Surgery treatment the infected wounds in experiments

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Abstract

Objectives: to invent the method of infected wound simulating with help of laser and to prove the efficiency of joint application of low-intensive laser radiation(LILR) and nanoparticles of copper in experiment.

Materials and methods: 30 white rats (weight=190-200g) were used for experiment of infected wound simulating. 100 similar rats were used for exploration of joint application of LILR and copper nanoparticles during the treatment of simulated infected wound.The expression of antimicrobial influence of laser radiation and copper nanoparticles was rated relative to Staphylococcus aureus according to McFarland Turbidity Standart. In the first series of experiment wound was irradiated by laser machine "Matrix";in the second – there was injection of copper nanoparticles;in the third - joint application of LILR and nanoparticles of copper.

Results: The worked out way with use of high-intensity laser (Lasermed 1001) permits to simulate the wound: in depth and in the area of lesion. And after injection of Staphylococcus aureus it allows to get infection of wound. In experiment in vivo next aspects were registered: low antibacterial activity of : LILR using and expressed bactericidal activity of copper nanoparticles. Experiment also allowed to discover the potentiation of antimicrobial effect during the joint application of copper nanoparticles and the laser. Combined local use of laser radiation and copper nanoparticles to infected wounds of experimental animals provided stopping of pathogenic microflora seeding by the 7th day, rise of granulation by the 4th day and wound epithelization by the 14th day.

Conclusion: New method of wound simulating was invented.Joint application of LILR and copper nanoparticles allows to reach antibacterial effect using less concentration of nanoparticles of copper during surgical treatment of infected

wound. At the same time probable toxic effect on organism descends.The time of total wound epithelization relative to standard methods of treatment also decreases.

Key-words: experiment, wound simulating, copper nanoparticles, low-intensive laser radiation, antimicrobial activity, joint treatment.

I. Intorduction. Use of laser and nanotechnologies is considered to be quite relevant and perspective direction in contemporary experimental medicine [1].Among variety of modern methods of modeling of infected wound the best method is use of high-intensive laser, that allows to simulate the wound of experimental animal: to get exact area and depth of lesion [2].There is a little number of reports about use of laser radiation and nanoparticles of metals in experimental surgery [3].In some researches of native and foreign authors the effectiveness of application of low-intensive laser radiation is high valued in combined treatment of infected wounds [4,5].It is showed that use of low-intensive laser for control of interstitial transport of metal nanoparticles allows to change hydropenetrability of tissue due to formation of canals and pores in 20 times [6], and laser influence boosts the penetration of metal nanoparticles in tissues [7].It is discovered that use of low-intensive laser radiation in treatment of infected wounds provides with antimicrobial effect, stimulates the process of regeneration, improves microcirculation in area of wound [8,9,10].It is proved by morphological examination, that use of low-intensive laser radiation stimulates macrophage reaction, activates biosynthetical function of fibroblasts, optimizes angiogenesis, promotes to faster maturation of granulation tissue and its fibrous transformation, which ends by 7-8th day [11]. In some foreign researchers' opinion, use of low-intensive laser radiation provides with faster wound purification, early formation of granulation, epithelization of wound defects and shortening period of treatment [12,13].

Nanoparticles of copper shows expressive bacteriostatic and bactericidal effect and this effect is prolongated and less toxic in comparison with copper salts [1,9]. In researches in vitro it is stated, that nanoparticles of copper have very expressive antibacterial effect on clinical culture of Staphylococcus aureus [8,14], and suspension of copper nanoparticles in concentration 100mg/ml has the highest antimicrobial influence on Gram-negative microorganisms.Concentration of nanoparticles more than 100mg/ml is toxic and it is potential dangerous for organism [15]. Wound healing effect of copper nanoparticles is proved.In comparison with antibiotics copper nanoparticles don't cause selection of resistant cultures.This conclusion allows to recommend nanoparticles of copper for treatment of infected wounds caused by polyantibiotic resistant cultures [16].

Combined use of nanotechnologies and laser radiation has not found its wide use in experimental medicine.There is a little number of reports about this developments [15,17].In our researches it is proved that combined use of low-intensive laser radiation and nanoparticles of copper provides with fast and effective suppression of pathogenic microorganisms' growth and acceleration of regeneration [1,3].

First results of potentiating effect of low-intensive laser radiation and nanoparticles of copper were reported by V.V.Alipov in Hannover in 2012 [18].This way, experimental justification of metal nanoparticles' efficiency, searching the way of strengthening of its bactericidal effect including in combination with low-intensive laser effect on wound is the actual concept in modern surgery during treatment of infected skin wounds and soft tissues.

II. Objectives: To develop the method of infected wounds' modeling and to validate the efficiency of combined use of low-intensive laser radiation and nanoparticles of copper during surgical treatment of infected wounds in experiments on laboratory rats.

III. Materials and methods. Corporative investigation with participation of four surgical collectives of SSMU were carried out at the Department of operative surgery and topographic anatomy of SSMU named after V.I.Razumovsky. During the investigation there was used European convention for the protection of vertebrate animals used for experimental and other scientific purposes [ETS N123, Strasbourg, 18.03.1986]. Before the beginning of exploration there were received the recommendation and approval from Ethics committee of SSMU named after V.I.Razumovsky (protocol №1 of 5 september 2011). During all manipulations animals were injected using intramuscular anesthesia (Sol.Zoletili 0,5 %) in combination with local injection of Solution of novocaine 0,5%.

IV. Modeling of infected wound. In experiment on 30 white laboratory rats (weight=190-200g) we developed original method of infected wound modeling [2], reached by bringing the adapter of high-intensive laser "Lasermed 1001" made in the form of copper plate with the required configuration and size (4000mm^2) to the chosen area of the skin. For infection of the wound caused by laser, the bottom of the wound was single sprayed by suspension with laboratory culture of Staphylococcus aureus.For that there was made suspension out of daily agar cultures according to McFarland Turbidity Standart in physiological solution NaCl with terminal concentration 3×10^7 cfu/ml and suspension with volume 0,1ml of the suspension.

V. Production of copper nanoparticles. Copper nanoparticles with dispersion=60-80nanometer were produced according to plasmous technology out of large-dispersed powder type.

For production of the copper nanoparticles suspension ether sunflower oil (sterilized) was mixed with 1mg of copper nanoparticles in concentration from 1000microgram/ml to 1microgram/ml.

Oil suspension was being carried on the surface of infected wound in stated portions daily to every animal.

VI. Analysis of low-intensive laser radiation influence. We defined parameters suitable for use of low-intensive laser radiation both isolated and in combination with suspension of copper nanopowder.There was used laser apparatus "Matrix": frequency - 80Hz, power of radiation- 15-30 milliwatt, wavelength – 630-650 nanometer.

At the first stage we examined antibacterial effect of combined use of LILR and copper nanoparticles in experiment in vitro [8]. We used suspension of daily culture of Staphylococcus aureus ($9*10^8$ cfu/ml every) standardized with McFarland Turbidity Standart. Suspension was mixed step-by-step with sterile physiological solution up to concentration $3*10^5$ cfu/ml. In the first series during two minutes there was LILR of microorganisms' culture carrying out in conditions: Frequency – 80Hz, power of radiation- 30 milliwatt, wavelength –0,63 nanometer.

In the second series of experiments suspension of copper nanoparticles on 0,2ml with terminal concentrations 1000, 100, 10 and 1,0 microgram/ml was injected in microorganisms' culture. In the third series there was combined use of LILR and copper nanoparticles. We discovered the potentiating antimicrobial effect of combined use of LILR and copper nanoparticles, that allowed to decrease the concentration of copper nanoparticles in experiment in vivo up to 1 microgram/ml.

VII. Combined use of LILR and copper nanoparticles during surgical treatment of infected wound. After infected wound had been modeled animals were carried out the next treatment during 14 days: LILR on the wound (1 series), injection of the copper nanoparticles' suspension (series 2), combined use of laser and copper nanoparticles (series 3), comparative drug – unguent "Levomicol"(series 4). Control group was presented by animals (series 5) who had only surgical treatment: pus evacuation, extraction of infected gauze tissue paper, removal of necrotic tissue and

wound lavage with antiseptic.Necrosectomy and wound lavage had been done before extensive inflammatory effect appeared. This surgical treatment due to necrotic tissues' removal provided for reduction of microbial contamination and reduction of autolytic enzymes number in wound.

Second phase of of special treatment was started on the 3rd day after surgical stage.On 2nd , 5th, 7th, 10th, 14th days of treatment health of animals was estimated with help of planimetric and microbiological observation of wounds.Qualitative composition of microbial agents and numerical calculation of microorganisms were estimated during bacteriological research. Features of clinical course of wound process were characterized by the nature of inflammatory reaction, condition of wound borders and its bottom, term of wound's purification out of necrotic tissues, term of appearance of granulation tissue, wound epithelization. In 12 days of treatment animals were brought out the experiment, there was the intake and coloring of paraffin slices with Hematoxylin and Eosin and the fragments of tissues out of the center and circumference of the wound with picrofuchsin.

Statistic processing of the results was carried out by variation-statistical method with use of programs «Statistica 6.0» (StatSoftInc., USA) for Windows XP. We used the criterion of Shapiro-Wilka, criterion of Fisher-Snedecor, Mann-Whitney criterion and Vilkokson. Distinctions were considered as statistically equal at $p<0,05$.

VIII. Results. Conducted investigation showed that in modeled method an infected wound formed in interscapular region of animals with all classical features of purulent inflammation.There were perifocal edema and hyperemia of the skin, local hyperthermia and fluctuation. Clinical criteria of wound festering was confirmed by bacteriological sowing of wound content. Before the phase of post-operative treatment the pollution of the wound by Staphylococcus aureus was 4300±512 cfu/ml of wound content. Bacteriological research of content out of animal wounds showed that during

combined treatment(series 3) there was step-by-step linear more expressed reduction of number of microorganisms in the wound in comparison with other groups of observation.

In evaluating of planimetric and morphological results of combined treatment of wounds in series 1-2 it was pointed that wound application by the copper nanoparticles' suspension had more effective medicinal effect than laser radiation of the wound.Thus in case of solitary use of LILR area of modeled infected wound (400mm^2) reduced up to 91±8,0 mm^2 by 14th day; in case of use of nanocopper suspension – up to 72±4 mm^2; in case of treatment by "Levomicol" – up to 75±18 mm^2 ; in case of combined use of LILR and nanocopper suspension – up to 11±14 mm^2. In the group of control there was reduction of wound area only up to 181±13 mm^2.

Evaluation of morphological structure of wound process showed up the following.After 3 days of treatment with use of LILR against a background edema and lymphocytic infiltration new scar with fibroblasts, collagen fibers and vascular loops formed. In the second series of experiments by using copper nanoparticles' suspension there were noted : subepithelial tissue edema, dilatation of vascular loops in other words all features of unstopped inflammation.Similar histological structure was marked in case of "Levomicol" using. By the third day in the region of defect generated attributes of reparative process formed of animals of 3 series: collagen fibers, dilatation and plethora of vessels, leucocytal infiltration.

On the 7th day in all series of experiments there were formation of formed granulation tissue, collagen fibers and anew generated vessels. During laser radiation granulation tissue which was on the border of the wound appeared.

There were regions of fibroblasts, fibrin aggregation of 2nd series animals which suspension of copper nanoparticles was applicated to. There were features of formed granulation tissue and full vascular rete during combined treatment against a background minor tissue edema.In wounds of control group of animals there were

features of remaining inflammation: tissue infiltration, big number of macrophages and neutrophil leucocytes. Purification of wound surface due to granulation tissue forming during local treatment in series of experiments was stated in the next order:use of laser radiation of the wound – 10th day, use of "Levomicol" – 10th day, application of nanocopper suspension – 9th day.The process of regeneration was the most expressive during combined use of LILR and nanocopper suspension – by 6-7th days of treatment.

IX. Discussion. Conducted experiments are evidence that solitary use of LILR has no sufficient effective antibacterial influence.By 14th day of post-operative period during local use of LILR area of modeled infected burning wound reduced only by 75%, and in case of use of copper nanoparticles – by 82%.Bacterial pollution of wound was liquidated by 11th day of laser radiation treatment and by 9th day of use of copper nanoparticles. Partial epithelization of wound was noted in these series only in 13-14 days of treatment.

We ascertained, that during combined use of LILR and nanocopper suspension photochemical reaction with turning on a trigger mechanism of strengthening of antibacterial activity occurred.In the first phase of wound process during use of combined treatment there were lowering of tissue edema, cell compactness of inflammatory infiltration and shortening of regeneration period. In the second phase there were intensification of proliferative processes and acceleration of granulation tissue formation.In the third phase, in case of combination of LILR and nanocopper suspension, there was maximal activity of reparative processes. Important difference of combined treatment is simultaneous influence on proliferation processes of epithelial cells, which are the structure of cicatrizing wound. Described processes of wound regeneration we connect with synergism of antimicrobial effect of LILR and nanocopper due to the absence of microbial pollution of tissues, which was stated on 2,0+0,7 days earlier in comparison with 1,2 and 4th series of experiments. Thus term of

wound granulation and epithelization(features of epithelization were noticed by 10th day of treatment) was shortened at 1,5 times.

X. Resume. As a result of conducted investigation with use of surgical laser «Lazermed 1001» it was discovered the experimental model of the wound controllable in depth and in the area of affection.

Local application of copper nanoparticles transcends the efficiency of laser influence, and in combination with laser irradiation speeds stopping of infectious process in wound on 6 days and wound epithelization, which is noted by 10th day of treatment.

It was experimentally validated the appropriateness of combined use of LILR and copper nanoparticles' suspension during surgical treatment of infected burning wounds, which was vindicated by shortening of term of treatment at 22,0%

References:

1. Nikolenko V.N., Alipov V.V. Perspective nanotechnologies in experimental medicine. Nanotechnics. 2009.- № 19.- P. 66-68.
2. KolsanovA.V.,AlipovV.V.,LebedevM.S,DobrejkinE.A.,LimarevaL.V. Method of simulating of thermal skin burn injury of laboratory animals. Samara State Medical University.Russian Federation
Published: 10.01.2013 Bulletin. № 1.

3. Alipov V.V., DobrejkinE.A., Urusova A.I., Belyaev P.A. Experimental laser nanosurgical technologies.First results and perspectives. Bulletin of experimental and clinical surgery. 2011. - V. 4.- № 2. P. 330-333.
4. Heinitz A.V. Laser therapy of infected wounds. Medicine-1988.- page 150 .

5. Tuchin V. V., Terentyuk, G., S., Maslyakova, G. N., Suleymanova, L. V., Khlebtsov N. G. and Khlebtsov, B. N. Laser-induced tissue hyperthermia mediated by gold nanoparticles: toward cancer phototherapy.V.V.Tuchin.J. Biomed. Optics. 2009 - № 14(2).- P.21016(1-9).

6. Nikiforova T.E. Magnetic guidance of ferromagnetic nanoparticles dispersing in biotissues during laser modification of form. Encyclopedia of Chemical Engineer. 2009.- № 5.- P. 19–22.

7. Omelchenko A.I. optomechanical tests of hydrotized biotissues during laser modification of theirs proportions and forms. Quantum electronics. 2008.- №38(3).- C. 269–272.

8. Alipov V.V. , DobrejkinE.A., Urusova A.I., Belyaev P.A Experimental substantiation of combined use of copper nanoparticles and low-intensive laser radiation during surgical treatment of modeled infected burn wounds of skin. Bulletin of experimental and clinical surgery. 2013. - № 4.- P. 411-417.

9. Babushkina I.V. Metal nanoparticles in treatment of experimental infected wounds. Saratov scientific medical journal. 2011.-№. 3.- C. 530-533.

10. Gadjiev E.A. Morphological features of infected wound healing during traditional method of treatment and its potentiation. Laser medicine. 2009.- № 13.- P.35-39.

11. Gadjiev E.A. Low-intensive laser and pulse-inductive magnet influence – method of potentiation of infected wounds traditional therapy Laser medicine 2009. - №4. - P.21-28.

12. Nishimori H., Kondoh M., Isoda K., Tsunoda S.-I., Tsutsumi Y. and Yagi K. Silica nanoparticles as hepatotoxicants. European Journal of Pharmaceutics and Biopharmaceutics .2009.- №7.- P. 496–501.

13. Tuner J., Hode. L.Laser therapy in dentistry and medicine. J.Tuner. PrimaBooks.-2006.- 236 p .

14. Rahmetova A.A. Wound-cicatrizing effect of copper nanoparticles according to their physicochemical characteristics. Russian nanotechnologies. 2010.- № 3-4.- P.102-107.

15. Bystrzejewska-Piotrowska G., Golimowski J., and Urban P. L. Nanoparticles: Their potential toxicity, waste and environmental management.Waste Management. 2009.-P. 2587–2595.

16. Doronin S.Y., Alipov V.V. S ynthesis and bactericidal properties of superdispersed powder of copper. Proceedings of Saratov University.New series.Series: Chemistry.Biology.Ecology. 2011. - V.11. - № 1.- P. 18-22.

17. Terentyuk G.S., Maksimova I.L., Tuchin V.V., Zharov V.P.,, Bogatyrev V.A., Dykman L.A. N. G. Application of gold nanoparticles to x-ray diagnostics and phototermal therapy of cancer.Saratov Fall Meeting: ConherentOptics of Ordered and Random Media VII Ed. D.A. 2007.-P. 6536- 6537.

18. Alipov V.V. Lazer nanotechnology in experimetal surgery. International Kongress « EuroMedica 2012».- Hannover. 2012-P.-22-23.