MICROBIOLOGY AND IMMUNOLOGY

Effect of Ribothyme on Delayed-Type Hypersensitivity and Wound Process in Mice with Surgical Trauma and Burn Disease

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Immunological and morphological evaluation of immunotherapy with ribothyme in experimental surgical and thermal injuries in mice showed its positive effect on posttraumatic reparative processes.

Key Words: *immunotherapy; ribothyme; delayed-type hypersensitivity; burn; surgical injury*

Pathogenesis, clinical picture, pathomorphology, and therapy of severe mechanical and thermal injuries attracted special attention in Russia and abroad during recent decades. Lethal outcomes of such injuries are caused by impairment of body's defense mechanisms and infectious complications. Patients develop defects in nonspecific inflammatory and specific immune response [2]. Severe injuries inhibit [1,7] or stimulate [3] the delayed-type hypersensitivity (DTH) reaction.

Traditional surgical and therapeutic treatment of traumatological patients is now supplemented with immunocorrecting therapy [5]. A ribonuclein drug sodium nucleinate stimulates DTH reaction [4] and anti-infective immunity in mice with burns [6]. Search for new effective drugs with immunomodulating effects is in progress.

We investigated the efficiency of immunotherapy in surgical and burn injuries in mice. Immunotherapy was carried out with a ribonuclein drug ribothyme, isolated in our laboratory from cattle thymus by an original method. Parallel morphological and immunological studies helped us to evaluate the effect of immunotherapy on posttraumatic reparative processes (by the level of DTH).

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MATERIALS AND METHODS

Experiments were carried out on 150 female BALB/c mice weighing 22-25 g. Median laparotomy (24-mm long) was performed by a standard method [9] under anesthesia. The wound was sutured layer-by-layer with silk.

Burns were inflicted by a standard method [8]. Full-layer burns of the back (25% of body surface) were inflicted under hexenal anesthesia by plunging the mice into boiling water for 7 sec. Mortality after this procedure was less than 5%.

Ribothyme was injected subcutaneously in a dose of 2.5 mg/kg in 0.1 ml normal saline during 4 days after injury.

DTH was evaluated routinely [4]. The mice were immunized intraperitoneally with sheep erythrocytes (2×10^7). On day 6, a challenging dose of sheep erythrocytes (10^8) was injected in the hindpaw pad (experiment) and an equivalent volume of isotonic NaCl was injected into the contralateral paw (control). The DTH index was calculated on day 7 by the formula: (experimental paw weight – control paw weight)/control paw weight×100%.

Biopsy specimens for histological analysis were collected on days 7 and 21 after laparotomy and thermal injury.

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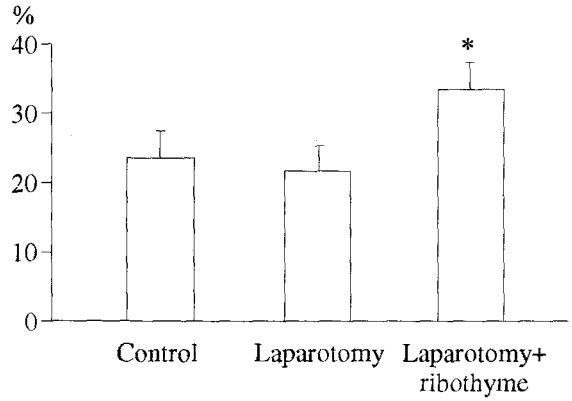

Fig. 1. Effect of ribothyme on delayed type hypersensitivity index in mice after laparotomy. $p<0.05$ *vs.* the control.

Differences were considered significant at $p<0.05$ by the Wilcoxon—Mann—Whitney U test.

RESULTS

Figure 1 shows the effect of ribothyme on DTH in mice with surgical injury (laparotomy). Laparotomy as a traumatic factor caused no statistically significant suppression of DTH. However, immunotherapy with ribothyme stimulated DTH, *i.e.* the drug stimulated T-cell immunity.

Histological analysis showed a beneficial effect of ribothyme on wound healing. By day 7, a wide clinoid defect involving the entire abdominal wall thickness

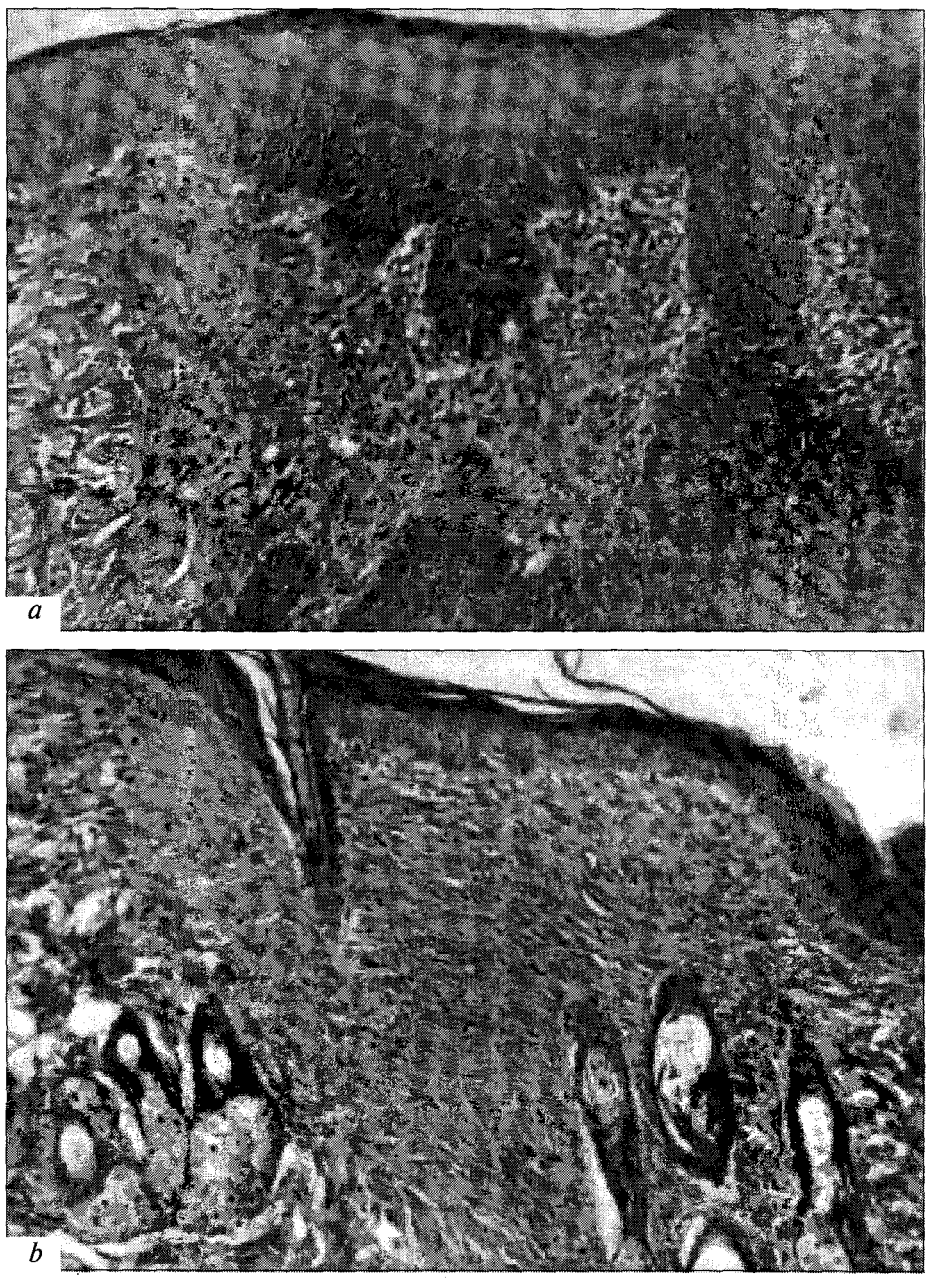

Fig. 2. Histological changes in skin wound on day 21. Hematoxylin-eosin staining, ×100. *a*) control, fibrous cicatricial transformation of the granulation tissue, complete epithelialization of the wound defect. *b*) after ribothyme: formation of full-value regenerate, with newly formed hair follicles.

was observed in animals subjected to laparotomy and receiving no ribothyme; the wound was covered with thickened epithelium with submerged epithelial growth at some sites. The wound channel contained young granulation tissue and fragments of necrotic tissues.

In animals treated with ribothyme, confluent epithelialization without submerged growth of epithelium and more mature and less bulky granulation tissue (with less abundant vessels and greater macrophage count) were seen by day 7. Edema of the underlying tissues was less pronounced.

On day 21 after laparotomy, the wound defect in the control group was completely covered with thickened epithelium, fibrous-cicatricial granulation tissue, neutrophil and macrophage infiltration of the surface layers were observed.

In animals treated with ribothyme almost complete recovery of damaged tissues was accompanied by the formation of new hair follicles; no coarse cicatrices were seen (Fig. 2).

Thermal injury of 25% body surface caused a 5-fold suppression of DTH (Fig. 3). The level of DTH in animals with burns treated by ribothyme was virtually the same as in animals with burns receiving no immunotherapy, though there was a trend to recovery of this parameter. Therefore, immunotherapy with ribothyme did not restore immune functions in these animals. This can be attributed to the fact that immunomodulator was administered after burn injury disturbing the earliest stages of DTH reaction: antigen binding and processing by antigen-presenting cells, cell-cell interactions, and the formation of the effector cell pool [1]. Unfortunately, DTH test does not allow to detect precisely the effect of burn on immune system in other periods of experimental burn injury. We conclude that DTH test does not adequately reflect the efficiency of immunotherapy in injury.

Morphological findings indicate a beneficial impact of immunotherapy on reparative processes in the wound. By day 7, a large wound defect covered with a thick crust with bacterial aggregates was seen in animals with burns not treated with ribothyme. Edema and necrotic processes in subcutaneous fat and muscles and hemorrhagic foci were observed. No macrophages were present. Slight neutrophil infiltration developed. Destructive forms of neutrophils, congestive plethora in capillaries and venules, and erythrocyte stasis were observed. No granulations were seen.

By contrast, in animals treated with ribothyme, pronounced neutrophilic infiltration of primary and secondary necrotic foci was observed by day 7. Destroyed neutrophils were scanty, bacterial contamination was

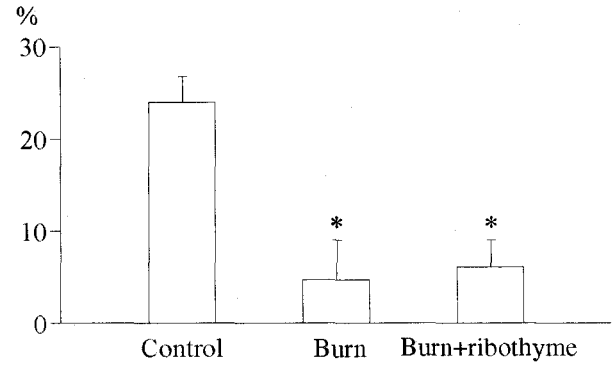

Fig. 3. Effect of ribothyme on delayed type hypersensitivity index in mice with 25% body surface burns. *$p<0.01$ *vs.* control.

less pronounced than in the control; it was obvious that demarcation inflammation forming the interface between new and necrotic tissues appeared earlier.

On day 21, granulation tissue appeared in the control. There was abundant neutrophilic and round-cell infiltration, many destructive forms of leukocytes, plethoric, erythrocyte stasis, and signs of fibroblast activation.

In animals treated with ribothyme granulation tissue developed more actively: there were many new vascular elements, macrophagal infiltrates appeared.

The study demonstrated a positive therapeutic effect of ribothyme on reparative processes after laparotomy and thermal injury: more mature granulation tissue (macrophagal infiltration, vessel formation, *etc.*) and acceleration of reparative processes. The drug affected T cell-dependent DTH. Optimal protocols of immunotherapy for practical clinical use based on the phasic pattern of reparative processes are to be determined.

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