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PATHOPHYSIOLOGICAL STUDY OF THE EFFECT OF COLLAGEN ON THE GRANULATION PROCESS IN PURULENT WOUNDS

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KEYWORDS

wound infection, wound healing, collagen, probiotics, alpha-interferon

ABSTRACT

The results of treatment of 60 patients with purulent wounds of various origins and localization using a collagen sponge containing the bacteria Bacillus subtilis, alfalfa extract, and α-interferon were analyzed. The effectiveness of treatment was assessed by clinical, histological, cytological and bacteriological methods. The use of this method made it possible to accelerate wound healing and reduce the time of hospital treatment.

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Target. Modern principles of purulent surgery suggest differentiated use of dressings depending on the phase of the wound process. In recent decades, biodegradable preparations based on collagen 1–7 have been increasingly used from this perspective. Collagen and its breakdown products in the wound play a major role in platelet aggregation and activation of the production of platelet-derived growth factor, which enhances the proliferation of fibroblasts; chemotaxis of neutrophils and macrophages producing cytokines - fibroblast growth factors and collagen synthesis (interleukin 1, tumor necrosis factor, etc.); fibroblast proliferation. The reparative function of exogenous collagen can be enhanced by adding medicinal regeneration stimulants (methyluracil, honsuride, cytochrome C, sea buckthorn oil, etc.) to the composition of the preparations 5.

We studied the effect of collagen coatings containing the bacteria Bacillus subtilis, alfalfa grass extract, and human leukocyte interferon on a purulent wound. Fundamental research of modern science has made it possible to develop and put into practice many probiotics, the basis of which are live microbial cultures (Bactisporin, Biosporin, Bactisubtil, etc.). There are reasons for their use in the treatment of purulent wounds 3, 4. The substance of alfalfa herb extract and drugs based on it have anti-inflammatory, antioxidant, anabolic, antibacterial and immunomodulatory effects 2. Many researchers note the wound-healing and antibacterial effects of human leukocyte interferon (α-interferon) 6.

Material and methods. The main group included 60 patients in whose treatment collagen coatings were used. Collagen sponges were placed into the cavity of a purulent wound or onto the wound surface: in the first phase of the wound process - coatings containing the bacteria Bacillus subtilis, in the second - alfalfa extract and interferon. The sponges were changed as biodegradation progressed. 60 patients who received traditional treatment using proteolytic enzymes, antiseptics, and polyethylene glycol-based ointments formed the control group. General drug therapy was the same in these groups. The distribution of treatment options in the main and control groups of patients was carried out using stratified randomization. Purulent wounds in 40 patients (33%) arose after trauma, in 51 (43%) - as a result of surgical treatment of purulent-inflammatory diseases of soft tissues, in 16 (13%) - after suppuration of a postoperative wound, in 13 (11%) - as a result trophic disorders in diabetes mellitus or chronic venous insufficiency. The age of the patients ranged from 16 to 74 years.

Results. The results of treatment of patients with purulent wounds were assessed based on the dynamics of the clinical picture (general and local symptoms of the purulent-inflammatory process), laboratory parameters, and data from bacteriological, morphological and cytological studies. We assessed the effectiveness of local treatment by visual characteristics of the wounds (the nature and amount of wound discharge, the condition of the tissues in the wound, the presence and severity of inflammation), the severity of pain in the wound area during dressings and at rest. In patients of the main group, perifocal inflammatory phenomena subsided already on days 3–4, and the amount

of wound exudate significantly decreased (Table 1), which acquired a serous purulent character. In patients in the control group, the corresponding condition was observed only on days 5–7. Collagen coatings provided a good drainage effect, dressings were carried out painlessly in most cases, since there was no need to remove them from the wound. In the control group, when using the ointment, many patients observed the bandage sticking to the wound. Removal of gauze dressings was accompanied by tissue trauma and severe pain, which required the administration of analgesics before dressing and complicated the procedure. Side effects when using collagen coatings were noted in 5% of cases. These were mainly cases of short-term burning and itching in the wound area. In the control group, side effects reached 10% and were manifested by rash and itching. The complex of clinical assessment of the effectiveness of local treatment included the following parameters: reduction in the size of the wound surface, cleansing of the wound from purulent-necrotic discharge, the appearance of granulations, the beginning of marginal epithelization. The data obtained indicate that wound cleansing and the onset of the second phase in all parameters characterizing the wound process occurred faster when using collagen coatings than in the control group (Table 2).

The rate of wound healing (WHR) as a percentage was determined according to the method of L. N. Popova (1942):

$$WHR = \frac{(S-S_n)\times 100}{S\times t} \qquad (1)$$

where S - is the value of the wound area at the previous measurement;

Sn – current area value;

t – number of days between measurements

Table 1

Dynamics of the amount and nature of wound discharge in patients with purulent wounds

Type of discharge	Main group		Control group	
	1 day	3-4 day	1 day	3-4 day
Amount of wound discharge				
No	-	-	-	-
Meager	6	16	5	7
Moderate	20	39	19	31
Abundant	34	5	36	22
Character of wound discharge				
Purulent	54	17	55	43
Serous-purulent	6	40	5	17
serous	-	3	-	-

Table 2

Dynamics of healing of purulent wounds in the main and control groups of patients

Group	Acute purulent process (phlegmon, etc.)		Chronic wound (trophic ulcers)	
	Main	Control	Main	Control
Number of patients	54	55	6	5
Frequency of dressings	1 time every 2-4 days	daily	1 time every 3-5 days	1 time every 2 days
Wound cleansing (24 hours)	4.8±0.2*	5.6±0.2*	7.8±0.7**	10.4±1**
Granulation and beginning of epithelization (days)	5.9±0.9*	7.3±0.8*	11.5±1.1**	15.2±1.3**
Subjective sensations (number of patients)				
Pain	0	18	1	2
Burning	3	4	0	0
Itching	0	1	0	1
Duration of treatment (days)	11.3±0.3*	13.6±0.4*	19.2±0.6**	22.6±0.5**

Note: * and ** – statistically significant differences between the corresponding indicators of the main and control groups ($p < 0.05$)

The rate of wound healing in the main group was 7.4 ± 0.6, in the control group – 5.9 ± 0.4% per day. The duration of treatment in the main group was 11.3 ± 0.3 days, while in the control group it was 13.6 ± 0.4 days. On average, the treatment time in the main group was 2 days shorter. A bacteriological study showed that the main causative agents of the purulent process in patients of the main and control groups were staphylococci, less often - gram-negative flora. In the second phase of the wound process, secondary wound infection was observed, mainly in the control group of patients. Staphylococci, Pseudomonas

aeruginosa, Proteus, etc. were sown both in monoculture and in association. In 75.0% of patients in the main group and 10.0% of patients in the control group, a progressive decrease in bacterial contamination was noted. In the majority of patients in the control group, microbial contamination remained at a high level for a long time and by the fifth day amounted to 105 microorganisms per 1 g of tissue. In the main group of patients, on the 5th day of treatment, the number of microorganisms in the wound was at the level of 103 per 1 g of tissue (Table 3), and in 20.0% of patients their growth was not observed. In the first phase, Bacillus subtilis bacteria were detected in the wound in an amount of 104–105 per 1 g of tissue (the first 7–10 days).

Table 3

Dynamics of the number of microorganisms in purulent wounds

Duration of treatment	Number of microorganisms in 1 g of tissue	
	Main group	Control group
1 day	$4.1 \pm 0.8 \times 10^7$	$3.8 \pm 1.0 \times 10^7$
5 day	$2.9 \pm 0.4 \times 10^3$*	$2.3 \pm 0.7 \times 10^5$*
10 day	No growth	$1.6 \pm 0.3 \times 10^3$

* – statistically significant differences between indicators ($p < 0.01$)

A morphological study of the preparations obtained on the first day showed that edematous tissue and pronounced infiltration of leukocytes (mainly neutrophils) and microorganisms were detected at the bottom of the wound (Fig. 1). Disorders of microcirculation in the form of hemolymphostasis, swelling of endothelial cells, and tissue edema are noted. The death of some groups of fat cells and their resorption by macrophages is observed.

On the fourth day, in patients of the main group there was a decrease in the number of leukocytes in the tissues surrounding the wound, the number of macrophages increased, young fibroblasts were detected and the content of mature fibroblasts increased. Inflammatory changes (increased vascular permeability, edema, leukocyte infiltration) were less pronounced than in the control group of patients. Islands of granulation tissue with fibroblasts appeared, which gradually filled the wound defect. By the eighth day, there was a further increase in the number of fibroblasts and maturation of granulation tissue, normalization of the microcirculation system - vascular permeability and infiltration of walls with leukocytes decreased. By day 12, the maturation of granulation tissue progressed, marginal epithelization was noted, and the content of fibroclasts involved in the restructuring of connective tissue increased.

Table 4

Dynamics of cytological characteristics of wounds

Cytogram type	Main group			Control group		
	1 day	4 day	8 day	1 day	4 day	8 day
Necrotic	28			27	2	
Degenerative inflammatory	2	1		3	20	1
Inflammatory		27			7	9
Inflammatory regenerative		2	3		1	15
Regenerative			27			5

In the control group, on the fourth day, microcirculation disorders persisted in the form of hemo-lymphostasis, loosening of basement membranes, and the formation of microthrombi. Differentiated forms of fibroblasts are few in number; young fibroblasts predominate. Islands of granulation tissue are detected only on the eighth day. By day 12, in most cases, the inflammatory reaction persists with further fibrosis and epithelization. Smear impressions were assessed in 30 patients in the main group and 30 in the control group (Table 4). On the first day, the cytogram of patients in both groups is represented by a large number of neutrophilic leukocytes, microorganisms and necrotic detritus. On the fourth day, small numbers of neutrophilic leukocytes, lymphocytes, single eosinophils, and macrophages with phagocytosed microbial cells were found in the smears and prints of patients in the main group. Young fibroblasts appear in large numbers. On the eighth day, no microbial cells are found in the cytogram.

Profibroblasts and fibroblasts predominate, and active growth of granulation tissue is noted. The cellular composition is also represented by macrophages, single phagocytic leukocytes, endothelial cells, and epithelial cells. The smears and fingerprints of patients in the control group on the fourth day contained large numbers of microorganisms and neutrophilic leukocytes. Macrophages are less common than in the main group. Single profibroblasts appear. On the eighth day, the cytogram still contains microbial cells and many phagocytic leukocytes. There are significantly fewer connective tissue cells than in smears and fingerprints of patients in the main group.

Thus, it was found that the studied combined collagen coatings have the following advantages compared to traditional methods of local treatment:

- accelerate the time for cleansing wounds from pus and necrotic masses by 2–3 days;
- more effectively stimulate the development of granulation tissue;
- have fewer adverse reactions and are better tolerated by patients;
- reduce the consumption of dressing material;

- actively influencing regeneration and epithelization, reducing treatment time, collagen coatings can be effectively used to prepare the wound surface for autodermoplasty with large areas of the wound surface;

-collagen preparations are easy to use, their use can be done both in a hospital and on an outpatient basis.

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