Effect of Local Application of Superoxide Dismutase on Dielectric Parameters of Cooled Skin in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 11, pp. 523-525, November, 2008
Original article submitted February 7, 2008

The effect of on Changes in dielectric parameters of the skin (modulus of complex dielectric permittivity |ε| and dielectric loss tangent tgδ) were studied on rats with local surface contact cooling followed by treatment with various cream formulations. Addition of antioxidant superoxide dismutase (SOD) to the cream significantly prevented the shifts in these parameters, which attested to less pronounced changes in the water balance in SOD-treated skin. Application of SOD during the early terms after cooling accelerated wound healing. Histological examination performed on posttraumatic day 60 revealed better integrity of the skin structures (hair follicle, sweat and sebaceous gland), which indicates ability of SOD to prevent and ameliorate the degree of cold-induced damage in the skin.

Key Words: *cooling; frostbite; UHF dielectrometry; superoxide dismutase; antioxidants*

Frostbites belong to the most severe traumas often leading to disability. Ointments containing animal fat, glycerol, and herbal preparations were tried as the preventive means against frostbites [1,2]. The emergency care procedures during frostbite of the limbs are well substantiated and developed. To this end, heat insulation bandages are applied to the affected limbs, thereafter a complex intraarterial and intravenous infusion therapy is carried out to eliminate vasospasm, prevent thrombosis, and improve rheological properties of the blood. Adequate therapy can be performed only in hospitals. There is a need to develop simple and efficient first aid facilities that could be used by the victims themselves. At present, there are no formulations that could efficiently moderate the degree of damage produced by cooling of the skin. One of the avenues of investigation is the use of antioxidants such as SOD.

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Our aim was to study the possibility of local application of antioxidant enzyme SOD to treat frostbites at the early terms after traumatic accident.

MATERIALS AND METHODS

The experiments were carried out on male albino rats (n=6) weighing 200-220 g. The dorsal surface of the body was shaved under inhalation ether anesthesia. The frostbites were produced with a cryoablator on 4 cutaneous sites, the area of each site being no less than 1.5 cm^2. The tip of the cryoablator (diameter 1 cm) was applied normally to the skin for 30 sec.

Measurements of skin permittivity *in vivo* were carried out using contact reflection UHF-dielectrometer at a probe frequency 60 GHz and energy flow density no more than 5 $\mu W \times cm^{-2}$. Permittivity was measured before frostbite, immediately after it, and 30 and 60 min after treatment of the affected area with the test cream compositions. The cream base was the same in any formulation, but the experi-

0007-4888/08/1465-0588

mental cream contained recombinant human SOD (0.1 mg/ml). The affected skin sites in the shoulder girdle were treated with experimental (SOD-containing) cream. Skin areas located caudally serves as the control.

In 60 days, the biopsy samples were taken for histological examination. The skin patches were fixed in 10% neutral formalin, passed through alcohols of increasing concentrations, and embedded into paraffin. The deparaffinized sections (7 μ) were stained with hematoxylin and eosin, and with picrofuchsin according to Van Gieson method.

RESULTS

Table 1 shows the modulus of complex dielectric permittivity |ε| and dielectric loss tangent tgδ of rat skin (Table 1).

On the traumatized sites treated with experimental (SOD-containing) cream, the developed epidermis was thinned insignificantly as compared to the intact skin. There were local foci of atrophy in the papillary layer of the derma, while its collagenation was poorly expressed. Skin appendages were completely preserved. The control sites of the skin were treated with SOD-free cream. In some of them, the thickness of the epithelial layer varied. Foci of pronounced atrophy were also observed. The derma had sites of pronounced fibrosis, indurations, and homogenization of collagen fibers. Despite complete epithelization of the damaged area, there were pronounced alterations in the structures of skin appendages with focal atrophy, formation of cyst in hair follicles, and cyst-like widening of the glandular ducts.

The increase in the modulus of complex dielectric permittivity of the skin after termination of cold exposure in any tested part of the body indicates elevation of tissue hydration. In other words, frostbite increased water content in the affected skin regions. In the first minutes, dielectric loss tangent slightly decreases, which reflected the increase in the content of predominantly non-structured (extracellular) water. Changes in this parameter probably indicate the development of interstitial edema. It is noteworthy that the skin in scapula region is more vulnerable to the action of frostbite than the skin in pelvic girdle, because cutaneous hydration is more pronounced in the former region. The observed phenomenon was statistically proved by pair sample methods, and it indicated a stable dependence of water content and sensitivity to extreme exposures on anatomic localization of the skin site.

The dynamics of hydration differed qualitatively in the control and experimental (SOD-treated) sites. In experimental sites, the progress in hydration was accompanied with further decrease in tgδ, which attested to impairment of water structural organization and aggravation of interstitial edema. In control sites, hydration increased less pronouncedly than in the experimental sites, but tgδ increased or stabilized itself indicating predominantly accumulation of intracellular water. Since animal cells are much more resistant to dehydration than to hyperhydration, interstitial edema is more favorable than cell edema. The indices of water metabolism in damaged tissues treated with SOD attest to more optimistic prognosis of vitality despite more pronounced indices of total hydration measured immediately after cooling of the skin in scapula in comparison with the skin in pelvic area.

One hour after application of the cream, water content in experimental sites decreased and dielectric loss tangent increase, *i.e.* edema became less severe. In the control site, the corresponding reparative alterations were less expressed despite smaller degree of initial damage.

It can be concluded that during functional shifts in skin water metabolism, the correlation between dynamics of total water content (|ε|) and dielectric loss tangent (tgδ) is negative, but it becomes positive after the development of organic disturbances in the skin.

The chosen protocols of cooling induced the surface frostbites that did not involved entire depth

TABLE 1. Effect of Frostbite of the Skin with Liquid Nitrogen on Cutaneous Dielectric Parameters and Their Dynamics after Surface Application of SOD-Containing Cream

Time	Control site		Experimental site	
	|ε|	tgδ	|ε|	tgδ
Before cooling	19.55±0.23	2.64±0.17	19.64±0.20	2.89±0.15
Minutes 5-7 after cooling	19.80±0.15*	2.60±0.17	20.47±0.34*	2.60±0.19*
Minute 30 after SOD application	19.94±0.21*	2.79±0.18*	20.96±0.26*	2.42±0.14*
Minute 60 after SOD application	19.73±0.24*	2.73±0.16	20.06±0.22*	2.54±0.18*

Note. *$p<0.1$ compared to previous measurement (method of paired samples).

of the skin. Respectively, healing of the wound proceeded self-maintained due to preserved appendages of skin. These preserved skin appendages also provided proliferation of epidermis. During this process, scab rejection can occur at various terms.

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