METHODS

Experimental Validation of the Clinical Use of New Surgical Lasers

S. V. Grachev, S. V. Eliseeva, N. D. Egorova, A. N. Nasedkin, A. N. Novikova, A. M. Prokhorov, and B. I. Denker

UDC 615.849.19.03.076.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 7, pp. 96-100, July, 1993
Original article submitted December 3, 1992

Key Words: *lasers; experiment; morphology; bioenergetics; enzymes; biosynthesis*

CO_2 surgical laser (Coherent) has been used at the Research Center of the I. M. Sechenov Medical Academy since 1982. This laser has been widely used to eliminate the factors inducing laryngeal or tracheal stenosis in children and to prevent restenoses [5,6]. There is no question but that the application of CO_2 lasers in endoscopic surgery of the larynx and trachea has raised clinical treatment to a higher level. Surgical CO_2 lasers are in many respects superior to both the traditional surgical microinstruments and surgical ultrasound. Still, even this method fails to solve quite a number of problems. Despite the obvious advantages of CO_2 laser over the traditional methods of treatment of laryngotracheal cicatricial tissue this laser may induce a postoperative edema and an inflammatory reaction, which, as the first phase of the regeneration process, determines the course of healing and may lead to restenosis. Edema and inflammation after exposure to CO_2 lasers even of the minimal power required for dissection develop because such a type of laser radiation induces thermal burn, carbonization, and profound warming of the tissues adjacent to the wound. Bearing these data in mind, we have studied, in cooperation with scientists from the Institute of General Physics of the Academy of Sciences, the effects on biological tissues of CO_2 laser (λ=10.6 μ) and of lasers hitherto not used in medicine: YAG-erbium (λ=2.94 μ) and glass-erbium (λ=1.54 μ). These lasers are rigid-body and operate in a pulsed periodic mode. The pulse length of YAG-Er laser is about 2 msec, that of glass YAG-Er laser about 1 msec. The pulse energy of these lasers from 1 to 10 J permits their use as surgical instruments. The cutting characteristics of these YAG-lasers and their effects on the tissues were studied, as were the terms of laser wound healing. To elucidate the effects of radiation on biological tissues we studied laser effects on the energy, biosynthetic, and proliferative processes in liver tissue and measured the activities of transamination enzymes. Although these problems are interesting from both a theoretical and a practical viewpoint, only a few papers have dealt with them, describing exposure to lasers with different wavelengths [1,7,8].

Laboratory of Emergency States and Laboratory of Restoration Surgery of the Larynx and Trachea, I. M. Sechenov Moscow Medical Academy; Institute of General Physics of the Academy of Sciences

MATERIALS AND METHODS

White outbred rats were used in the experiments. For the morphological studies the abdominal white line tissue was exposed to laser; the presence of

0007-4888/93/0007-0886$12.50

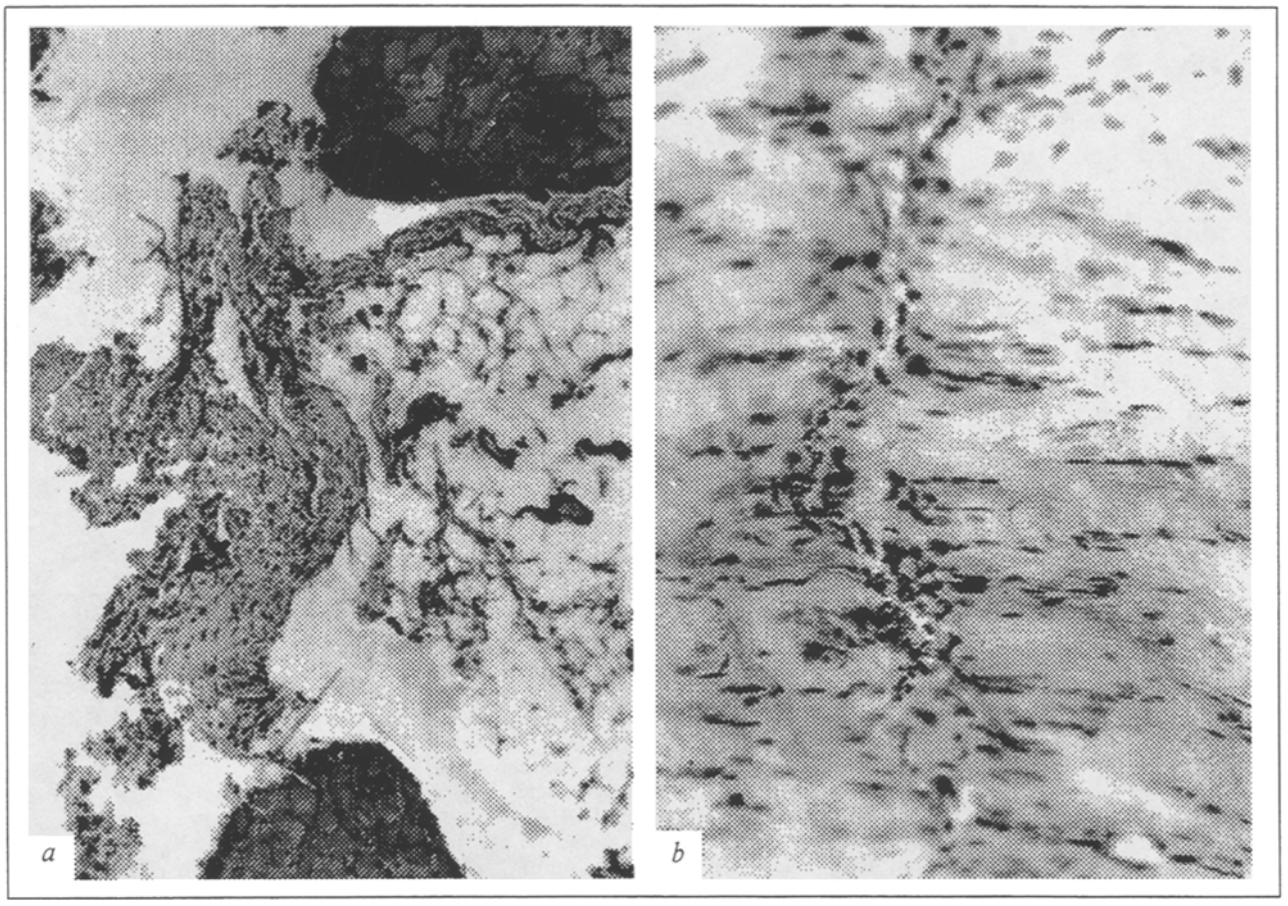

Fig. 1. Tissues of rat anterior abdominal wall after treatment with surgical laser. *a*) funnel-shaped laser wound after application of CO_2 laser with the formation of an extensive zone of injury. ×80; *b*) wound canal after application of YAG-Er laser, surrounded by virtually intact tissues. ×200.

large quantities of connective tissue in this line permitted us to regard it as an adequate model of a linear cicatrix. The skin was dissected under inhalation anesthesia. The abdominal white line tissue then was laser-treated, resulting in the formation of a laser wound on the anterior abdominal wall. For assessment of the cutting properties of the lasers, tissue sites exposed to irradiation were dissected immediately after the treatment, while for assessment of the time course of wound healing, portions of tissue were dissected on days 1, 3, 5, and 7. The tissue was placed in 10% formalin solution, after which the material was embedded in paraffin and histological micropreparations were made. The preparations were stained routinely: with hematoxylin-eosin and after Van Gieson. For a study of the effects of laser on bioenergetics, aminotransferase activities, and regeneration processes in tissues, the left lobe of the liver was bombarded with laser (5 pulses) immediately after decapitation of the rat and opening of the abdominal cavity (5 animals per group). The liver was then placed in 0.9% KCl solution and cooled to 0°C for 40 min (time of transportation of the irradiated liver). The intact left lobe of the liver was examined for control. The effects of laser on energy processes were studied in mitochondria (MC). A homogenate was prepared from the whole liver lobe. The mitochondria were isolated according to the routine method, which was slightly modified [12]. Mitochondrial respiration was recorded with an LP-60 polarograph (Czechoslovakia) at 20°C. The rates of oxygen uptake by the mitochondria were studied in various metabolic states: at rest, in the presence of only the oxidation substrate in the incubation medium (V_2); in an active state, after addition of adenosine diphosphate (ADP) in a concentration of 200 μM (V_3); at rest after ADP phosphorylation (V_4); and for mitochondrial respiration disturbance with 2,4-dinitrophenol (V_{DNP}). The oxygen volumes used for phosphorylation of the added ADP (ΔO_p) and the time of its phosphorylation (t_p) were measured. Then the phosphorylation rate (V_p = ADP/t_p/mg MC protein), phosphorylation efficacy ($P/O=ADP/\Delta O_p$), respiration stimulation ($RS=V_3/V_2$), and respiratory control ($RC=V_3/V_4$) were calculated. The protein content in the mitochondrial suspension was estimated af-

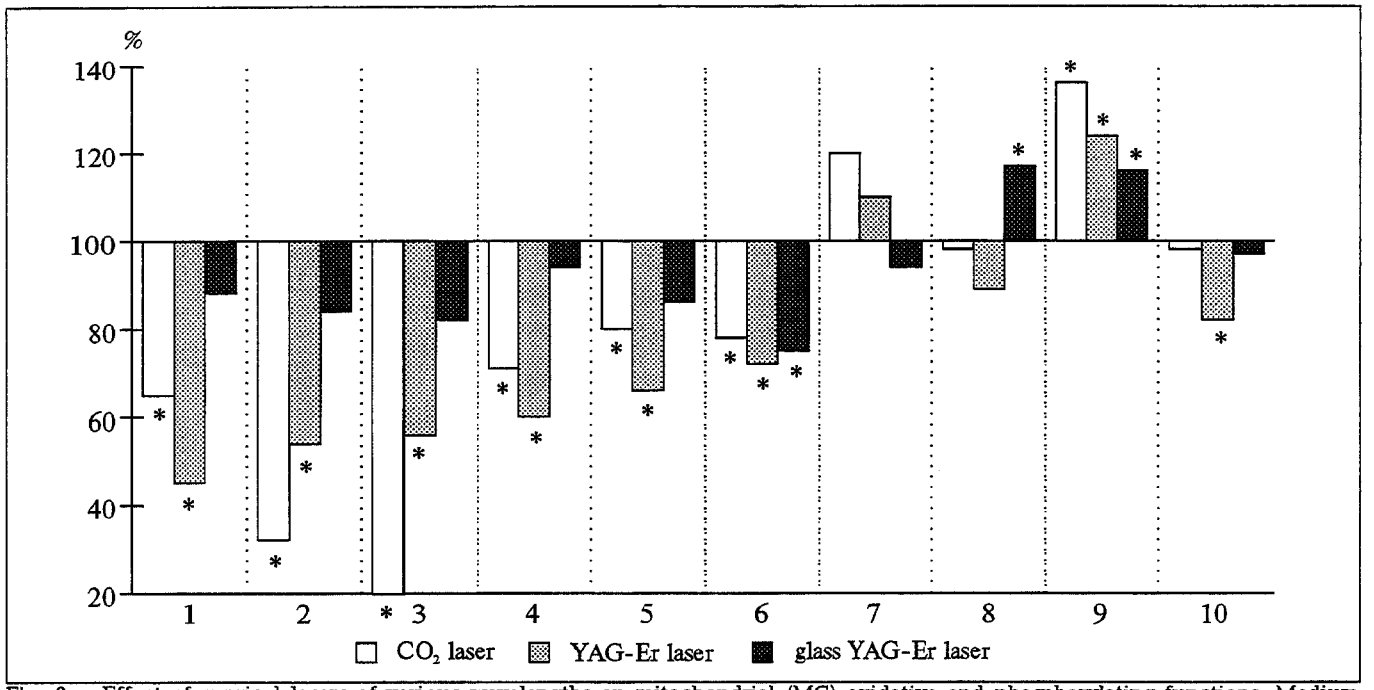

Fig. 2. Effect of surgical lasers of various wavelengths on mitochondrial (MC) oxidative and phosphorylating functions. Medium for MC isolation: sucrose 0.320 M, tris 0.020 M, EDTA 0.001 M (pH 7.5). Incubation medium: sucrose 0.240 M, $MgCl_2$ 0.005 M, KH_2PO_4 0.010 M. Succinate + DNP (*1*), αKG + DNP (*2*), βHOB + DNP (*3*): rates of 2,4-dinitrophenol-disturbed succinate (5 mM), α-ketoglutarate (4 mM), and β-hydroxybutyrate (7 mM) oxidation with mitochondria. V_2 (*4*), V_3 (), and V_4 (*6*): rates of mitochondrial oxidation of succinate (5 mM) at rest, in activity, and at temporary rest (respectively). RS (*7*): respiration stimulation induced by ADP (200 μM). RC (*8*): respiratory control. P/O (*9*): phosphorylation efficacy. V_p (*10*): phosphorylation rate. The values of mitochondrial function parameters are presented in percent vs. the control, taken as 100%. Here and in Figs. 3-4: an asterisk denotes a significant difference from the control.

ter Lowry, Rosebrough, Farr, and Randall [9]. Aspartate and alanine aminotransferase (AST and ALT) activities in the liver tissue homogenates were measured after Frankel and Reitman [11]. RNA and DNA levels in the liver tissue homogenates were assessed according to Schmidt and Tanhauser's method, modified by Trudolyubova [4].

RESULTS

Assessment of the cutting characteristics of the tested lasers showed good cutting properties of YAG-Er laser, that seem to be due to abnormally high absorption of its radiation with water (α - 10^4 cm^{-1}), whereas the same characteristic of CO_2 lasers is two orders lower. The YAG-Er laser-induced wound differed markedly and fundamentally from the wound after CO_2 lasers treatment. The morphological picture of the CO_2 laser-induced wound consisted in the emergence of several zones of injury: complete destruction of the tissue in the focus of the beam, a loose and compact layer of necrosis, and a zone of perifocal inflammatory edema. Exposure to YAG-Er laser resulted in minimal injury of the biological tissues: the edges of the wound canal were tightly closed and just negligible dystrophic changes (cytoplasm vacuolization and nuclear chromatin redistribution) were found in the cellular elements adjacent to the dissected area. No changes in the blood vessels or microvessels were detectable (Fig. 1). Microhemorrhages occurred only as a result of a direct hit of the vascular wall. Visually blood was seen on the surface of the tissue after YAG-Er laser treatment, this indicating the absence of a coagulation effect.

Comparative assessment of the rate and course of wound healing in rats after treatment with CO_2 and YAG-Er lasers showed the following scheme of wound healing after CO_2 lasers bombardment: augmentation of the reactive symptoms up to days 3-5 after treatment and abatement of these symptoms by days 5-7. Morphologically a pronounced inflammatory focus with the typical symptoms was seen on day 5 at the site of the CO_2 laser-induced wound. Healing of the wound inflicted with YAG-Er laser was fundamentally different. The signs of an inflammatory reaction were minimal the very first day after treatment, and on day 3 the wound was quite undetectable. Similar data were obtained in studies of the effects on biological tissues of low-intensity radiation of glass YAG-Er laser. It is noteworthy that this laser, with good dissecting properties, was characterized by a marked hemostatic effect as well.

Morphological studies have demonstrated the ability of laser radiation at λ 1.54 and 2.94 μ to destroy biological tissue while causing a minimal inflammatory reaction, this being conducive to a smooth, rapid healing of the laser wound. Due to this property, these lasers compare favorably with the well-known and widely used CO_2 laser.

Solving problems of surgery with the aid of these lasers necessitates comprehensive studies of their effects on biological tissues. The aspects of laser energy interaction with energy processes in the mitochondria appear to be the most interesting to us, for these processes ultimately determine the body's resistance to various agents. The results of studies of laser radiation at various wavelengths on the oxidative and phosphorylating functions of the hepatocyte mitochondria are presented in Fig. 2. Treatment with CO_2 lasers resulted in a marked (in comparison with the control) reduction of the rate of 2,4-dinitrophenol-disturbed oxidation of both α-ketoglutarate and β-hydroxybutyrate, as well as succinate by the mitochondria. This indicates electron transport inhibition at the NAD- and PAD-dependent sites of the mitochondrial respiratory chain. Succinate oxidation was associated with a significant reduction of V_2, V_3, and V_4. This was not paralleled by a reduction of the phosphorylation rate, whereas the phosphorylation efficacy was enhanced. Respiration stimulation and respiratory control values were virtually unchanged. The rate of oxygen uptake was reduced as compared to the control in all metabolic states of the mitochondria in succinate oxidation during exposure to both YAG-Er and CO_2 lasers. The reduction of the respiration rate was attended by a significant dimi-

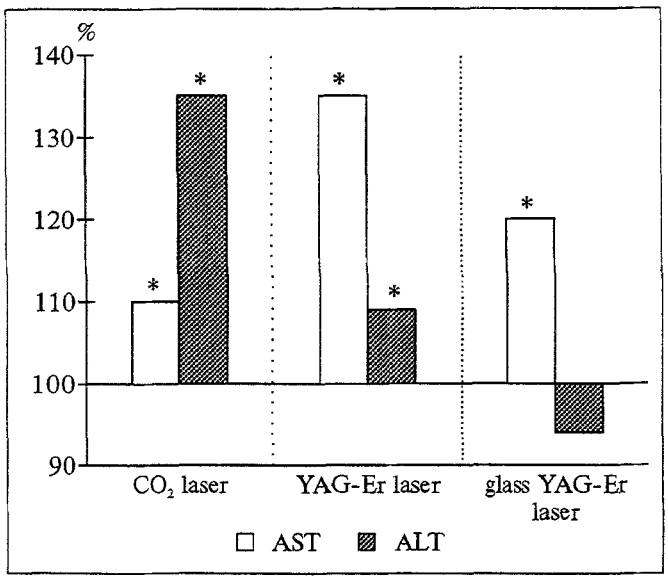

Fig. 3. AST and ALT activities in liver tissue exposed to different lasers, % vs. the control.

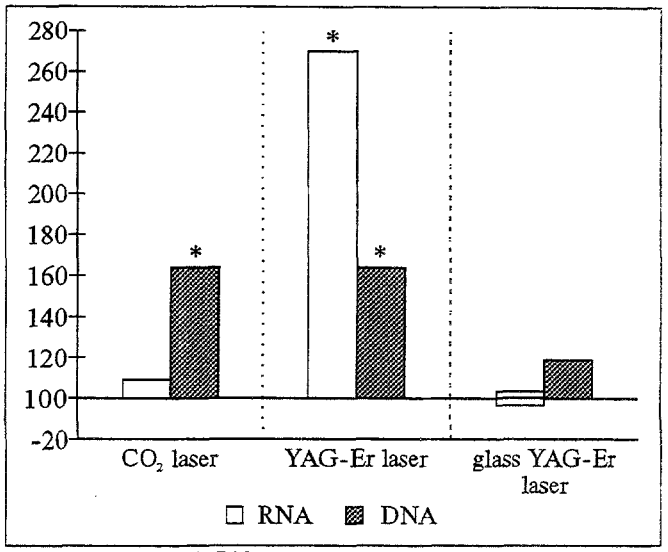

Fig. 4. RNA and DNA content in liver tissue homogenate exposed to different lasers, % vs. the control.

nution (by 20%) of the phosphorylation rate, the P/O coefficient increasing but the respiration stimulation and respiration control values being virtually unchanged. At the same time, treatment with YAG-Er laser was associated with a lesser reduction of V_{DNP} in oxidation of α-ketoglutarate and β-hydroxybutyrate - by 1.8 and 1.7 times, respectively, as against that in exposure to CO_2 lasers - by 3.0 and 4.0 times, respectively. This indicates a less expressed (almost twofold) depression of electron transport at the NAD-dependent site of the respiratory chain of the mitochondria during treatment with YAG-Er laser as against CO_2 laser. Exposure to glass YAG-Er laser did not result in any noticeable change of the V_{DNP} in oxidation of succinate, α-ketoglutarate, and β-hydroxybutyrate in the mitochondria. V_2, V_3, V_p, and the respiration stimulation values were unchanged in succinate oxidation, but the respiration rate at rest was significantly reduced, this resulting in an increase of the respiratory control value. The P/O coefficient increased as well. Hence, with surgical laser treatment the mitochondrial respiration rate was reduced in all metabolic states, whereas phosphorylation efficacy increased because lower amounts of oxygen were required to phosphorylate the same ADP portion. Note that the reduction of the rate of mitochondrial respiration in various functional states under the action of glass YAG-Er laser was negligible, that is, such exposure induced less marked changes of mitochondrial functions.

The results of a remarkable study by Kondrashova [3] demonstrated the interaction between mitochondrial respiration and transamination processes in various functional states of tissue. Therefore, the next stage of our study was to measure

the activities of alanine and asparaginic aminotransferases. Figure 3 presents the results of ALT and AST activity measurements in liver tissue bombarded with laser at various wavelengths. Exposure to CO_2 laser significantly enhanced the activities of ALT and AST. The AST activity increase was not more than 10%, whereas ALT activity increased by 38.5% as against the control. Treatment with YAG-Er laser also resulted in an increase of AST and ALT activities, AST activity growing sharply (by 39.6%) and ALT activity increasing by only 12.8% as compared to the control. Glass YAG-Er laser elevated AST activity but left ALT activity at the lower threshold of the normal range. Thus, exposure to glass YAG-Er laser resulted in the least marked changes of enzymatic activity and mitochondrial functions.

Keeping in mind the relationship between the energy and regeneration processes in the cell, we studied the content of RNA and DNA in liver tissue. The results of RNA and DNA measurements in the liver irradiated by the studied lasers are presented in Fig. 4. Exposure to CO_2 lasers resulted in a significant rise of the DNA level (by 68%), while the RNA level remained unchanged. Treatment with YAG-Er laser raised the levels of both DNA (by 68.7%) and RNA (by 172.7%). Exposure to glass YAG-Er laser was not attended by noticeable changes in the DNA and RNA levels, indicating that such action induced virtually no changes in the proliferative and biosynthetic processes in the cell, since DNA is the indicator of the proliferative processes [2] and RNA of the biosynthetic processes in the cell [10].

Our findings evidence that even a brief local exposure to the studied lasers changes, albeit to various degrees, the parameters of energy processes, the activities of transamination enzymes, and the RNA and DNA content in the entire lobe of the liver. The data demonstrate a specific relationship in the changes of the parameters of bioenergetics, enzymatic activities, and regeneration processes. The most pronounced bioenergetic changes observed for the action of CO_2 and YAG-Er lasers were attended by notable changes in the activities of aminotransferases and in the content of RNA and DNA. Irradiation of the liver with glass YAG-Er laser induced the least noticeable changes in the cell. The majority of mitochondrial functions were either unchanged or improved under its effect: specifically, energy regulation of respiration and phosphorylation efficacy were enhanced. This laser did not affect the biosynthetic or proliferative processes, and induced no marked changes in aminotransferase activities. Such action of glass YAG-Er laser on the cell probably accounts for its favorable effect on the time course and quality of wound healing.

The results of the experimental studies led to the creation of working models of surgical lasers (λ= =1.54 μ) that are at present undergoing clinical trials.

REFERENCES

1. S. M. Zubkova, I. B. Laprun, Z. A. Sokolova, and V. I. Popov, *Biol. Nauki*, № 4, 24-31 (1981).
2. M. Sussman, *Developmental Biology: Its Cellular and Molecular Foundations*, Prentice-Hall (1973).
3. M. N. Kondrashova, *Biokhimiya*, **56**, № 3, 388-405 (1991).
4. M. G. Trudolyubova, in: *Current Methods in Biochemistry* [in Russian], Moscow (1977), pp. 313-316.
5. D. G. Chireshkin, *Laryngeal Diseases in Children* [in Russian], Moscow (1982), pp. 88-92.
6. D. G. Chireshkin, A. M. Dunaevskaya, and G. E. Timen, *Laser Endoscopic Surgery of the Upper Respiratory Tract* [in Russian], Moscow (1990), p. 192.
7. Tiina Karu, *Proc. Spec. Symp. Matur. Technol. Emerg. Horizons Biomed. Eng.*, New Orleans, № 4-7, 34-35 (1988).
8. Tiina Karu, *Laser Life Sci.*, **2**, № 1, 53-74 (1988).
9. O. H. Lowry, H. J. Rosebrough, A. S. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265-276 (1951).
10. Pekka H. Maeupaa and Juhani Ahonen, *Biochem. Biophys. Res. Commun.*, **49**, № 1, 179-184 (1972).
11. S. Reitman and S. Frankel, *Amer. J. Clin. Pathol.*, **28**, 56 (1957).
12. W. C. Schneider and G. H. Hogeboom, *J. Biol. Chem.*, **183**, 123 (1950).