Immunomodulation of Reparative Processes in the Skin with Ribomunyl

V. V. Bazarny, I. E. Valamina, O. N. Selyanina, E. A. Tikhonina, O. Yu. Beresneva, A. V. Klein, and A. I. Isaikin

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A stimulatory effect of intradermal injections of ribomunyl on healing of a skin wound was shown in rats. The effect is based on activation of glycosaminoglycan synthesis and stimulation of neoangiogenesis in newly formed granulation tissue.

Key Words: *skin wound; ribomunyl*

Technologies aimed at skin defect replacement and employing cell cultures, recombinant growth factors, artificial skin equivalents, *etc.*, are widely used in the treatment of skin wounds in recent years [6-8]. At the same time, potentialities of the development of new trends in stimulation of epidermal cell proliferation based on the important role of the immune system in skin homeostasis regulation are not yet exhausted [2,5].

Here we studied the possibility of stimulating repair processes in the skin with ribomunyl, an immunomodulator capable of inducing specific immune response and activating nonspecific immunoreactivity.

MATERIALS AND METHODS

The study was carried out on 48 male Wistar rats aged 3-4 months (120-150 g). The animals were kept under standard vivarium conditions. Skin wound (1 cm^2) on the dorsal surface was inflicted surgically under aseptic conditions.

Ribomunyl aqueous solution (Pierre Fabre) diluted in 0.5 ml procaine was injected to animals with skin wounds for 3 days after surgery for immunomodulation of the repair process in the skin. The animals were divided into 4 groups, 12 per group. Experimental rats received ribomunyl intraperitoneally (0.001 mg) or intradermally (0.001 or 0.0005 mg, to a depth of ~1 mm around the wound). Control rats received the same volume of procaine. Our preliminary study showed that the main laboratory values and the duration of skin wound epithelialization were virtually the same in animals receiving intradermal and intraperitoneal injections of procaine.

The animals were decapitated under ether narcosis on days 7 and 15.

Skin regeneration was evaluated by a complex of clinical morphological parameters, in particular, by the time of crust detachment. Histological sections were prepared routinely, stained with hematoxylin and eosin and with picrofuchsin after van Gieson. The ratio of aci to neutral glycosaminoglycans (GAG) in the connective tissue extracellular matrix was evaluated by standard staining (after Hail, PAS reaction).

Computer morphometry on a SIAMS-610 videocomputer complex was carried out for objective evaluation of quantitative differences.

Tissue preparations of the skin wound were prepared routinely and analyzed using a Jeol-200C electron microscope at accelerating voltage 80 kV and working magnification 7300.

The results were statistically processed using methods of variation statistics (nonparametric tests) [1]. The differences between the groups were evaluated using Kruskal—Wallis nonparametric dispersions analysis.

Central Research Laboratory, Ural State Medical Academy, Ekaterinburg. ***Address for correspondence:*** bazarny@okbl.ru. V. V. Bazarny

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TABLE 1. Morphometric Characteristics of the Zone of Skin Regenerate ($M \pm m$)

Group	Volume percent of cells unit area	Volume percent of lymphocytes per unit area
Control	15.8±0.4	3.2±0.1
Ribomunyl intraperitoneally, 0.001 mg	16.2±0.2	4.3±0.1*
Ribomunyl intradermally, 0.001 mg	22.5±0.4*	6.5±0.2*
Ribomunyl intradermally, 0.0005 mg	20.3±0.4*	5.2±0.2*

Note. *$p<0.05$ compared to the control.

RESULTS

The effect of ribomunyl on skin wound healing was well demonstrated by the time of crust detachment: 13 days in the control group, 11 in the group injected with ribomunyl intraperitoneally, and 9 days in the group receiving intradermal treatment ($p<0.05$); no differences in the effects of different intradermal doses were detected.

Histological study on day 7 showed proliferative reaction in the wound in all groups. In the control group, the wound was filled with newly formed granulation tissue (GT) with numerous fine-wall vessels and inflammatory infiltration cells. Acid GAG predominated in the GT matrix; neutrophil infiltration and edema indicated persisting exudative reaction.

Signs of exudative inflammation were less pronounced in animals receiving intraperitoneal injections of ribomunyl. Histiocytes and fibroblasts with PAS-positive cytoplasm, lymphocytes, and plasma cells predominated in the inflammatory infiltrate. The ratio of acid/neutral GAG in the matrix and the presence of fine collagen fibers attested to GT maturation. "Crawling" of newly formed epithelium on GT was observed in 50% cases by this term. No epithelialization of the wound defect was detected in control animals during this period; only thickening of the epithelium at the wound edges due to proliferation of basal layer cell just started.

The impact of the administration route and dose of ribomunyl for the proliferative activity of regenerating tissue was evaluated by computer morphometry of skin regenerate cell counts.

Intradermal injection of ribomunyl led to a 42% increase ($p<0.05$) in regenerate cellularity, the maximum dose was more effective (Table 1). Intraperitoneal injection did not cause any appreciable increase in the studied parameters. The total count of cells in the regenerating epidermis correlated with lymphocyte content, which attests to an important contribution of these cells to regeneration. The data indicate that intradermal injection of ribomunyl in a dose of 0.001 mg produced the maximum stimulatory effect on skin regeneration.

Analysis of subsequent dynamics of skin wound regeneration in different groups showed that drug injection led to the formation of less coarse connective tissue cicatrix and accelerated reparative regeneration of the epithelium.

The positive effect of ribomunyl on skin wound regeneration was confirmed by electron microscopic findings. In controls, discomplectation of cellular and tissue elements in all parts of the skin wound was associated with necrobiotic changes in its central part. Highly osmiophilic epithelial cells and separate collagen fibers were seen in the peripheral part of the wound. Plasma cells and new capillaries in moderately edematous environment were seen in the center of the skin wound after injection of ribomunyl. Mononuclears, keratinocytes, and collagen fibers were seen in some visual fields.

Hence, ribomunyl stimulated reparative processes and accelerates skin wound healing. Intradermal injections were more effective. The data suggest that this effect of ribomunyl is realized via several routes: through activation of GAG synthesis in newly formed GT and through stimulation of neoangiogenesis. Presumably, activation of skin lymphoid elements producing cytokines and stimulating cellular mechanisms of reparation makes an important contribution into these processes.

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