Hyperthermal effect of laser osteoperforation in a treatment of experimental acute purulent osteomyelitis

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ABSTRACT

We suggest to use laser osteoperforation as a new approach to surgical treatment of acute haematogenous osteomyelitis. In the present work this approach is studied in experiment on 30 rabbits with an emphasis on a role of hypertermia. The osteomyelitis was produced with the help of an original technique by percutaneous introduction of microorganisms 'Staph. Aureus' in medullar channel. The animals were divided into 2 group, experimental (17 rabbits) and control (13). On the 7th day of the illness all animals were subjected to the osteoperforation, with Nd:YAG laser (1064 nm) in the first group and with mechanic drill in the second. During the laser osteoperforation the dynamic control of temperature was performed simultaneously in 5-7 points in medullar channel. The original measuring-computational system designed for temperature measurements in the presence of intense laser radiation was used.

Significant increase of temperature in medulla was fixed near laser channels, in the range up to 2 mm. In the experimental group the perforation results rapid positive clinical changes and convalescence of all animals confirmed by clinical, roentgenological, and hystological tests. In the control group there was no positive dynamics, there were intermuscular phlegmon and pyo-inflammatory changes in medulla, sequestra were formed. The reason of such an advantage of laser perforation consists in new factors of action in addition to decompression of medullar channel (available in both methods). The laser produces hypertermia of the medulla which kills a significant part of microorganisms, and it creates intensive radiation field which, possibly, stimulates reparative processes.

Keywords: osteomyelitis, laser, osteoperforation, hyperthermia, thermometry.

1. INTRODUCTION

Last years the increasing frequency of diseases caused by a surgical infection, in particular, acute hematogenous osteomyelitis in adults and children, has made this problem extremely important in the social and hygienic aspects\textsuperscript{2}. The treatment of acute hematogenous osteomyelitis became one of the important problems in modern purulent surgery because of the significant increase of microorganisms antibioticresistant forms and because of the immunity level reduction in many people. The pathogenic properties of gramnegative flora and its association with aerobes requires a careful and proper choice of antibiotics\textsuperscript{3}. In many cases the treatment of acute hematogenous osteomyelitis is delayed. The disease often turns into a chronic stage, the number of late relapses and infection generalisation increases. It leads to terms and cost of treatment increasing, occurrence of physical defects resulting in invalidisation of the patients in spite of new methods of early diagnostic development, duly surgical operation and application of highly effective antibiotics. The treatment of osteomyelitis is still an expensive one\textsuperscript{4}. All this requires to look for more effective means and methods of this pathology treatment\textsuperscript{5}.

In the modern medicine the energy of various kinds of high-energy lasers is widely used. Laser light causes local hyperthermia, destruction of pathogenic microorganisms and, hence, sanation of pathology focus\textsuperscript{6}, destruction of pathological tissues and stimulation of reparative processes. So one can hope for an effective use of laser radiation for osteomyelitis treatment.

The purpose of the present research was to develop a new way of acute purulent osteomyelitis treatment in the experiment, based on the local laser hyperthermia.
2. MATERIALS AND METHODS.

The experiment was carried out in 30 pubertal rabbits, 2.5 - 3 kg weight. The structure and blood supply of a rabbit femur is similar with those of a man and it's size provides high speed and accessibility during an operative intervention, which makes this animal good for the experiment. The animals were kept in a vivarium on a standard food ration. All operations and painful manipulations were carried out in experimental operation room in aseptic conditions under intramuscular anesthesia. The animals were removed from experiment by an ether overdosage on 1-7, 14-22, 23-30 and 38-43 day after operation with the following morphological study. In isolated series the study of experimental acute purulent osteomyelitis clinic and technical characteristics of laser osteoperforation was carried out in 27 animals.

The experimental acute osteomyelitis was produced according to an original technique by percutaneous intraosteal introduction of 0.5 ml physiological solution, containing 5 ml Staph. aureus. This amount of Staph. aureus can cause the development of purulent process in the medullar channel: the greater amount causes the generalisation of infection, the less cannot develop the purulent process in the bone. Aseptic coagulative necrosis was formed locally before the infecting of the bone marrow by the preliminary injection of 96° spirits and hot physiological solution into the medullar channel. This procedure blocked the way of Staph. aureus to blood flow and thus prevents the generalisation of infectious process on the early terms. It was confirmed by medullography with transcutaneous intramedullar injection of 0.5 ml water-soluble contrast (Urographin 76%).

Chromogenic mannitpositive Staph. aureus with fibrinolytic, plasmocoagulant and hemolytic properties was isolated from a pathological material of a patient with osteomyelitis. Microorganism was identified, cultured and tested in bacteriological laboratory of clinic of general surgery of Chelyabinsk state medical academy. The development of acute purulent osteomyelitis was confirmed by clinical, bacteriological, morphological and roentgenological data (Fig. 4a).

All experimental animals were divided into 2 groups. The first, control, group consisted of 13 animals with experimental acute purulent osteomyelitis, was treated with well-known method of a surgical treatment - mechanical osteoperforation. Mechanical osteoperforation was carried out with the help of electric drill; the frequency of rotation 3 000 rev/min, the diameter of drill 0.4 mm. The second, experimental, group consisted of 17 animals with experimental acute purulent osteomyelitis was treated with decompressive osteoperforation by high-energy laser irradiation. Nd:Yag laser "Raduga-1" (λ=1.064 μm, maximal power 30W) was used for laser osteoperforation. The laser beam was delivered to the bone through quartz monofiber (d = 0.4 mm).

The preliminary experiments were carried out to choose the optimal parameters of laser radiation for osteoperforation with subsequent irradiation of the medullar. We tried the continuos wave and pulsed modes with changing power, frequency and regularity of pulses.

The operative access to the infected femur in experimental and control groups was carried out in aseptic conditions after local depliation on the 6-7 days after beginning of the experiment. After the section of the skin and removing of the lateral group of muscles the distal metadiaphysis was osteoperforated in 4-5 points with 2-4 mm distance from each other. It took us about 30 sec to make 4-5 laser osteoperforations per each experimental animal. Postoperative wounds after laser and mechanical osteoperforations were sutured with surgical gut and silk. In some cases the laser osteoperforation was carried out in transcutaneous way. During clinical observation, treatment and study antibiotics and other medicaments were not applied in all experimental animals.

The dynamic control of temperature was performed during laser osteoperforation in 5 - 7 points of medullar channel and the nearest soft tissues simultaneously. The termadetectors were placed in the medullar channel at the distance 2, 3, 5 10, 15 mm from a place of laser osteoperforation, in soft tissues on the opposite side of the bone and in muscles of extremity at the distance 15-20 mm (Fig. 1).
Fig. 1. Localization of thermodetectors in the medullar channel and in soft tissues on the opposite side of the bone.

The original measuring-computational system consisting of 8 miniature semi-conductor detectors (7 temperature and 1 radiation), 8-channel amplifier, 8-channel analog-digital converter and a computer with a special complex of the programs for registration, recording and mathematical processing of detector signals (Fig. 2) was used for thermometry of tissue. The main problem was to estimate the temperature difference between detectors and tissues in contact with them. This difference occur because of the presence of intensive laser radiation around the detectors, causing the significant their own absorption of the radiation. The method of thermometry applied here is described in detail in the work (we expect it is published in this book).

Fig. 2. The experimental device.
1. Nd:YAG laser
2. Translucent prism
3. Laser irradiation detector
4. Light fiber
5. Thermodetectors
6. Amplifier block
7. Analog-digital converter
8. Computer

Dynamic osteotonometry in the medullar channel of the infected femur at the moment of laser osteoperforation was done with the graduated capillary (point 0.001 ml) fixed in the lumen of the channel.

Every day after the operation the activity, behavior reactions, appetite and weight of the animals were controlled. We examined the postoperative wound condition and measured the rectal temperature. The white blood cells, erythrocyte sedimentation rate and differential blood count were quantified.

The bacteriological study of blood from the marginal vein of the rabbit ear 1 and 24 hours after infecting, suppurative discharge from the medullar channel and bone marrow on 7 and 35 day of illness were carried out by inoculation on 5 % blood agar medium with the subsequent study of the colony. The studies of the blood and medullar culture were done using the same tests that were applied for testing the infected culture. The quantitative determination of microorganisms in 1 ml of medullar channel contents used inoculation of homogenized infected bone marrow on 7 and 35 days of illness in both experimental groups and in untreated animals.

Before the removing of the animals from experiment the arteriography of the lower extremities was carried out on the 21, 28, 34, 50 day at the 1st and the 3rd second after the injection of 3-3.5 ml water-soluble contrast (Urographin 76 %) through microcatheter placed in infrarenal aorta.

The morphological study were performed on the first day, 8-14 day, 21-29 day, 30-37 day and 45-50 day after aseptic destruction and following infecting of the bone marrow. The visual examination of internal organs and macropreparations of the infected bone and then morphological researches followed the death of animals. 0,5 cm fragments of infected femur from the point of laser and mechanical osteoperforation
and in some distance, and specimens of internal organs (left ventricle of the heart, lung, liver, spleen, kidney, intestine, brain and pia mater) were taken for microscopic research.

The material was fixed at room temperature in neutral 10 % formalin solution within 48-72 hours. The part of bone fragments was fixed in a Carnya liquid within 1-2 hours. After the decalcification of bone fragments by formic acid and processing of all specimens with spirits of growing concentration, paraffin blocks were made. We stained 8 micron histological sections with hematoxylin-eosin for common microscopy and picrofuchsin method to reveal collagen fibres. To find out bacteria in preparations the Gram’s staining was used. The stained preparations were put into Canadian balsam and plastic for subsequent microscopy. Histological study of preparations was carried out by the light microscopy method. The photographing of the preparations was carried out in the automatic mode (microscope "Oxiophot", Carl Zeiss).

The morphological changes of bones and internal organs of animals, treated with mechanical and laser osteoperforation were compared with changes in the animals with experimental acute purulent osteomyelitis without any treatment. The compact bone, endosteum, periosteum, bone marrow and nearest soft tissues were examined. The morphological evaluation of necrotic, inflammatory and reparative processes in these tissues was based on the following features: the count of not differentiated hemopoetic and reticular cells, WBC, macrophages, histiocytes, fibroblasts, mononuclears, fat cells and osteoblasts on a standard area unit. Besides the size of the necrosis and hemorrhage, thickness of periosteum, sizes of bone marrow sinusoids, and also the presence of sequestrum, their size, and the new growth of bone tissue.

### 3. RESULTS AND DISCUSSION

The new experimental model of acute osteomyelitis in rabbits permitted to decrease the rabbit’s lethality from 55.3% to 12% because of prevention of purulent process dissemination due to preliminary formation of thermal and chemical aseptic necrosis in the place of subsequent intramedullar injection of infection agent. This model was used for study of the influence of high-energy laser radiation on progress of acute purulent osteomyelitis and detailed characteristic of temperature fields in the place of laser osteoperforation.

The preliminary experiments had allowed us to choose optimal mode and parameters of laser radiation. We preferred the impulse modes because the usage of continuos wave with the power 15 W for perforating of femur diaphysis caused the significant heating of bone and neighboring tissues, thus the compact bone perforation was not fulfilled in some cases. The increase of the exposition time to 30-60 sec gave the same effect, but the bone and soft tissues were heated even more considerably (Fig. 3a). Changing frequency, period of pulses and laser power, we worked out the optimum parameters for laser osteoperforation. The best effect for 0.4 mm diameter osteoperforation gave the power = 10 W and the pulse frequency = 10 Hz for the period of 3-5 sec.

![Fig. 3 Temperature of medium around detectors 1,2,3,4,5,6, in a rabbit medullar channel irradiated by Nd:YAG laser.](image_url)


This parameters have allowed us to form the compact laser channel through all bone layers with the subsequent irradiation of medullar in all cases for a short period of time. The experiment revealed the precise dependence of temperature changes in tissues from the power and the Nd:YAG laser exposition time.
The significant rise of temperature in the medullar channel only in the point of laser osteoperforation and in 2 mm distance from it was displayed with the help of original measuring-computational system thermometry. We had no possibility to measure the temperature at the place of laser beam action, but one could see charring of the tissues on the way of the beam. The maximal temperature of the neighboring tissues on the path of the beam reached 75°C. On 5 mm and more distance from monofiber the temperature rose up to 38-39°C and did not influence essentially on neighboring tissues during the short-term action. The graphic of the temperature curves revealed the certain regularity of temperature change during the laser osteoperforation (Fig. 3b). When the monofiber have been fallen down into the medullar channel the propagation of laser radiation along the channel was registered.

The dynamic osteotonometry did not revealed any significant fluctuations of intraosteal pressure near the monofiber. Postoperative period in the experimental group of animals was without complications and suppuration of the wound. The blood tests on the 7-8 days after infecting of animals has revealed the WBC increasing up to $11 \times 10^6$ and then its reduction up to the normal figures to the 10 day after the laser osteoperforation. Bacteriological study of medullar on the 7 day revealed $10^6-10^7$ microorganisms in 1 cm$^3$, on the 35 day - $10^5$ in experimental group and $10^5$- in control.

Roentgenological study of bone in early terms (on the 3, 7, 14 day of illness) had not revealed changes. Significant periosteal reaction, thickening of periosteum, narrowing of the lumen and unclear outlines of the medullar channel (Fig. 4b) were observed on the control group femur roentgenograms at the time of removing animals from experiment. In the experimental group roentgenograms the periosteal reaction was poorly expressed, the changes of the medullar channel lumen were insignificant (Fig. 4c).

The morphological study on the 9-14 day after animals infecting revealed that in untreated animals the necrotic zone of compact bone occupied a significant area, periosteum was thickened and fibrillared. Between the fibres one can see the compact groups of decaying WBC with some macrophages, histiocytes and thin fibroblasts bundles. In some sights the

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**Fig. 4. Roentgenogramm of rabbit bones.**

*a.* 48 day of disease without treatment: the distinct changes of right femur bone, periosteum and medullar channel.

*b.* 34 day of disease after mechanical osteoperforation: periosteum reaction, moderate osteoporosis. The medullar channel is narrowed.

*c.* 34 day of disease after laser osteoperforation: moderate osteoporosis of right femur bone.
formation of bone tissue out of endosteum and periosteum could be defined. The necrotic centers with hemorrhage or without them could be seen in the medullar. The count of not differentiated hemopoetic cells was moderate. The abrupt paretic widening of sinusoids was registered. The neighboring transversostriate muscular tissue was loosened, fibrillated and infiltrated with decaying segmented leukocytes with some macrophages and mononuclears. There were centers of colliquative necrosis of myocytes (Fig. 5a).

The study of bone sections of rabbits after mechanical osteoperforation revealed, that necrotic centers in bone had smaller sizes in comparison with untreated animals, it was marked loosening and infiltration of it and surrounding tissues with segmented leukocytes and some macrophages and mononuclears. The formation of bone tissue was presented by thin strias of small extent. We could determine the centers of bone destruction extending from the medullar channel up to periosteum in some preparations. Compact bone sequester were found in the nearby soft tissues and in medullar channel. Great number of fatty cells and islands of hemopoetic tissue and some macrophages could be seen in medullar.

![Fig. 5. Microphoto (x56) of rabbit bone.](image)

a. Septic destruction of periosteum.
b. Endosteal reaction in the zone of laser osteoperforation: proliferation of bone tissue in endosteum with osteoblasts and tight fitting of mature granulations from the side of medullar channel.

After the laser osteoperforation the amount of necrotic centers in bone directed from medullar channel up to periosteum was less, than in control group. The walls of the centers were presented by indurative necrosis with polygonal black particles. Periosteum thickening was insignificant and only a few leukocytes were in it and in surrounding soft tissues. The necrosis centers of medullar were not in all preparations and occupied small sites. Paretic widening of sinusoids with blood overfilling was marked. The amount of fatty cells in medullar was moderate, a lot of macrophages, hemopoetic tissue occupied the significant areas. Comparative morphological researches of bone in the distance of 0.5, 1.0 and 1.5 cm from the point of mechanical and laser osteoperforation demonstrated significant difference with the control group where inflammatory changes prevail (Fig. 5b). Quantity and localization of reticular cells, fibroblasts in all cases were similar. Fibroblasts were arranged along the vessels of middle and large size. Reticular cells formed reticular areas, containing fatty and ripening hemopoetic cells.

At study of internal organs of the animals of control group the morphology of septicopyemia was revealed in many cases. There were focuses of serodesquamative bronchopneumonia in lungs. In liver the focal interstitial hepatitis and albuminous dystrophy was revealed in many cases. In brain paretic and plethoric venous and capillary bed, edema of brain substance and pia mater encephali were found out. The acute circulatory disorders prevailed in the myocardium and the foci of cardiomyocytes fragmentation were revealed. The spread of pyemical foci were found out in kidneys regularly. However the pyemical foci in kidneys were marked only in isolated cases and were subjected to reparative changes in the animals after laser osteoperforation. The focal interstitial hepatitis and the serodesquamative bronchopneumonia were observed rarely in this group.
Thus, the new method of surgical treatment of acute purulent osteomyelitis based on the new experimental model is developed. It proposes the decompression and sanation of the medullar with the help of the high-energy laser osteoperforation with Nd: YAG laser.

Comparative clinical and morphological researches revealed that the Nd: YAG laser osteoperforation with radiation power of 10 W in the impulse mode produces minimal traumatization of a bone and soft tissues and has a number of advantages in comparison with mechanical osteoperforation.

The purulent inflammation in a bone is blocked quickly due to local hyperthermia and antibacterial properties of Nd: YAG laser. The number of microorganisms in 1 cm³ of medulla after laser osteoperforation has decreased from $10^{6.7}$ to $10^5$, while after mechanical osteoperforation in the same terms the level of dissemination was more ($10^7$). Vaporization and quick laser decompressive effect in inflammatory focus of medullar channel prevent the dissemination of infection, and strictly local hyperthermia provides safety for surrounding tissues. At the same time the reduction of hyperthermia in some distance from the focus of laser destruction up to 40-41°C apparently promotes the reparative processes and stimulation of osteogenesis. That was confirmed by the minimal signs of exudative and inflammatory reaction in tissues and osteoblasts prevalence in the bone after laser osteoperforation.

The opportunity to sanate the purulent focus in the bone by the way of transcutaneous Nd: YAG laser osteoperforation without significant surgical intervention and wide sections prevents the development of rough cosmetic defects, promotes fast recovery of extremity function and considerably reduces probability of turning of acute purulent osteomyelitis into a chronic form.

The developed method of treatment of purulent osteomyelitis on the basis of local hyperthermia produced with high-energy laser radiation can be recommended for introduction in clinical practice, that will allow us to reduce the terms and costs of treatment, and also to reduce number of failures.

4. CONCLUSIONS


2. The sanative effect of laser osteoperforation is caused by both mechanical decompression of the bone marrow and by local hyperthermia (possible partial vaporisation of pathological tissues and bactericidal effect).

3. Exudative and inflammatory characters of the disease cut short after laser treatment in comparison with mechanical osteoperforation, that reduce the terms of treatment of acute purulent osteomyelitis.

4. Laser radiation and local hyperthermia stimulate the reparative processes in bone tissue and medullar.

5. REFERENCES