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The Effective Use of Programmed Barbotage Method for Wound Sanitation in Experiments.

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ABSTRACT

Treatment of wounds remains an urgent multidisciplinary problem of surgery, requiring new highly effective approaches, including those aimed at reducing the risks of developing infectious and cosmetic defects. The aim of the study was to develop a method of programmed software barbotage sanitation (PBS) for soft tissue wounds and to study the effectiveness of its application in an experiment. Studies were performed on 192 white rats in 2 blocks of the study, in which the effects of PBS on the course of the wound process in aseptic and purulent wounds, respectively, were studied. In the control groups, aseptic dressing were used to treat wounds. In the experimental groups, an additional 0.9% PBS of sodium chloride solution was applied to the wounds for 3 minutes. The PBS method was carried out using a special device, whose operation is based on combined application of gas and hydrodynamic effects realized by passing through the solution of gas bubbles. These gas bubbles, when in contact with the wound surface, improve the quality of sanitation and improve blood circulation. Clinical, metrical, histological and histochemical methods were used to assess the course of the wound process. The use of the PBS method in aseptic wounds allowed to accelerate the reduction of edema and hyperemia by more than 20%, to shorten the time of exudation by 50% compared to the control group. In the treatment of purulent wounds, the PBS method made it possible to accelerate the change in the hydration and dehydration phases by an average of 1.5-2 times.

Keywords: programmed barbotage sanitation (PBS), wounds of soft tissues.

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RELEVANCE

Complex treatment of the wound process continues to be one of the urgent problems of surgery, which is confirmed by the high frequency of occurrence and the absence of a tendency to reduce this pathology (1, 2, 4, 5). Comorbital pathology, immunosuppressive states, antibiotic resistance and other causes lead to a delay in repair, suppuration of wounds, reduce of the efficacy of treatment, increase the cost of treatment, and lead to cosmetic and functional defects (5, 6). In the complex treatment of wounds, modern methods based on the use of hydropressive, ultrasound, cryogenic, laser and other technologies have shown high efficiency (3, 7). But in many works there has been an emphasis of the importance of searching for new means and methods of stimulating reparative processes, eliminating wound infection taking into account the current level of development of science and technology (7).

The aim of the study was to develop a method of software barbotage sanitation (PBS) for treating soft tissue wounds and to study the effectiveness of its application in an experiment.

Experimental studies were carried out in 2 blocks of research on 192 mature male white rats. Their choice was based on their susceptibility to modeling of the wound process and easy of handling (Table 1).

Table 1: Experimental groups of the first and second blocks of the study

Groups	№ of animals	Characteristics of groups
First block of study		
Control	48	Intact animals
Experimental	48	PBS method once a day for 3 minutes.
Second block of study		
Control	48	Change of aseptic dressings 2 times a day
Experimental	48	PBS method 1 time per day for 3 minutes. Change of aseptic dressings 2 times a day.

The first block of research was aimed at studying the influence of PBS on the course of the wound process in aseptic wounds and included two groups of 46 laboratory animals: control and experimental. Simulation of the aseptic wound was performed under anesthesia on a previously shaved area of the body (the outer surface of the middle third of the thigh), where a linear cut of the skin, subcutaneous tissue, fascia, muscles 1 cm long was made. The soft tissues were hooked, edges and bottom of the wound were pressed by a surgical clamp. The treatment was started immediately after hemostasis. In the control group, the aseptic dressings were applied and replaced 2 times a day with intervals of 8-10 hours. In the experimental group, apart from the aseptic dressings, PBS session was performed for 3 minutes in a 0.9% solution of sodium chloride during the morning dressing.

The second block of studies was aimed at studying the effectiveness of PBS system in the treatment of purulent wounds; two groups of 46 animals were included: control and experimental. Modeling of purulent wounds was performed after the production of the aseptic wound process according to the scheme described above. A wound gauze weighing about 0.5 grams with daily culture of St. Aureus was added to the wound in a dose of 1010 microbial bodies in 1 ml of 0.9% sodium chloride solution. 1-2 adaptation sutures of silk thread were punctured on the skin. On the third day after initiating the infection, the edges of the wound were cut with the removal of the gauze tampon. By the third day, a purulent wound with an average size of 1.0 x 0.5 cm was formed. The treatment was started on the 3rd day after modeling the wound with the evacuation of pus and rinsing of the wound. After that, medical measures were done in accordance with the plan of experiments. In the control group, the treatment consisted of changing dressings twice a day at intervals of 8-10 hours. An aqueous solution of chlorhexidine bigluconate 0.05% was used. In the experimental group, during the morning dressing, a PBS session was performed for 3 minutes in a 0.9% solution of sodium chloride. Antibiotic therapy and general treatment in the study groups were not prescribed.лась.

The PBS system was carried out with the help of a special device developed at the Department of General Surgery of the State Medical University in Voronezh, Russia VSMU. N.N. Burdenko of the Ministry of Health of the Russian Federation. The work pf the special devise was based on combined application of gas

and hydrodynamic influences, by passing gas bubbles through the solution, which, when in contact with the wound surface, makes it possible to strengthen the mechanical separation of dense and viscous necrotic masses and improve blood circulation.

Experimental studies were carried out in strict accordance with the existing ethical norms, the principles set forth in the European Convention for the Protection of Vertebrates used for experiments or for other scientific purposes (Strasbourg, France, 18.03.1986), and also the Order of the Ministry of Health of the Russian Federation of 19.06 .2003 №267 "On approval of the Rules of laboratory practice". The statistical processing was carried out with the help of variation statistical methods, Student's criteria (the difference was considered reliable for p≤0,05), Wilcoxon and Mann-Whitney (for comparing of unrelated samples), Spearman's analysis (for evaluating of the relationship between the signs), the chi –square (for verifying the hypothesis).

METHODS OF RESEARCH

To assess the course of the wound process, we used the following methods: clinical methods (the nature of the inflammatory reaction, the timing of purification and the appearance of granulations, the onset of epithelization, wound closure, the nature of the granulation tissue, the measurement of wound area and the general state of the animals), planimetric method, histological and histochemical methods. In order to study the dynamics of morphological changes on the 1st, 3rd, 5th and 7th days from the beginning of the treatment, an incision of a single block of tissues was made; it contained all the structural elements in the center. For histological assessment, the material was fixed in a 10% solution of neutral formalin; it was also fixed to an automatic histogram AT-4M to remove excess fluid. The resulting samples were primed with Hystomix solution. Then paraffin sections with a thickness of 6 µm were prepared, which were later stained with hematoxylin-eosin and enclosed in Biomount medium. To carry out histochemical studies, the material was frozen in petroleum ether, and then cooled with liquid nitrogen. The sections, of 12 µm thickness, obtained in a cryostat at -20 ° C, were placed for 3 minutes in a cooled mixture of acetone and chloroform (1: 1) at + 4 ° C for the extraction of lipids. The activity of alkaline phosphatase was detected with the help of £ - naphthyl phosphate and the diazonium-strong blue PP salt. Histochemical preparations were encapsulated in glycerol-gelatin and stored in the dark. A qualitative assessment of the histochemical reaction took into account the nature of the distribution of precipitated residue. The number of structures active in AFP was determined by a stereometric method; we used an increase in the lens of x40 and eyepiece x7 using an ocular mesh.

Results obtained in the control and experimental groups of the 1-st block of research.

On the 1st day, palpation of the wound area caused the great anxiety in the animals of the control and experimental groups; we noted the appearance of edema and a scant serous discharge. In the experimental group, there was an insignificant tendency in the decrease the inflammatory reaction, which was proven by a decrease in edema and hyperemia.

On day 3, palpation in the projection of the wounds of the animals did not cause any significant anxiety. In the experimental group, there were no signs of inflammation, in the control group, we noted an insignificant serous discharge.

Table 1: Objective signs of wound process progression in the experimental and control groups, per day

Groups of the research	Characteristics of the groups in research	Objective signs		
		Edema	Hyperemia	Exudate[1]
Control	Intact animals	2,2±0,3	1,8±0,6	2,7±0,4
Experimental	Receiving PBS method	1,8±0,3	1,5±0,4	1,8±0,3*

[1] – decrease in the amount of exudate to a scant amount; * - authenticity of different symptoms in comparison with the control group p<0, 05.

By the end of the 3rd day from the moment of modeling the aseptic wound in the animals of both groups, there were no signs of edema and hyperemia, the healing took place under the strip of the scab. In the

experimental group, the edema stopped on the 1.8 ± 0.3 day, hyperemia of the skin - on the 1.5 ± 0.4 day, the amount of exudate decreased to a scant level on the 1.8 ± 0.3 day ($p < 0.05$). In the control group, the studied parameters were as follows: 2.2 ± 0.3, 1.8 ± 0.6 and 2.7 ± 0.4 days respectively (Table 1).

We also studied the dynamics of changes in wound area during the healing process (Table 2).

Table 2: Dynamics of the changing area of the wounds of animals in the experimental and control groups, mm²

Groups of the research	Characteristics of the groups in research	Timing after remodeling the wounds			
		On the moment	Day 1	Day 3	Day 7
Control	Intact animals	26,3±0,5	19,5±0,3[1]	8,8±0,4[1]	3,2±0,2[1]
Experimental	Receiving PBS method	25,9±0,4	13,3±0,5[1,2]	6,2±0,5[1,2]	1,8±0,4[1,2]

[1] - authenticity of the differences in comparison to the first day,
[2] - authenticity of differences in comparison with the control group.

In the control group, the area of wounds on the 1st day was 19.5 ± 0.3 mm², on the 3rd day - 8.8 ± 0.4 mm², on the 7th day - 3.2 ± 0.2 mm². In the experimental group, the studied parameters were as follows: 13.3 ± 0.5, 6.2 ± 0.5 and 1.75 ± 0.38 mm², respectively. By day 11, it was not possible to measure the area of wounds; in all animals, wound closure was observed with scar formation. On day 1 wound area reduction in the control group was 25,7 ± 1,7%, while in experimental - 48,6 ± 2,4%; from the 1st to 3rd day - 27.6 ± 0.4 and 26.8% ± 0.39%; from the 3rd to 7th day - 15.8% ± 0.4, and 17.9 ± 0.3% a day, respectively.

Table 3: Dynamics of changes in the area of the wound in the experimental and control groups,% per day

Groups of the research	Characteristics of the groups in research	Timing after remodeling the wounds		
		1st day	Day 1-3	Day 3-7
Control	Intact animals	25,7±1,7	27,6±0,4	15,8±0,4[1]
Experimental	Receiving PBS method	48,6±2,4[2]	26,8±0,4[1]	17,9±0,3[1, 2]

[1] - authenticity of the differences in comparison to the first day,
[2] - authenticity of differences in comparison with the control group.

The results of histological studies in the control and experimental groups of the 1 st block of the study showed that in the control group samples, by the 7th day, there was a diverse picture: along with individual cases of the healing process, a weaker, inconsistent development of granulation tissue was observed in most of the samples; the inflammatory infiltration persisted both at the edges of the wound and in the thickness of the granulations. In the samples of the experimental group, the wound defect was filled with a more uniform granulation of the tissue to the level of the papillary dermis layer; there were no signs of inflammation, epithelization happened on time.

The data obtained in the analysis of the results of objective and planimetric methods in the first block of the study speak about the safety and effectiveness of the application of programmed barbotage sanitation (PBS) in the treatment of aseptic wounds of soft tissues in an experiment. The use of the PBS method made it possible to accelerate the reduction of edema and hyperemia by more than 20%, to shorten the exudation time by 50% in comparison with the control group.

Results obtained in the control and experimental groups of the 2nd block of research.

On the 1st day, palpation in the projection of the wound, in the animals of both groups, caused high level of anxiety of the animals; there was paravual edema and hyperemia. In the experimental group, there was a slight decrease in the inflammatory reaction. On the 2nd day, the animals in the experimental group became more active and by the 3rd day practically did not differ from healthy ones; palpation in the projection of the wound did not cause any significant concern; in some animals a small amount of serous exudate was

noted. In the control group, the normalization of the general condition of the animals was noted on the 5th-6th day from the beginning of treatment. In the control group, necrolysis appeared on the 2.1 ± 0.2 days, hyperemia - 2.5 ± 0.2 days, edema - 2.4 ± 0.2 days, appearance of granulations - 2.2 ± 0.3 days. In the experimental group, the studied parameters were as follows: 1.4 ± 0.2, 1.7 ± 0.2, 2.0 ± 0.2 and 1.5 ± 0.2 days, respectively (Table 4).

Table 4: Objective signs of wound process progression in experimental and control groups of the study, per day.

Groups of the research	Characteristics of groups	Necrolysis	Hyperemia	Edema	Appearance of granulations
Control	Wound dressings	2,1±0,2	2,5±0,2	2,4±0,2	2,2±0,3
Experimental	PBS + wound dressing	1,4±0,2	1,7±0,2	2,0±0,2*	1,5±0,2

* - authenticity of different symptoms in comparison with the 1st control group p<0,05.

The duration of fibrinolysis in the control group was 3.4 ± 0.3, the beginning of epithelialization was 3.5 ± 0.4, the reduction of discharge to minimum level was 4.3 ± 0.4 days. In the experimental group, the studied parameters were as follows: 2.6 ± 0.2, 3.0 ± 0.3 and 3.2 ± 0.3 days, respectively (Table 5).

Table 5: Objective signs of wound process progression in experimental and control groups, per day

Groups of the research	Characteristics of groups	Fibrinolysis	Beginning of epithelization	Amount of discharge
Control	Wound dressings	3,4±0,3	3,5±0,4	4,3±0,4
Experimental	PBS + wound dressing	2,6±0,2*	3,0±0,3	3,2±0,3*

* – authenticity of different symptoms in comparison with the 1st control group p<0,05.

When studying the dynamics of bacteriological seeding, the following results were obtained (Table 6). On the 1st day, the level of microbial bodies in exudate, taken before PBS was 109-1010. This was followed by a decrease by day 3 to 104-105, by day 5 - to 103-105, by day 7 - to 103-104 Microbial bodies per gram of tissue in the control group.

Table 6: Dynamics of bacteriological contamination in the control and experimental groups of the study, microbial bodies per gram of tissue

Groups of the research	Characteristics of the groups	Bacterial seeding			
		Day 1	Day 3	Day 5	Day 7
Control	Wound dressing	10^9-10^{10}	10^4-10^5	10^3-10^5	10^3-10^4
Experimental	PBS + wound dressing	10^9-10^{10}	10^3-10^4	10^2-10^3	10^2-10^3

In the experimental group, at the indicated times, the number of microbial bodies per gram of tissue was 109-1010, 102-103, 102-103, and 102-103, respectively. The decrease in microbial contamination of up to 102-103 occurred 40% faster. The area of wounds in the control group on the 1st day was 19.4 ± 0.4 mm², for the 3rd day - 13.4 ± 0.4, on the 5th day - 10.2 ± 0.4, on the 7th day - 7, 7 ± 0,4; in the experimental group - 25,9 ± 0,5, 17,3 ± 0,5, 10,4 ± 0,3, 7,0 ± 0,3 and 4,7 ± 0,4 mm², respectively. On the 11th day, a scar was formed in all groups (Table 7).

Table 7: Dynamics of changes in the area of the wound surface in the control and experimental groups of the study, mm²

Groups of the research	Characteristic of the groups	Area of wounds after wound remodeling, mm²				
		immediately	Day 1	Day 3	Day 5	Day 7
Control	Wound dressing	26,2±0,5	19,4±0,4*	13,4±0,4*	10,2±0,4*	7,7±0,4*
Experimental	PBS + wound dressing	25,9±0,5	17,3±0,5*	10,4±0,3*	7,0±0,3*	4,7±0,4*

* - reliability of differences in comparison with the initial size of the wound in the group, p < 0,05.

When studying the dynamics of closure of a wound defect, we obtained the following results (Table 8).

Table 8: Dynamics of changes in the area of the wound surface in the control and experimental groups of the study, %

Groups of the research	Characteristic of the groups	Percentage of wound closure,% of initial size			
		Day 1	Day 3	Day 5	Day 7
Control	Wound dressing	26,0±0,3	48,9±0,5[1]	61,1±0,6[1]	70,6±0,5[1]
Experimental	PBS + wound dressing	32,9±0,4	59,7±0,6[1]	72,9±0,8[1]	81,8±0,5[1]

[1] - reliability of the differences in comparison with the data of 1 day, $p<0,05$

On the 1st day in the control group, the wound defect decreased by 26.0 ± 0.3%, on the third day - by 48.9 ± 0.5, on the 5th day - by 61.1 ± 0.6, on 7th day - by 70.6 ± 0.5%. In the experimental group, within the indicated periods, the studied parameters decreased in the following manner: 32.9 ± 0.4, 59.7 ± 0.6, 72.9 ± 0.8, 81.8 ± 0.5%, respectively.

In a comparative analysis of histological samples of control and experimental groups, it can be concluded that while in the control group the lengthening of the terms of the inflammatory phase of wound healing, the prolonged preservation of necrosis foci in the muscle layer and the uneven development and maturation of the granulation tissue, the use of programmed barbotage sanitation led to a more rapid and qualitative cleaning of the wound from microorganisms and products of tissue disintegration; it also led to the uniform development of complete granulation tissue and earlier enclosure of the wound defect.

The analysis of the results, of objective and planimetric study methods in the second block of the study, made it possible to establish that the change in the phases of the course of the wound process from hydration to dehydration in the experimental group was observed on the average by the 2nd day, while in the control group - 4th day.

CONCLUSIONS

- The conducted experimental studies showed the safety and effectiveness of the application of programmed barbotage sanitation (PBS) in the treatment of aseptic wounds of soft tissues.
- The use of the PBS method in aseptic wounds allowed to accelerate the reduction of edema and hyperemia by more than 20%, to shorten the exudation time by 50% in comparison to the control group. In the treatment of purulent wounds, the PBS method made it possible to accelerate the change in the hydration and dehydration phases by an average of 1.5-2 times.
- In the study of histoarchitectonics of soft tissues in the control group, the lengthening of the terms of the inflammatory phase of wound healing, the prolonged preservation of necrosis foci in the muscle layer, and the uneven development and maturation of the granulation tissue were noted. The use of barbotage sanitation led to a faster and qualitative purification of wounds from microorganisms and products of tissue disintegration, uniform development of full granulation tissue and earlier closure of the wound defect.

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