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Cytokine Therapy of Experimental Purulent Wounds

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The effect of naturally occurring cytokine complex on aseptic and purulent wounds is examined in guinea pigs. Healing of aseptic wounds treated with the complex is 1.4 times faster than in the control: the rate of wound contraction reaches the maximum after 5-6 days *vs.* 10-11 days in the control. Healing of these wounds occurs without pronounced inflammatory processes. Healing, epithelization, and wound cleansing are significantly faster in cytokine treated wounds than in the control. Histological analysis reveals formation of delicate cicatrices in cytokine-treated wounds, while rough cicatrices are observed in the control.

Key Words: *cytokines; purulent wounds; guinea pigs*

Regulation of reparative and inflammatory processes is an important problem in modern surgery. Local application of new preparations based on cytokines (naturally occurring cellular regulators) is a prospective approach to the solution of this problem. Recombinant cytokines: interleukin-1 (IL-1), interferons, and fibroblast growth factors have been used in the treatment of experimental wounds [3,4,11].

We have developed a method based on local application of natural combinations of cytokines secreted by autologous lymphocytes isolated from peripheral blood [5]. Natural cytokine complex (NCC) accelerates wound healing [1], prevents formation of rough cicatrices [6], and reduces inflammatory processes. Presumably, these effects are due to the presence of IL-1 and IL-2, factor inhibiting migration of macrophages, and the leukocytin-inhibiting factor IL-6 in the NCC [2]. However, the activity and spectrum of cytokines secreted in pathological states change considerably. This should be taken into consideration in the treatment with autologous cytokines.

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In this study we examined the effect of heterologous NCC on healing of aseptic and purulent wounds by morphological and bacteriological methods.

MATERIALS AND METHODS

Purulent wounds were modeled in guinea pigs of both sexes weighing 200-400 g as described elsewhere [8]. A standard wound (area 200 mm^2) was produced and contaminated with *St. aureus* strain P209.

Natural cytokine complex was isolated from stimulated cultures of pig peripheral blood leukocytes [6]. The preparation (0.2-0.3 ml, 1000 mg protein/ml) was applied on the wound daily within a 12-19-day period.

Wound healing was assessed by the terms of a) granulation tissue formation, b) epithelization of wound edges, c) wound cleansing from purulent-necrotic detritus, and d) complete epithelization.

The wound area was measured as described [9]. The number of microorganisms per gram tissue was determined on days 3, 5, 7, and 10 [7]. The wound was regarded as clean from microbes if their content was less than 10^5 cells/g tissue.

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Histological studies were carried out days on 3, 7, 10, and 19. Sections were stained with hematoxylin and eosin.

RESULTS

Application of NCC onto aseptic wounds shortened the period of complete epithelization from 15.2±2.0 days (control) to 11.2±1.5 days ($p<0.001$), and the rate of contraction of NCC-treated wounds reached the maximum on days 5-6 *vs.* days 10-11 in the control.

The parameters characterizing the healing of purulent wounds are summarized in Table 1.

Macroscopically, the purulent-inflammatory processes were less pronounced in NCC-treated animals, and wound healing took a more abortive course, particularly during the hydration phase. On the first days, the wound edges were moderately hyperemic and edematic. Wound began contracting on day 1; it was rapidly cleansed from purulent-necrotic detritus, and on day 7 was filled with granulation tissue without active inflammation and with good epithelization of the edges.

Pronounced inflammation with acute edema, hyperemia, infiltration, and strong exudation was observed in the control group. Complete epithelization and formation of a rough cicatrix occurred on day 19, while in NCC-treated animals a delicate cicatrix was formed. It should be noted that NCC-treated wounds healed predominantly by edge epithelization, while control wounds healed by edge retraction.

Taking into account the prospects of NCC use in clinical practice, we performed a series of experiments with combined application of the preparation and Dioxycol, an ointment on a water-soluble basis. At the present time this ointment is the most effective means of local treatment of wounds.

The animals were divided into 3 groups (6 in each). In group 1, NCC was applied during the first phase of healing (before wound cleansing), and the ointment Dioxycol was applied after 10-15 min. After wound cleansing (phase II), only NCC was used. In group 2, Dioxycol was applied during phase I and medium 199 during phase II. In group 3 only medium 199 was applied onto the wound. Groups 2 and 3 served as the control.

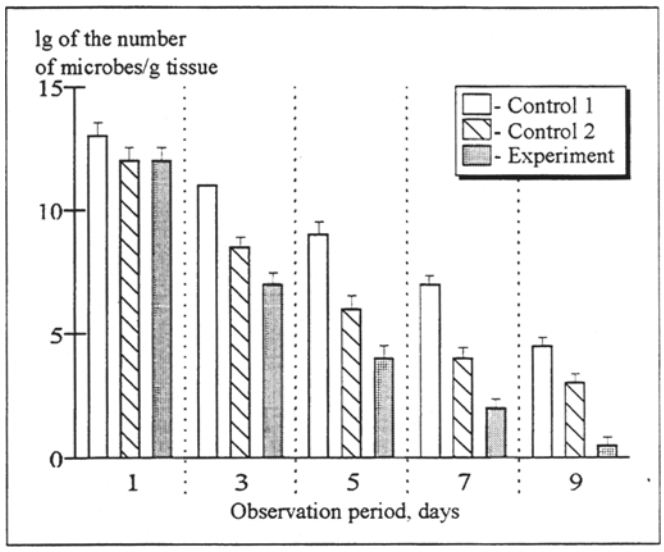

Fig. 1. Reduction in the degree of bacterial contamination of the wound.

There were no pronounced inflammation in animals treated with the NCC—Dioxycol combination. The rate of wound healing increased significantly in comparison with both control groups (Table 2). The period of wound cleansing from *St. aureus* was much shorter than in the control groups (Fig. 1).

The dynamics of wound contraction was similar in all groups (Fig. 2, *a*, *b*).

There were practically no differences in the histological appearances of purulent wounds treated with NCC and NCC—Dioxycol combination. The outgrowth of granulation tissue with chaotic arrangement of capillaries against the background of active infiltration (predominantly by neutrophils) and necrotic foci was observed in experimental animals on day 3. In control animals, the wounds were covered with purulent-necrotic detritus containing occasional islets of granulation tissue and pronounced neutrophil infiltration.

On day 7, in experimental guinea pigs the wound was filled with granulation tissue; moderate cellular infiltration, occasional necrotic foci, and active epithelization of wound edges were seen.

TABLE 1. Parameters of Healing of Purulent Wounds

Assessment of wound process	Control	Experiment
Appearance of granulation tissue, days	4.2±1.0	2.1±0.5***
Epithelization of wound edges, days	5.3±1.0	3.1±0.8***
Wound cleansing from *St. aureus*, days	9.3±2.0	7.3±1.8*
Complete epithelization, days	19.5±2.0	14.9±1.5**

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control.

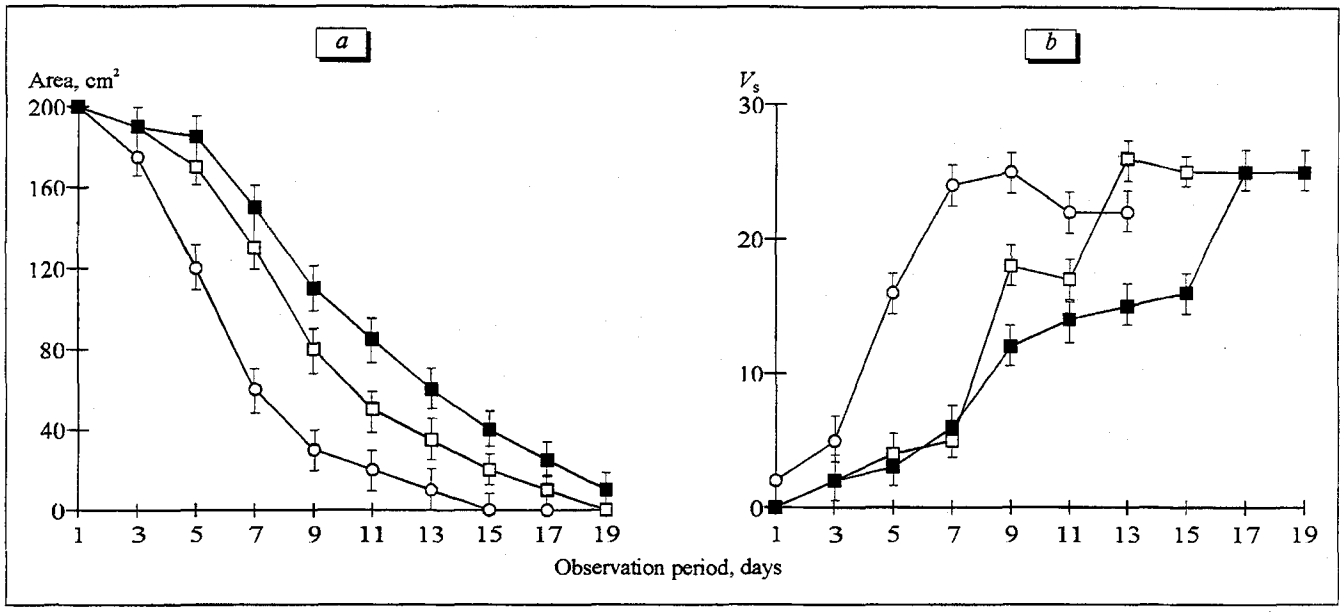

Fig. 2. Contraction of purulent wounds. Dynamics of wound contraction (*a*) and rate of contraction (*b*). *1*) experiment, *2*) control 1, *3*) control 2. $V_s=(S—S_N)\times100/(S\times t)$, where t is the time (days), S is the initial area of the wound, and S_N is the area on the given day.

In control animals, pronounced cellular infiltration, extensive necrotic foci, and initial epithelization of wound edges with microabscesses under outgrowing epithelium were observed.

On day 10, lymphocytic infiltration was observed in wounds treated with NCC and Dioxycol. The arrangement of capillaries became orderly, numerous fibroblasts appeared, and a network of collagen fibers was formed. Epithelium was actively outgrowning over granulation tissue, and the detritus almost disappeared.

Outgrowth of granulation tissue was observed in the control group. Numerous fibroblasts were arranged chaotically; the wound was covered with considerable amounts of detritus with microabscesses.

In experimental group, histological investigation of macroscopically healed wounds (day 19) revealed no inflammatory processes. The wound was evenly covered with granulation tissue with slight cellular infiltration, fine collagen network, and a delicate cicatrix was formed.

In control group, the wound was covered with epithelium of varying thickness, which was accompanied by preservation of microabscesses and cellular infiltration and formation of a rough cicatrix.

From these observations it can be concluded that NCC acts as a regulator at all stages of wound process. Active migration of neutrophils and an increase in their functional activity occur at the early stages (first 7 days). Complete cleansing of the wound from microorganisms is observed during this period (Fig. 1). At the late stages (10-19 days) NCC potentiates the remodeling processes: numerous fibroblasts appear, collagen fibers are formed, and epithelium is outgrowing from the wound edges. This is confirmed by experimental data on activation of fibroblast proliferation *in vitro* [1]. At the same time, the mechanisms responsible for suppression of the connective tissue outgrowth are triggered. For example, it is known that IL-1 and tumor necrosis factor-α stimulate the production of collagenase [10]. These processes prevent the development of rough cicatrix.

In general, activation of wound process can be related to a cascade of effects induced by cytokines

TABLE 2. Parameters of Healing of Purulent Wounds Treated with NCC—Dioxycol Combination

Assessment of wound process	Control 1	Control 2	Experiment
Appearance of granulation tissue, days	3.5±1.0	4.3±1.2	2.0±0.5**
Epithelization of wound edges, days	5.0±1.1	5.2±1.2	3.0±1.0**
Cleansing from *St. aureus*, days	6.8±1.8	9.8±2.0	4.7±1.5****
Complete epithelization, days	16.8±2.7	19.6±2.7	13.5±1.8****

Note. *$p<0.05$, **$p<0.01$ compared with the control 1, *$p<0.01$, **$p<0.001$ compared with control 2.

of the natural cytokine complex. Combined application of NCC with ointments potentiates their effects.

Thus, our results point to the prospectiveness of NCC use in the treatment and prevention of purulent-inflammatory pathologies.

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Benzamide and Its Derivatives Are Active Against Botulinal Intoxication

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A high antibotulinal activity of benzamide injected for prevention and of 3-N-butyrylaminobenzamide injected for treatment was observed in mice with intoxication caused by type A botulin toxin. 3-aminobenzamide and 3-N-butyrylaminobenzamide did not inhibit botulinal toxin *in vitro*.

Key Words: *experimental botulinal intoxication; botulinal neurotoxin; benzamide; benzamide derivatives*

Specific antibotulinal sera at late stages of botulism are often ineffective and can induce allergic reactions. The search for pharmacological antagonists of botulinal toxin (BT) is difficult because little is known about the mechanism of BT action. Damaging effect of BT is explained by its capacity to block mediator release in the cholinergic myoneural synapses, which results in paralysis and, in severe cases, in death [10].

Guanidine [9], 4-aminopyridine and its derivatives [5,6], tusendanine [8], and other agents are capable of blocking botulinal neurotoxin. However, therapeutic effectiveness of these agents is low, therefore, new agents for treating botulism are required.

Recently, the inhibitors of endogenous ADP-ribosylation in body tissues and isolated cells have been studied [7]: primarily benzamide and its 3-substituted derivatives, i.e., structural analogs of the nicotinamide moiety of NAD whose ADP-ribosyl fragment is transferred onto the acceptor proteins by mono-ADP-ribosyltransferases [11]. Some of these substances can decrease the intensity of endogenous ADP-ribosylation in experimental animals by 90-95% [2], which permits us to regard them as potential pharmacological antagonists of some bacterial toxins.

In this study we assessed antitoxic effectiveness of benzamide, 3-aminobenzamide (3-ABA), and 3-

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