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Regulatory Effects of Ribotim on Functional Activity of Neutrophils and Wound Healing during Experimental Burn Trauma

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We studied the effects of ribonucleic immunotropic preparation Ribotim obtained from the thymus on functional activity of peripheral blood neutrophils and wound healing after burn trauma. Immunotherapy normalized immunological parameters, attenuated demarcation inflammation in burn wound, and promoted epithelialization of the wound surface preserving skin structures.

Key Words: *Ribotim; neutrophils; burns*

Much attention to the functional state of neutrophils during burn trauma is due to their pronounced positive effects in posttraumatic processes. Neutrophils are involved in eradication of pathogenic microorganisms in damaged tissues [10], provide antioxidant protection [14], and regulate reparation of the skin and subcutaneous structures in the wound area [6,11]. However, hyperactivated neutrophils produce adverse effects. After burn trauma, peripheral blood neutrophils can damage intact tissues (lungs, liver, gastric mucosa, *etc.*) [13].

Immune correction modulates functional state of phagocytes and promotes healing of burn wounds. Here we studied the efficiency of new immunotropic preparation Ribotim obtained from the thymus and containing low-molecular-weight RNA (12-60 kDa). Immunotropic properties of Ribotim were studied previously [12]. Biological activity of this preparation is determined by the presence of regulatory RNA molecules [3].

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MATERIALS AND METHODS

Experiments were performed on 79 male Wistar rats weighing 220-240 g. Burn trauma involving 20% total body surface area (IIIA) was routinely produced under hexenal anesthesia [15].

Ribotim in a dose of 1 mg/kg was injected daily subcutaneously for 4 days after trauma and then 2 times a week over 10-12 weeks. Control animals received isotonic NaCl.

Functional activity of phagocytes was studied spectrophotometrically by estimating adhesive properties of blood leukocytes and activity of bactericidal enzymes, acid phosphatase (AP) and myeloperoxidase (MPO), before and after stimulation with opsonized zymosan [8]. The formation of reactive oxygen species (ROS) during phagocytosis was studied by luminol-dependent chemiluminescence [9].

Histological preparations were stained with hematoxylin and eosin and examined using an Avtandilov grid. The number of points in 1 sample was estimated using a Weibel curve and counted under a microscope (×400) [1]. The results were analyzed by Mann—Whitney *U* test. Morphometrical data were analyzed using methods of variation statistics.

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RESULTS

Functional activity of rat blood phagocytes underwent phasic changes after burn trauma, which reflects the general adaptive syndrome in the immune system described by H. Selye (Table 1).

The phase of anxiety developed 4 days after trauma and was characterized by mobilization of protective mechanisms, enhanced adhesive and enzymatic activities of phagocytes, and ROS excretion by phagocytes. The phase of resistance developed by the 9th day after trauma. Despite the presence of stress factor (burn wound) and strain of physiological processes in the body, functional activity of blood phagocytes was stabilized (data not shown).

Exhaustion of adaptive capacities developed by the 15th day after trauma (Table 1). In control animals, MPO and AP activities sharply decreased. ROS generation in response to stimulation with opsonized zymosan decreased against the background of progressive bacterial invasion in burn wound. The 2nd week after burn trauma was characterized by secondary bacteremia. Previous studies showed that exhaustion of reserve capacities of the immune system is accompanied by progression of bacterial infection [5].

Thirty days after burn (convalescence) most animals adapted to novel conditions (data not shown). Ribotim prevented exhaustion of functional capacities of peripheral blood neutrophils on day 15 after burn trauma (Table 1).

It was previously demonstrated that Ribotim regulates skin regeneration after burn and surgical traumas in mice [12]. In our experiments, Ribotim stimulated epithelialization of the wound surface and recovery of skin structures in rats. By day 33 after trauma, the wound area in control animals looked like a whitish scar with central weeping ulcers occupying 39.9± 3.7% wound area. In rats treated with Ribotim, this nonepithelialized region occupied only 4.1±2.1% wound area (9.7-fold lower than in the control, $p<0.05$). In Ribotim-treated animals, hairs were recovered in 64± 23% wound area, which 9.1-fold surpassed the control (7±2% wound area, $p<0.05$). Differences between control and Ribotim-treated rats persisted for 77 days after burn trauma.

Histological assay revealed differences between the control and Ribotim-treated animals in the formation of demarcation inflammation (Table 2). Accumulation of neutrophils was seen in all skin layers and subcutaneous tissues of all burned animals. In control rats, a diffuse line of demarcation involved all skin layers. We found diffuse inflammation in subcutaneous fat and neutrophil infiltrates in the muscle layer. In Ribotim-treated rats, demarcation inflammation was seen at the boundary between subcutaneous fat and necrotized dermal tissues and was not accompanied by accumulation of neutrophils in subcutaneous fat and muscle layer.

Thus, Ribotim reduced the severity of secondary damages to the skin and subcutaneous tissues, which promoted rapid epithelialization of the wound area and preserved viability of germinative cells in hair follicles and skin glands.

The efficiency of Ribotim is related to the presence of regulatory RNA playing an important role in

TABLE 1. Functional Activity of Peripheral Blood Phagocytes in Rats (*M*±*m*)

Parameter	Before burn (*n*=21)	After burn, days			
		4		15	
		control (*n*=10)	Ribotim (*n*=9)	control (*n*=10)	Ribotim (*n*=9)
Adhesion, %	53.33±3.21	72.45±4.23*	35.64±6.02*+	71.67±6.45*	60.18±4.49
MPO activity, 10^{-4} U					
spontaneous	4.16±0.65	5.90±0.81*	7.97±0.98*+	2.45±0.28*	5.42±0.96
induced	4.34±0.70	5.90±0.88	7.73±0.81*+	2.43±0.23*	5.71±0.97
MPO reaction index	1.04±0.02	0.99±0.04	0.99±0.04+	1.02±0.05	1.08±0.05
AP activity, 10^3 optical density units					
spontaneous	74.89±2.81	106.16±8.51*	125.25±20.71*+	43.80±4.45*	88.67±5.00*+
induced	84.00±5.08	120.60±11.05*	158.13±23.03*+	70.40±8.18*	110.44±5.58*+
AP reaction index	1.08±0.02	1.14±0.04	1.33±0.13*+	1.63±0.13*	1.26±0.07*
Chemiluminescence, cpm/10^5 neutrophils	1506±162	1881±125	1033±132*+	745±118*	1140±99+

Note. Here and in Table 2: $p<0.05$: *compared to parameters before burn, +compared to the control.

TABLE 2. Volume Ratio of Neutrophils (%) Forming the Demarcation Line in Various Skin Layers on Day 7 after Burn ($M \pm m$)

Tissue layer	Before burn (n=7)	After burn	
		control (n=7)	Ribotim (n=8)
Deep dermal layers	0.93±0.48	19.41±0.75*	15.75±4.07*
Boundary between the derma and subcutaneous fat	0.50±0.27	15.89±5.36*	22.50±6.51*
Subcutaneous fat	0.75±0.31	24.43±3.54*	2.14±1.08*+
Muscles	0.13±0.10	18.5±6.5*	1.25±0.56*+

skin reparation. The preparation probably produces a direct or indirect effects on skin regeneration by modulating neutrophil activity. In mature neutrophils, proteins are not synthesized, and RNA synthesis is extremely low [7]. RNA synthesis is maximum in *in vivo* activated neutrophils migrating into the wound area [11]. Wound neutrophils undergo rapid destruction accompanied by the disappearance of granules and other cytoplasmic structures. However, even after destruction neutrophils intensively synthesize regulatory RNA. Previous studies showed that RNA synthesis in activated neutrophils is not followed by protein synthesis [7]. Neutrophils probably synthesize RNA performing regulatory functions not only inside, but also outside the cell [11]. This assumption is indirectly confirmed by the data that exogenous RNA induces differentiation, regeneration, and adaptation in the bone tissue, liver, myocardium, and nerve fibers [4]. High-molecular-weight RNA undergoes partial endonucleolysis (fragmentation) [2,3]. It can be hypothesized that specific low-molecular-weight RNA is involved in the regulation of various processes at the cellular, tissue, and organism levels. These RNA molecules irrespective of their origin produce similar effects, which determines high efficiency of Ribotim in wound healing.

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