studying the effects of LT in the yellow spectral range, which has not been earlier used for internal irradiation of blood, additional precautions were taken to decrease the number of experiments. To this end, a major part of measurements was done near the expected line center, being within 586–587 nm range, according to the action spectrum obtained in the experiments on erythrocytes irradiation,[9] as well as at the assumed line boundaries.

Figure 2 shows a cytodiffractometric factor as a function of time of irradiation at 586 nm wavelength with different level of output average power. It has the same shape as in the experiments with cells. It is clearly seen that as the power increases, the curve's maximum shifts toward shorter period t, what clearly indicates that the optimal dose is reached faster.

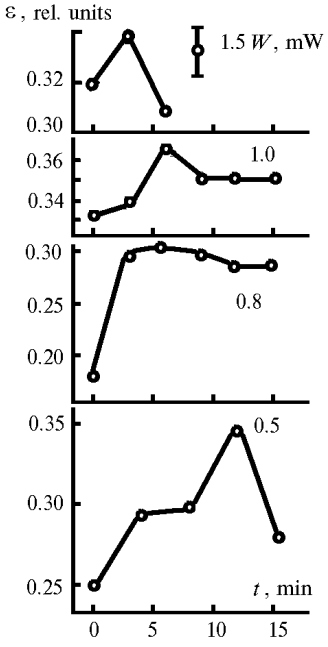

FIG. 2. The cytodiffractometric factor ε *as a function of the irradiation time at* 586 nm *wavelength for different average power of laser radiation W.*

Exposure to radiation at 592 nm with the average power of 5 mW had no marked effect on the factor during 15 min of internal irradiation. Marked variations in cytodiffractometer indications under exposure to radiation at another line's boundary, near 578 nm, were not observed too. In this case we used the radiation of the yellow line of a Cu-vapor laser, and the radiation power at the waveguide output was increased up to 20 mW.

Based on the data obtained, the linearity characteristics and the action spectrum reduced to the average power of 1 mW were constructed (see Fig. 3). Although the latter has only three experimental points, their positions are in full agreement with the data from Ref. 9.

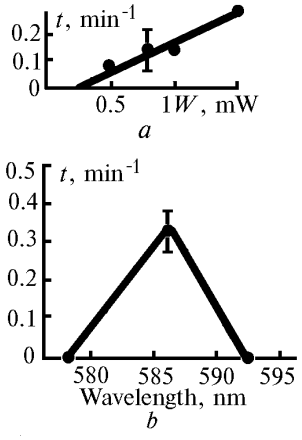

FIG. 3. The linearity characteristics of the cytodiffractometer at 586 nm *wavelength (a) and the action spectrum for laser therapy (b) in the* 578–592 nm *wavelength range.*

It should be emphasized that the absence of a therapy effect at $\lambda = 592$ or 578 nm cannot be interpreted as a proof of lack of any prospects for the use of radiation at these wavelengths for the purposes of LT. This simply shows that this radiation in small doses is ineffective for blood irradiation. However, it is noted in Ref. 16 that the radiation at $\lambda = 578$ nm, being used for external irradiation (with other mechanism of action), gives some helps for patients.

When working in the 627–650 nm range, researchers less apprehended. Some examples of cytodiffractometer readings as a function of irradiation time are shown in Fig. 4.

Irradiation at $\lambda = 633$ nm was carried out with two different lasers operating in the repetitively pulsed and continuous wave modes, with the same average power. Coincidence of t values in both cases demonstrates the property of accumulation of biomolecule modifications under the action of PhSO reaction.[6]

The action spectrum in the 627–660 range, constructed within the linear section of $t(P)$ dependence, is shown in Fig. 5. It corresponds to the action spectrum for the *in vitro* experiments[8] and the absorption band profile of oxygen dissolved in freons.[17]

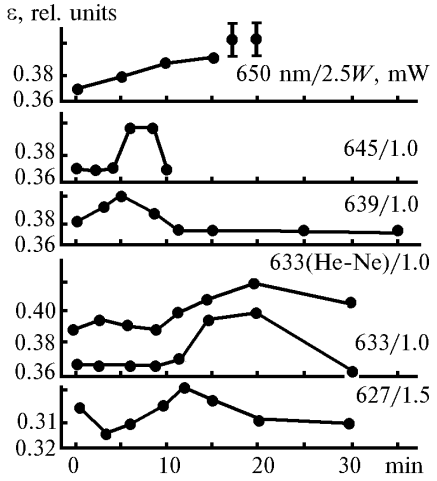

FIG. 4. The cytodiffractometer factor ε *in the* 627–650 nm *wavelength range.*

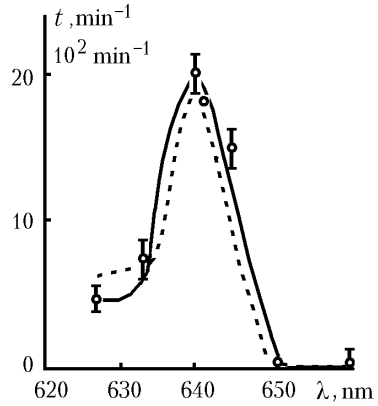

FIG. 5. The action spectrum of laser therapy (solid line) in the 627–660 nm *wavelength range and the absorption spectrum of oxygen dissolved in freons*[17] *(dotted line).*

Thus, when conducting the intravenous laser therapy with the use of laser operating at $\lambda = 633$ nm for irradiation, the therapeutic effect observed is due to photon absorption by the molecular oxygen dissolved in the blood. In addition, experimental results prove the analogy of the mechanisms of laser radiation action onto the blood, when laser operates in either continuous-wave or repetitively pulsed modes.

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