Ointments with Superoxide Dismutase and Interleukin-1β: Effect on Reparative Processes and Impedance of Burn Wound

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We found that tissue impedance can serve as a reliable criterion of the severity of wound process and efficiency of burn treatment. Ointment with superoxide dismutase effectively promoted wound reparation and recovery of tissue structure after thermal skin burn compared to ointments containing interleukin-1β or mixture of interleukin-1β and superoxide dismutase.

Key Words: *interleukin-1β; superoxide dismutase; burns; reparation; tissue impedance*

The development of methods for local therapy of burn wounds is an urgent problem of surgery. Recent experimental and clinical observations indicate that tissue damage during mechanical and thermal traumas is accompanied by overproduction of reactive oxygen species (ROS) and activation of lipid peroxidation (LPO) in the area of paranecrosis and surrounding tissues. ROS and LPO products are highly toxic for biological systems at the molecular, cellular, and organism levels. As regards the wound process, intensification of free radical oxidation and progression of oxidative stress are followed by damage, cytolysis, and death of partially viable and intact cells. These changes contribute to the development of secondary necrosis. Published data show that ROS and LPO have an adverse effect on tissue regeneration (including epithelial tissue) [1]. Enhanced production of ROS, high LPO intensity, and reduced antioxidant capacity of blood plasma decelerate wound healing [5,6]. The use of medicinal preparations suppressing generation or degrading ROS and LPO products is a pathogenetically substantiated approach. Local application of preparations with antioxidant enzymes and several cytokines stimulating wound healing holds much promise in this respect.

Histological examination of burned skin at different terms after trauma is one of the major approaches to studying reparative processes. There are only a few methods for noninvasive vital monitoring of changes in burned tissues, particularly those covered with burn eschar. Some methods are based on recording of blood flow in tissues and imply peroral or intravenous administration of dyes (disulfinate blue, Evans blue, patent blue, bromophenol blue, dimiphen blue, *etc.*) or radioactive substances (*e.g.*, technetium isotopes). However, these imperfect methods are not extensively used in practice. Physical methods of diagnostics (thermometry and thermography, infrared probing, Doppler sonography, and diffusion of ultrasound and laser radiation) require complex devices, have several methodical imperfections, and cannot be used in animal studies. We proposed a method of impedance measurement, which allows evaluating electrophysiological characteristics of the skin.

Reparative processes and changes in impedance of burned skin were studied in rats after local application of preparations containing proinflammatory cyto-

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kine interleukin-1β (IL-1β) and the major component of the cell antioxidant system superoxide dismutase (SOD).

MATERIALS AND METHODS

Experiments were performed on 36 male outbred albino rats weighing 200-220 g. The animals were intramuscularly anesthetized with calipsol and droperidol in doses of 70 and 1 mg/kg, respectively. Skin burn was produced by application of tubes with boiling water (100°C) to the depilated skin for 10 sec. Each rat had burns with a diameter of 1 cm in 4 skin areas. The wounds were treated with the test preparation (ointments with IL-1β, SOD, or mixture of IL-1β and SOD). Polyethylene oxide 1500 (PEO-1500, ointment base) was applied to the control area. Treated and control areas were arranged in the following order: upper left area, PEO-1500 (control); upper right area, IL-1β; lower left area, SOD; and lower right area, mixture of SOD and IL-1β. Burn wounds were covered with gauze fixed with loop sutures. The dressing was changed daily. Ointment preparations were repeatedly applied to the skin. We estimated external characteristics of the wound, presence and type of discharge, and state of granulations. The terms of eschar detachment and completion of epithelization were evaluated. Impedance of the skin was measured before and immediately after infliction of burn trauma and on days 3, 5, and 10 (50, 100, and 1000 Hz). Tissue impedance was measured using 2 needle electrodes with a diameter of 0.8 mm (interelectrode distance 4 mm, depth 3 mm). Impedance was estimated using a balanced system (alternate current bridge). The polarization coefficient was calculated as the ratio between impedance at low (50 Hz) and high frequency (1000 Hz). Biopsy specimens were taken from the wound and examined morphologically (light microscopy) on days 3, 5, 10, and 15 after trauma. For histological examination the samples of internal organs were fixed in 10% neutral formalin, treated with alcohols in increasing concentrations, and embedded into paraffin. Paraffin sections were stained with hematoxylin and eosin. The samples were photographed under a microscope equipped with an Opton attachment. The significance of differences was estimated by Student's *t* test.

TABLE 1. Terms of Eschar Detachment and Epithelization under Different Regimens of Local Therapy ($M \pm m$, n=9)

Preparation	Eschar detachment, days	Completion of epithelization, days
Control (PEO-1500)	8.9±0.6	17.0±0.9
IL-1β	6.8±0.7*	16.7±1.1
SOD	8.0±0.9	14.2±0.9*
IL-1β+SOD	7.2±0.9	15.2±1.3

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

RESULTS

Burn trauma was accompanied by the formation of a thin gray-white crust (surface eschar). The burned area looked like a slight prominence above the intact skin. Thickening and consolidation of the skin were revealed palpatory. The skin became less elastic. Eschar was rigid and difficult to become folded. One day after the incidence of trauma external characteristics of the burned surface did not differ between various animals. The wound was covered with a dense light-brown eschar; we revealed no discharge or signs of inflammation. Venous thrombosis under the eschar was seen in some animals. Application of the ointment with IL-1β accelerated wound cleansing from necrotic tissue, which occurred on days 6-7 after trauma ($p<0.05$). Detachment of the whole eschar usually exposed the wound surface covered with granulation tissue and minor discharge. Then the skin contracted and the wound healed via marginal epithelialization. Application of the ointment with SOD had little effect on the time of eschar detachment, but considerably improved epithelization ($p<0.05$, Table 1).

Electrophysiological study (*e.g.*, impedance measurement) is used to monitor the course of wound

TABLE 2. Polarization Coefficient of Burn Wounds under Various Regimens of Local Treatment ($M \pm m$, n=9)

Preparation	Immediately after burn	Time after burn			
		after 24 h	after 3 days	after 7 days	after 10 days
Control (PEO-1500)	1.784±0.030	1.40±0.04	1.384±0.020	1.33±0.03	1.59±0.03
IL-1β	1.784±0.030	1.776±0.070*	1.472±0.030*	1.38±0.02	1.96±0.06*
SOD	1.784±0.020	2.05±0.09*+x	2.05±0.05*+	1.91±0.02*+	2.76±0.04*+
IL-1β+SOD	1.784±0.030	1.77±0.02*	1.93±0.03*	1.96±0.04*	—

Note. $p<0.05$: *compared to the control; +compared to IL-1β; xcompared to IL-1β+SOD.

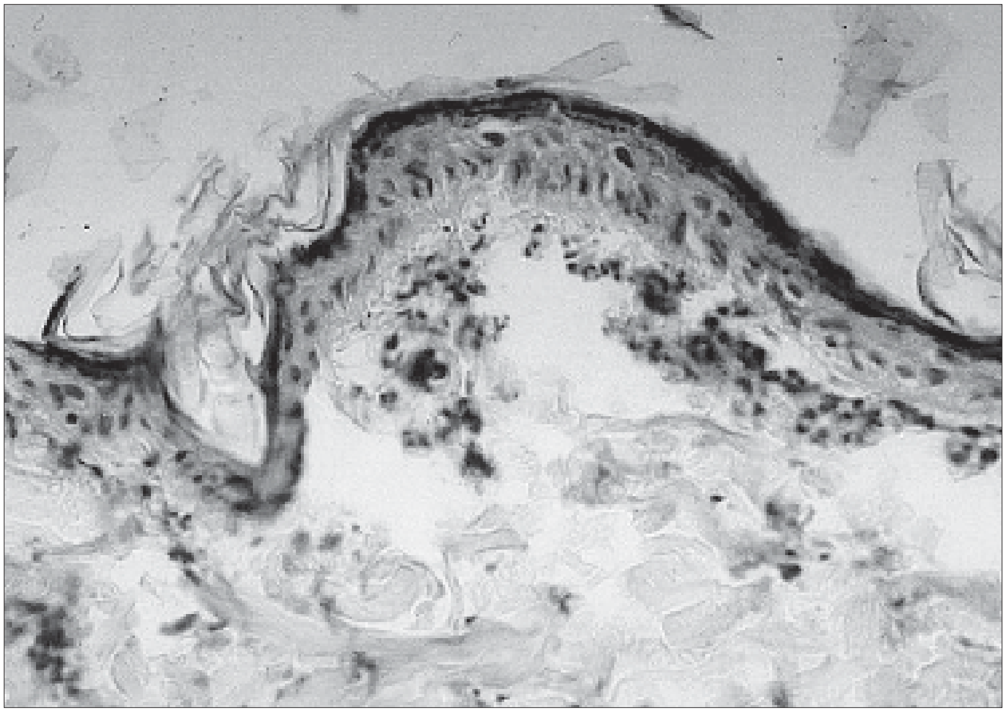

Fig. 1. Eschar detachment on day 10 after burn trauma and application of ointment with IL-1β. Staining with hematoxylin and eosin, ×180.

changes. Impedance is the integral resistance to alternating current. For biological tissues of particular importance are active (resistive) and capacitive components of impedance, because inductance of biological objects approaches zero and can be ignored. At low frequencies the resistance does not depend on frequency and remains constant. Capacitance reactance decreases with increasing the frequency. The higher is the capacity of biological objects, the greater is the effect of capacitance reactance on impedance. Polarization capacity of cells, fasciae, and tendinous intersections should be taken into account at a frequency of below 1000 Hz. Polarization capacity appears at the boundary between high-conductivity region (cytosol and interstitial fluid) and dielectric (membrane, fascia, and tendon sheath). The higher is the heterogeneity of tissue, the greater is the influence of frequency on impedance. Impedance decreases with increasing the frequency. Impedance of the subcellularly homogenous tissue little depends on frequency. The absolute

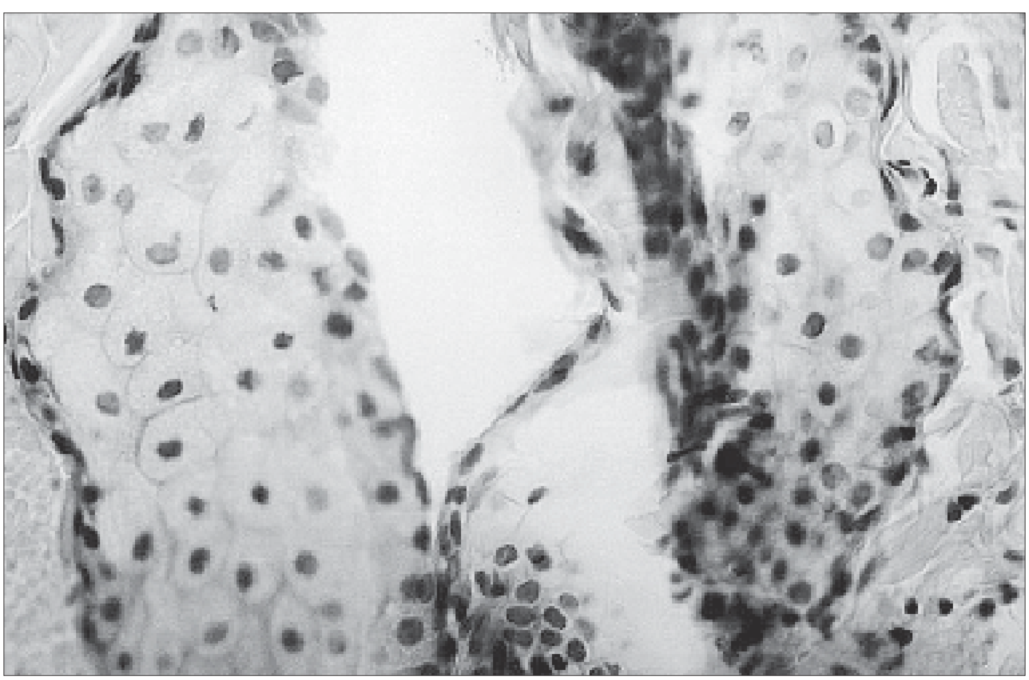

Fig. 2. Proliferation of epithelium of the sebaceous gland in surface dermal layers on day 10 after burn trauma and application of ointment with SOD. Staining with hematoxylin and eosin, ×240.

value of tissue impedance undergoes considerable variations and depends on the quality and shape of electrodes, interelectrode distance, and other factors. The dimensionless coefficient of polarization is the ratio between impedance at low and high frequency estimated by the same method of measurement. It was shown that tissue destruction (cytolysis, necrosis, degeneration, and atrophy) reduced the polarization coefficient.

Impedance measurement showed that death of surface layers in the skin after thermal burn is accompanied by a sharp decrease in the polarization coefficient (up to 1.784). Application of PEO-1500 to the wound further decreased this parameter, which reached minimum on day 7 (1.33). The polarization coefficient slightly increased on day 10 (1.59), but was lower than in other samples. PEO-1500 probably has osmotic activity and produces tissue dehydration. The polarization coefficient was low and only slightly increased on day 10 after application of ointment with IL-1β (1.96, Table 2).

Potent proinflammatory cytokine IL-1β activates the nonspecific resistance system and recruits activated neutrophils and macrophages to the wound [2,3]. Activity of these immunocompetent cells contributes to more rapid wound cleansing from necrotic tissues after local application of IL-1β (Fig. 1).

Treatment with the ointment containing SOD (antioxidant enzyme) rapidly decreased the severity of inflammation and accelerated the formation of burn eschar. These changes improved the follow-up period and accelerated wound healing (Fig. 2). The polarization coefficient was maximum after local application of SOD in various periods of observations. We compared the polarization coefficient of 4 areas in each specimen to exclude individual differences. The polarization coefficient increased in the following order: PEO<IL-1β<IL-1β+SOD<SOD.

Thus, impedance measurement confirmed high therapeutic activity of the ointment with SOD. The polarization coefficient rapidly returns to normal, which reflects structural recovery of the damaged tissue and acceleration of the posttraumatic regeneration.

REFERENCES

1. B. S. Vikhriev and V. M. Burmistrov, *Burns: Manual for Physicians* [in Russian], Leningrad (1986).
2. V. G. Konusova, A. S. Simbirtsev, S. A. Ketlinskii, *et al*., *Vestn. Ros. Akad. Med. Nauk*, No. 2, 27-29 (1993).
3. A. S. Simbirtsev, *Novosti Otorinolaringologii Logopatologii*, No. 4, 10-15 (1997).
4. B. N. Tarusov, *Bases of Biological Physics and Biophysical Chemistry* [in Russian], Moscow (1960).
5. Y. Kiyohara, K. Nishiguchi, F. Komada, *et al*., *Biol. Pharm. Bull*., **16**, No. 3, 1146-1149 (1993).
6. Y. Niwa, *Dermatologica*, **179**, Vol. 179, Suppl. 1, 101-106 (1989).