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PREVENTION OF OPERATION WOUND SEPSIS BY CO_2 LASER RADIATION: EXPERIMENTAL STUDY IN VIVO

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UDC 617-089.168-06:[617-001.4-002.3-084:615.849.19]-092.9

Key Words: CO_2 laser; prevention of sepsis; operation wound

Operation wound sepsis in emergency and cold abdominal surgery is due mainly to contamination of the subcutaneous cellular tissue by an infectious agent brought in during the operation from the infected peritoneal cavity or from the lumen of hollow organs. For instance, appendectomy for acute destructive appendicitis is complicated by operation wound sepsis in 26-94% of cases [4, 6, 7, 9]. In complicated cholecystitis removal of the gall bladder is accompanied by operation wound sepsis in every fourth patient [8, 10].

The degree of infection of the wound determines the frequency of sepsis: in clean operations sepsis arises in 2.2-5.5% of cases, but in operations with considerable bacterial contamination, in between 31.0 and 55.5% [1, 12].

There is as yet no method of preventing operation wound sepsis that is absolutely effective. By the use of known methods it is possible to secure more or less complete elimination of microorganisms from the wound surface. However, some microorganisms penetrate into the subcutaneous cellular tissue and become inaccessible for general methods of treatment [5]. A jet of physiological saline, pulsing under high pressure, has a more deeply penetrating action [11].

Considering the ability of radiation of a CO_2 laser to exert a thermal action not only on superficial, but also on deeper layers of tissues, we undertook experimental and clinical investigations of laser prevention of operation wound sepsis. The optimal conditions for laser scanning were 15-100 W/cm^2/sec. The efficacy of prevention of operation wound sepsis in acute destructive appendicitis was 100% [2, 3].

The aim of this investigation was to establish the theoretical basis for laser prevention of sepsis of contaminated wounds and to determine the parameters of effective action by lasers on microorganisms without any damaging action on the host's tissues.

EXPERIMENTAL METHOD

A production line model of the "Skal'pel'-1" laser surgical apparatus with continuous emission and with an output power from the light guide of 20 ± 2 W, was used for the investigation. Operations on the animals were performed under general ether

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 456-457, May, 1990. Original article submitted February 24, 1989.

0007-4888/90/0005-0602$12.50

inhalation anesthesia. The animals were brought out of the experiments by intraabdominal injection of a lethal dose of pentobarbital.

Strains of the bacterial cultures for investigation, namely *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus,* were isolated from patients admitted for treatment to the clinics of the Institute, and strain *Bacillus subtilis* 6633 was obtained from the Tarasevich State Control Institute for Medical and Biological Preparations.

Preliminary investigations in vitro, in which cultures grown on nutrient broth in Petri dishes were irradiated in a laser, showed that total destruction of the microorganisms took place after exposure to laser energy of 21.4 J/cm^2 or more.

For effective laser prevention of sepsis of contaminated wounds, laser energy of lower intensity is needed, for bacteria under conditions of vegetation in the host are exposed to the antimicrobial action of biological, including immunologic, factors of protection.

There were two series of experiments. Series I: laser irradiation of the above bacterial strains in a scalp wound on the dorsal aspect of rats. For each strain 24 rats were used (six animals, four different programs of laser irradiation). Skin on the animal's back was excised over an area of 40 cm^2. Cultures of bacteria were seeded on Hottinger's agar slopes and cultured (37°C, 18-20 h). A suspension was prepared from the growing cultures in physiological saline, with a density of 10^9 viable bacterial cells in 1 ml. The suspension was poured into a Petri dish, in which sterile paper applicators were placed (ash-free filters 7 cm in diameter). After the applicators were soaked with the bacterial suspension, they were applied to the wound surface, pressed against it tightly, and held in that position for 1 min. The applicators were then removed and the wound surface contaminated by microorganisms was irradiated by laser radiation for 1 sec under four programs: 5.2, 10.6, 16.0, and 21.4 W/cm^2 (density of laser energy 5.2-21.4 J/cm^2). Immediately after irradiation a replica was produced, for which purpose a sterile paper applicator, identical with that used to infect the wound, was wetted with physiological saline and pressed firmly against the wound surface, after which it was transferred to agar in a Petri dish, carefully spread out over it with a sterile spatula, and removed. The Petri dishes with the replicase were incubated (37°C, 18-24 h). A similar experimental technique was used on the control animals, which were not irradiated by laser.

Under the influence of laser radiation on nonspore-forming microorganisms (*E. coli, P. aeruginosa,* and *Staph. aureus,* a bactericidal effect was obtained and was absolute with all four conditions of irradiation (with the supplementary program of 2.5 J/cm^2 some of the microflora in the wound survived). On laser irradiation of the spore-bearing microorganism *B. subtilis* an absolute bactericidal effect was obtained with 10^6 J/cm^2 or more.

Series II: chronic experimental model of an infected wound. Experiments were carried out on rats and guinea pigs, six animals at each of five times of observation (up to 4-28 days). A triangular area of skin measuring 20 cm^2 was dissected from the subcutaneous fascia on the animal's back. A 18-h culture of *E. coli* was used for infection. To infect the wound sterile paper applicators (1.5 × 1.5 cm) were placed on the bacterial lawn on agar, and then pressed against the wound surface for 1 min. In the animals of the control group, after removal of the applicator the skin flap was applied to the wound and fixed with interrupted sutures. The wound surface in the experimental group was irradiated by a laser with a power of 10^6 W/cm^2 for 1 sec. The skin flap was then replaced and fixed with interrupted sutures.

For quantitative determination of the microflora in the wound before and after irradiation, a piece of tissue weighing 0.1 g was excised from the wound with scissors, weighed, treated with 0.5 ml of 0.14 M NaCl, and homogenized. The homogenate was diluted in physiological saline by 10^5 times and seeded on Hottinger's agar. The results of the seedings were read and the number of viable bacteria in 1 g tissue determined after incubation for 18-24 h at 37°C.

EXPERIMENTAL RESULTS

On macroscopic examination of the wounds in the control group (series I) marked edema of the soft tissues and accumulation of opaque fluid were observed on the 4th and 7th days. At these times the number of viable microorganisms reached $5 \cdot 10^5$ or $5 \cdot 10^6$ per gram of tissue. By the 14th day the signs of inflammation were reduced and the effusion and edema had disappeared, but on quantitative determination from 10^3 to 10^5 viable microorganisms were counted in 1 g tissue. By the 21st day, all signs of inflammation in the wound region had disappeared and no microflora could be found.

In the main group of animals, macroscopic investigation (series II) on the 4th day revealed moderately severe tissue edema, but no evidence of accumulation of fluid. On the 7th day very slight edema was present, and on the 14th day the wound was externally free from all signs of inflammation.

Quantitative determination of the microflora showed that the wound was sterile in 27 of 30 animals, but on the 3rd and 4th days, 10^3 viable microbial cells could be seeded, two orders of magnitude above the critical level. All wounds were sterile at the other times.

Morphologic investigations of tissue taken from the wound surface showed that the degree of severity of thermal changes depended on the density of the applied laser energy. Thus if thermal changes were absent, at 10.6 J/cm^2 – occasional areas of superficial necrobiotic changes were seen, and at 16.0 J/cm^2 the irradiated surface carried traces of moderately severe thermal necrosis up to 400-500 μ thick.

Experimental investigations to determine the effect of radiation from a CO_2 laser on microorganisms showed that an energy density of 10.6 J/cm^2 sterilizes a contaminated wound, and reduces the number of microorganisms in it below the critical level (10^5 microbial cells in 1 g tissue). Thermal injury to tissue under the conditions described above had no negative effect on regeneration, repair, or epithelization of the wound.

As regards the efficacy of laser radiation during bench tests, total destruction of microorganisms on nutrient media occurred with an energy density of 21.4 J/cm^2 or more. The difference in the quantity of laser energy necessary for destruction in investigations in vitro and in vivo indicates that the microbial flora in the wound is exposed to the synergic action of laser radiation and of biological factors protecting the host against infection. The host's cells, being connected with the microcirculation, also have greater resistance to laser radiation than the anatomically and physiologically independent cells of the infectious agent, with no outside protection.

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