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The Use of Vacuum-Dried Maral Blood in the Treatment of Purulent Wounds

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ABSTRACT

INTRODUCTION: Purulent wounds of soft tissues remain one of the main problems of modern surgery. More than 30% of patients in surgical hospitals and over 60–70% of primary requests for surgical care are patients with infectious complications of wounds. The use of various inexpensive biogenic growth factors, such as maral preparations, in particular vacuum-dried maral blood (VDMB), seems promising for potentiating reparative processes.

AIM: To study the effectiveness of using VDMB in treatment of purulent wounds in experiment.

MATERIALS AND METHODS: *Wistar* rats (n=90) standardized by sex, weight and age, were divided into 3 groups: group 1 (control) — no treatment, group 2 (control) — daily dressings using 0.01% benzyldimethyl-myristoylamino-propylammonium (BMP) solution, group 3 (experimental) — similar dressings supplemented with application of VDMB. Purulent wounds were modeled in rats with subsequent assessment of hyperemia, soft tissue edema in the defect area, type and amount of discharge, appearance of epithelialization and granulation, surface cleansing (fibrinolysis, necrolysis), wound area, histological and histochemical analysis of the skin dermis.

RESULTS: Utilization of VDMB in combination with 0.01% BMP solution resulted in reduction in the time of stopping local inflammatory reactions. In group 1 the average wound area on day 7 was (34.4±4.8) mm^2, in group 2 — (29.3±4.6) mm^2, and in group 3 — (20.7±4.7) mm^2. In group 3 reduction of microbial contamination on day 3 to 10^2–10^3 microbial bodies per 1 ml of exudate was recorded, versus 10^5–10^8 in the control groups. The morphological picture of the reparative processes indicated a more complete restoration of tissue histoarchitecture when using complex treatment in the experimental group, early potentiation of remodeling processes and activation of cellular elements.

CONCLUSION: The use of VDMB in the complex treatment of purulent wounds of soft tissues permitted to reduce the time of stopping local inflammatory reactions, accelerate the reduction of the wound area, decrease the activity of growth of bacterial microflora in the wound. There was also demonstrated a positive dynamic of cellular elements and connective tissue fibers, which evidences a more complete restoration of the dermis when using the proposed treatment method.

Keywords: wounds; purulent wounds; maral blood; growth factors; purulent wound treatment; wound process.

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Применение крови марала вакуумной сушки в лечении гнойных ран

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АННОТАЦИЯ

Введение. Гнойные раны мягких тканей остаются одной из главных проблем современной хирургии. Более 30% пациентов хирургических стационаров и свыше 60–70% первичных обращений за хирургической помощью составляют пациенты с инфекционными осложнениями ран. Перспективным для потенцирования репаративных процессов представляется применение различных биогенных факторов роста, имеющих невысокую стоимость, таких как препараты марала, в частности кровь марала вакуумной сушки (КМВС).

Цель. Изучить эффективность применения КМВС в лечении гнойных ран в эксперименте.

Материалы и методы. Крысы линии *Wistar* (n=90), стандартизированные по полу, весу и возрасту, были разделены на 3 группы: 1-я (контрольная) — без лечения, 2-я (контрольная) — ежедневные перевязки с использованием 0,01% раствора бензилдиметил-миристоиламино-пропиламмония (БМП), 3-я (опытная) — аналогичные перевязки были дополнены нанесением КМВС. Проведено моделирование гнойных ран у крыс с последующей оценкой гиперемии, отечности мягких тканей в зоне дефекта, характера и количества отделяемого, появление эпителизации и грануляций, очищение поверхности (фибринолиз, некролиз), площади раны, гистологического и гистохимического анализа дермы кожи.

Результаты. При применении КМВС в сочетании с 0,01% раствором БМП отмечалось снижение сроков купирования местных воспалительных реакций. В 1-й группе средняя площадь раны на 7 сут составила (34,4±4,8) мм^2, во 2-й — (29,3±4,6) мм^2, в 3-й — (20,7±4,7) мм^2. Снижение микробной обсемененности зарегистрировано в 3-й группе на 3 сут исследования до 10^2–10^3 микробных тел на миллилитр экссудата против 10^5–10^8 в контрольных группах. Морфологическая картина репаративных процессов свидетельствовала о более полном восстановлении гистоархитектоники тканей при применении комплексного лечения в опытной группе, раннем потенцировании процессов ремоделирования и активации клеточных элементов.

Заключение. Применение КМВС в комплексном лечении гнойных ран мягких тканей позволило сократить сроки купирования местных воспалительных реакций, ускорить сокращение площади раны, снизить активность роста бактериальной микрофлоры в ране. Также продемонстрирована положительная динамика клеточных элементов и волокон соединительной ткани, что свидетельствует о более полноценном восстановлении дермы при использовании предложенного метода лечения.

Ключевые слова: раны; гнойные раны; кровь марала; факторы роста; лечение гнойных ран; раневой процесс.

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INTRODUCTION

Purulent wounds of soft tissues remain one of the main problems of the modern surgery [1–3]. More than 30.0% of patients of surgical hospitals and more than 60.0–70.0% of requests for primary surgical care are cases of infectious complications of wounds. According to prognosis, these complications will cause more than 10 million annul deaths, among other things, due to the growth of antibiotic-resistant microorganisms [4]. Treatment of infectious complications is also associated with a high incidence of disability and mortality and with high cost [5, 6]. To solve these problems, various methods of treating purulent wounds, wound dressings, medical drugs and antibiotics [7–10], biologically active drugs, for example, platelet-enriched autoplasma [11–13], are being actively developed.

The use of various biogenic growth factors, which have a low cost, seems promising for potentiating reparative processes [14]. Thus, preparations of maral, in particular, vacuum-dried maral blood (VDMB), are noted by a number of authors as a medicinal raw material with proven high effectiveness [15–17]. The concentration of various growth factors in them, such as IGF-I, IGF-II, TGF-β, EGF, group B, PP vitamins, amino acids, testosterone, growth hormone, makes these medicinal substances a potential stimulator of reparative processes in soft tissue injuries [18, 19]. However, the complexity and multifactorial nature of their composition do not allow a confident prediction of the extent of their effectiveness in reparative processes in various tissues.

The **aim** of this study to effectiveness of use of vacuum-dried maral blood in the treatment of purulent wounds in experiment.

MATERIALS AND METHODS

The experimental study was conducted from December 2022 to October 2023 of the Research Institute of Experimental Biology and Medicine (Voronezh). The study was conducted in strict accordance with the requirements of the Federal Law of the Russian Federation of May 14, 1993 No. 4979-1 'On Veterinary Medicine' (as amended on July 02, 2021), Directive 2010/63/EU of the European Parliament and of the Council of the European Union 'On the Protection of Animals Used for Scientific Purposes', GOST No. 33216-2014 'Guidelines for the Maintenance and Care of Laboratory Animals, Rules for the Maintenance and Care of Laboratory Rodents and Rabbits', and is based on the approval of the Study Protocol by the Local Ethics Committee of the N.N. Burdenko Voronezh State Medical University (Protocol No. 7 of December 13, 2022).

The study was conducted on 90 *Wistar* rats standardized by gender, weight and age, and randomized into three groups:

- in ***control group 1*** (n=30) — no treatment of wounds was carried out;
- in ***control group 2*** (n=30) — daily dressings were carried out with 0.01% benzyldimethyl-myristoylamino-propylammonium (BMP) solution,
- in ***experimental group*** (n=30) — daily dressings were carried out with 0.01% BMP plus application of VDMB at a dose of 0.7 g/cm2 (particle size 100–150 μm).

The ***purulent wound was modeled*** according to the following scheme. In the first stage, under inhalation narcosis, (isoflurane, induction dose — 3.0–5.0%, maintenance dose — 1.5–3.0%), the rear surface of the neck was shaved at first with a trimmer, then with a disposable shaving stick, the prepared surgical field was twice treated with 0.01% BMP solution [20]. In the second stage, using a plastic round template 1.3 cm in diameter, soft tissues and superficial fascia were excised with a scalpel. For contamination, *S. aureus* culture (1 ml, 10^9 microbial bodies) was applied on a cotton-gauze tampon and placed in the zone of wound defect. Then, 3–4 interrupted sutures were applied to the edges of the wound until the defect was completely closed. On day 2, pronounced hyperemia and edema in the wound area, and the appearance of tissue discharge were noted. On day 3, the sutures were removed, significant purulent exudation was detected, the wound surface was washed with 0.01% solution of BMP, non-viable tissues were removed and treatment was started. After modeling, all animals were kept in individual cages with the same nutrition and care conditions.

Animals were withdrawn from the experiment and biological material was collected for histological and histochemical examination on days 1, 3, 5, 7 and 14 after modeling.

Hyperemia, edema of soft tissues in the zone of defect, the kind and amount of discharge, appearance of epithelialization and granulation, surface cleansing (fibrinolysis, necrolysis) were visually assessed daily. Planimetric examinations with the calculation of the wound surface areas were performed using WoundVision personal computer software (IIMEDSCAN, Russia) and a RealSeance D415 camera (Intel, China). Morphological analysis of the material of the rat skin dermis was conducted with hematoxylin and eosin staining, Giemsa staining, impregnation with silver and toluidine blue. The dynamics of mast cells, collagen formation processes, and inflammatory infiltrate areas were also assessed.

Statistical processing of the obtained data was performed using the Statistica 10.0 (Stat Soft Inc., USA) and Excel 2010 (Microsoft, USA) software packages. The normality of distribution was checked and the following

descriptive statistics methods were used: calculation of the mean (M) of the obtained results, standard deviation (SD) within the study groups. To determine the reliability of differences, Student's t-test was used to evaluate indicators with a normal distribution, Mann–Whitney — indicators whose distribution did not correspond to the normal, Wilcoxon t-test — for dependent samples in case of non-compliance with the normal distribution. The significance level was taken as 5.0% (p <0.05).

RESULTS

The average time of relief of local inflammation signs in treatment of purulent wounds is given in Table 1, and the dynamics of reduction of the wound area is given in Table 2.

After modeling a purulent wound and removing the sutures, the microbial contamination was on average 10^{10}–10^{13} of microbial bodies per ml of exudate (Table 3). In the experimental group, a marked decrease in this parameter was noted on day 3 of the study to 10^{2}–10^{3} microbial bodies per ml of exudate, which indicates suppression of bacterial growth in the wound canal when using a combination of VDMB and 0.01% BMP solution.

In morphological analysis on day 3 of the study, in control groups 1 and 2, a pronounced inflammatory reaction, accumulation of neutrophilic-lymphocytic cells, mainly segmented and band neutrophils, and formation of microthrombi in the parietal vessels were determined (Table 4). In the experimental group, small foci of formed granulations, single fibroblasts and macrophages were visualized, indicating the onset of regeneration processes.

On day 5 of the study, the formation of epithelium at the wound edges was recorded in the experimental group. In control group 1 without treatment, inflammatory infiltrates were visualized not only in the tissue, but also in the walls of newly formed vessels, which may indicate the secondary bacterial dissemination due to untimely rejection of the scab. Also, among the key participants in fibrillogenesis and tissue remodeling after injury are mast cells, the growth of which was noted in the experimental group of the study (Table 5).

On day 7, with the use of a combined staining method toluidine blue and silver impregnation allowing for assessment of the remodeling process in the dermis structure in the experimental group using VDMB, formed collagen fibers were visualized coinciding with the morphological architectonics of the surrounding dermis outside the wound. In the control groups, the different thickness of the fibers and their chaotic arrangement was noticeable (Tables 4, 5). In the micropreparations of control group 1, there were single inflammatory infiltrates, mainly consisting of lymphocytes and macrophages (Table 4).

On day 14, in the experimental group, a complete epithelial layer was formed after the rejection of the scab, and in the dermis, there was a large number of hair follicles in the mature anagen phase, and newly formed sebaceous glands.

Table 1. Time (M±SD) of relief of local inflammation signs in the control and experimental study groups, days

Wound process symptoms	Study group			p
	1	2	3	
Necrolysis	4.3±0.2	3.9±0.2	3.6±0.2	p_{1-2}=0.075 **p_{1-3}=0.032** **p_{2-3}=0.041**
Skin hyperemia	4.7±0.3	4.5±0.2	3.8±0.2	p_{1-2}=0.064 **p_{1-3}=0.021** **p_{2-3}=0.038**
Edema	4.5±0.2	4.4±0.2	3.8±0.2	p_{1-2}=0.072 **p_{1-3}=0.043** **p_{2-3}=0.037**
Fibrinolysis	5.5±0.2	5.2±0.2	4.2±0.3	p_{1-2}=0.078 **p_{1-3}=0.029** **p_{2-3}=0.031**
Appearance of granulations	4.2±0.2	3.9±0.2	3.1±0.2	p_{1-2}=0.066 **p_{1-3}=0.019** **p_{2-3}=0.032**
Start of epithelialization	5.1±0.3	4.8±0.2	4.2±0.2	p_{1-2}=0.061 **p_{1-3}=0.024** **p_{2-3}=0.039**
Reduction of discharge to scanty	6.8±0.2	5.7±0.3	4.5±0.3	p_{1-2}=0.042 **p_{1-3}=0.014** **p_{2-3}=0.027**

Table 2. Dynamics of reduction (M±SD) of the wound area in control and experimental study groups, mm^2

Timepoint of study	Wound area after modeling			*p*
	1	2	3	
Day 1	93.7±6.1	88.4±5.9	81.2±5.8	p_{1-2}=0.057 **p_{1-3}=0.043** p_{2-3}=0.052
Day 3	54.2±6.3	53.5±5.4	45.7±5.6	p_{1-2}=0.073 **p_{1-3}=0.037** **p_{2-3}=0.046**
Day 5	47.3±5.6	41.5±5.1	29.5±5.2	p_{1-2}=0.053 **p_{1-3}=0.018** **p_{2-3}=0.024**
Day 7	34.4±4.8	27.3±4.6	20.7±4.7	**p_{1-2}=0.043** **p_{1-3}=0.014** **p_{2-3}=0.044**
Day 14	13.1±1.3	9.8±1.5	2.2±1.2	**p_{1-2}=0.035** **p_{1-3}=0.006** **p_{2-3}=0.012**

Table 3. Dynamics of bacterial contamination (M±SD) of wound surface in the control and main study groups, microbial bodies per milliliter of exudate

Timepoint of study	Study group			*p*
	1	2	3	
Day 1	10^9–10^{10}	10^9–10^{10}	10^8–10^{10}	p_{1-2}=0.087 **p_{1-3}=0.047** p_{2-3}=0.059
Day 3	10^5–10^8	10^5–10^6	10^2–10^3	p_{1-2}=0.063 **p_{1-3}=0.025** **p_{2-3}=0.039**
Day 5	10^5–10^7	10^4–10^5	10^2–10^3	**p_{1-2}=0.038** **p_{1-3}=0.021** **p_{2-3}=0.033**
Day 7	10^4–10^5	10^3–10^4	10^1–10^2	**p_{1-2}=0.044** **p_{1-3}=0.013** **p_{2-3}=0.019**
Day 14	10^4–10^6	10^1–10^2	10^1–10^2	**p_{1-2}=0.021** **p_{1-3}=0.010** p_{2-3}=0.086

Table 4. Dynamics of number (M±SD) of cells of inflammatory infiltrate and connective tissue fibers in the zone of wound defect in the control and experimental study groups

Timepoint of study	Клеточные элементы	Группа исследования			*p*
		1	2	3	
Day 3	Inflammatory infiltrate cells, U/mm^2	194.0±7.7	158.0±4.3	131.0±5.11	**p_{1-2}=0.036** **p_{1-3}=0.022** **p_{2-3}=0.027**
	Connective tissue fibers > 1 μm, %	8.3±0.2	9.8±0.4	14.2±0.2	**p_{1-2}=0.024** **p_{1-3}=0.018** **p_{2-3}=0.015**

Continuation of the table

Day 5	Inflammatory infiltrate cells, U/mm^2	147.0±9.5	102.0±7.3	84.0±11.4	p_{1-2}=0.023 p_{1-3}=0.016 p_{2-3}=0.028
	Connective tissue fibers > 1 μm, %	10.1±0.2	19.7±0.4	27.4±0.5	p_{1-2}=0.022 p_{1-3}=0.012 p_{2-3}=0.021
Day 7	Inflammatory infiltrate cells, U/mm^2	93.0±6.9	85.0±9.2	51.0±3.7	p_{1-2}=0.041 p_{1-3}=0.014 p_{2-3}=0.019
	Connective tissue fibers > 1 μm, %	15.5±0.2	31.2±0.3	39.7±0.3	p_{1-2}=0.020 p_{1-3}=0.009 p_{2-3}=0.037
Day 14	Inflammatory infiltrate cells, U/mm^2	41.0±3.4	33.0±1.2	15.0±5.6	p_{1-2}=0.032 p_{1-3}=0.017 p_{2-3}=0.013
	Connective tissue fibers > 1 μm, %	44.7±0.2	61.3±0.2	74.1±0.5	p_{1-2}=0.016 p_{1-3}=0.013 p_{2-3}=0.025

Table 5. Dynamics of the number (M±SD) of mast cells and fibroblastic differon cells in the wound defect zone and surrounding tissues in the control and main groups, units/mm^2

Timepoint of study	Клеточные элементы	Study group			*p*
		1	2	3	
Day 3	Mast cells	112.0±5.4	107.0±4.2	99.1±5.7	p_{1-2}=0.067 p_{1-3}=0.033 p_{2-3}=0.056
	Fibroblastic differon cells	11.0±2.3	15.0±5.4	21.0±5.3	p_{1-2}=0.074 p_{1-3}=0.042 p_{2-3}=0.059
Day 5	Mast cells	96.0±9.5	87.0±3.7	81.2±3.9	p_{1-2}=0.081 p_{1-3}=0.046 p_{2-3}=0.055
	Fibroblastic differon cells	17.0±4.3	25.0±3.3	36.0±1.8	p_{1-2}=0.037 p_{1-3}=0.023 p_{2-3}=0.029
Day 7	Mast cells	91.0±7.3	82.0±4.4	77.3±1.8	p_{1-2}=0.063 p_{1-3}=0.031 p_{2-3}=0.057
	Fibroblastic differon cells	27.0±3.2	34.0±5.7	29.0±4.1	p_{1-2}=0.044 p_{1-3}=0.082 p_{2-3}=0.077
Day 14	Mast cells	75.0±6.4	69.0±3.1	61.3±5.4	p_{1-2}=0.085 p_{1-3}=0.032 p_{2-3}=0.039
	Fibroblastic differon cells	26.0±4.2	23.0±3.2	17.0±3.6	p_{1-2}=0.089 p_{1-3}=0.026 p_{2-3}=0.052

In control group 1, the wound bed is in places devoid of the epithelial coating, however, in the dermis, single germs of hair follicles are visualized, the surrounding tissue is moderately infiltrated with lymphocytes. In control group 2, healing occurred with a predominance of fibroblastic differon cells over others, single hair follicles were formed (Table 4).

DISCUSSION

VDMB, containing high concentrations of various growth factors, hormones, vitamins and microelements, is of considerable interest for use in the treatment of soft tissue wounds.

The conducted study showed that the introduction of 1 ml of *S. aureus* culture at a concentration of 109 microbial bodies according to the proven method, led to the formation of a purulent wound with a considerable purulent discharge by day 3. In the model without treatment (control group 1), epithelialization of wounds started on average on day 5.1±0.3, the wound area after modeling reduced to (34.4±4.8) mm^2; microbial contamination of the exudate on day 7 reduced to 10^4–10^5 microbial bodies per ml.

Dressings with 0.01% BMP (control group 2) led to acceleration of epithelialization to (3.9±0.2) days, reduction of the defect area to (27.3±4.6) mm^2, reduction of microbial contamination of the exudate on day 7 to 10^3–10^4 microbial bodies per 1 ml.

Conducting standard treatment in combination with VDMB and 0.01% BMP solution (experimental group) led to a reduction in all the studied parameters when compared with both control groups 1 and 2. In particular, the onset of epithelialization decreased to (4.2±0.2) days, the area of the defect after modeling to (20.7±4.7) mm^2, microbial contamination of the exudate on day 7 reduced to 10^1–10^2 microbial bodies per ml of exudate. The obtained dynamics indicates an enhancement in regeneration processes with the use of VDMB, which can be explained by enhanced manifestations of local immunity confirmed by histological studies, and is one of the properties of VDMB.

CONCLUSION

The use of vacuum dried maral blood in complex treatment of purulent wounds of soft tissue made it possible to reduce the time of stopping the local inflammatory reactions, accelerate reduction of the wound area, reduce the activity of growth of bacterial microflora in the wound. Positive dynamics of cell elements and connective tissue fibers was also demonstrated, which indicates a more complete restoration of the dermis when using the proposed treatment method.

ADDITIONAL INFORMATION

Author contributions. N.O. Mikhaylov — conducting an experimental study, analysis and interpretation of data, writing the text; A.A. Andreev, A.A. Glukhov — concept and design of study, editing; V.V. Shishkina — analysis of morphological material, writing the text; O.V. Sudakov, D.V. Sudakov — analysis and interpretation of data, statistical processing. All authors approved the manuscript (the publication version), and also agreed to be responsible for all aspects of the work, ensuring proper consideration and resolution of issues related to the accuracy and integrity of any part of it.

Ethics approval. The study was approved from the Local Ethics Committee of the N.N. Burdenko Voronezh State Medical University (Protocol No. 7 of December 13, 2022).

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