NANOTECHNOLOGIES

Effects of a Nanocomplex Containing Antioxidant, Lipid, and Amino Acid on Thermal Burn Wound Surface

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Dihydroquercetin (flavonoid of plant origin) immobilized with an amino acid in lecithin nanoparticles promotes reduction of inflammatory reactions in the wound after thermal burn. The use of a liposomal complex in burn injury stabilizes endogenous antioxidant system and limits the secondary necrotic zone in the wounds. The treatment was associated with intensification of skin regeneration processes and reparation of hairy follicles and sebaceous glands.

Key Words: *antioxidants; oxidative stress; thermal burn; skin structure; regeneration*

The search for effective means for the treatment of thermal and chemical burns is a pressing problem of medicine. Recent experimental and clinical studies showed that tissue injuries are associated with phagocyte migration to the focus of damage and their subsequent activation leading to the development of oxidative stress [4]. Activation can be paralleled by excessive production of reactive oxygen forms, cytotoxic for normal and damaged cells [2,3,8]. Antioxidants can correct the negative aftereffects of phagocyte hyperactivation. A drug containing an antioxidant is to be nontoxic and easily applied to the damaged surface and the dosage form is to be long acting. It is therefore advisable to use antioxidants in the liposomal form preventing reactive inflammation and LPO and stimulating regeneration processes. We selected a drug containing natural flavonoid dihydroquercetin (DHQ, this substance is a highly active antioxidant due to the presence of five hydroxyl groups) and is immobilized in the phospholipid container with glycin. This nanocomplex was developed by Flamena research company and its commercial name is Flamena.

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MATERIALS AND METHODS

The effects of Flamena on healing of wounds after thermal burns were studied on male Wistar rats (220-250 g). The animals were handled in accordance with Regulations for Studies on Experimental Animals. The animals were divided into 2 groups: 1) rats with thermal burns (control; n=10) and 2) rats in which the wounds after thermal burns were treated with Flamena 4 times per day until decapitation (experimental group; n=30).

Before the experiment, the hairs on the upper dorsal scapular area were removed by shaving and depilation. Thermal burn of the skin was inflicted with a metal rod (1 cm^2) heated to 100±5°C. As a result, the animals had II degree burns.

The concentration of DHQ in Flamena is 4 mg/ml liposomes. Other components of the drug are 10% lecithin (membrane-forming phospholipid) and 5% glycin (amino acid) solution in 0.1% water/ethanol. The liposomes obtained by interphase separation varied in size from 20 to 300 nm.

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The wound process was controlled by objective criteria: planimetry of the wound defect area and dynamic morphological analysis of longitudinal histological sections of the skin from the wound and adjacent area of control and experimental animals. Skin specimens (wound and adjacent skin) for histological studies were fixed in 10% formalin. The sections (5-7 μ) were prepared by the standard method, membrane structures and cell cytoplasm were stained with hematoxylin and eosin after Ehrlich [5].

Histological sections were analyzed under an Axiovert 200 microscope. The rate of burn wound epithelialization was evaluated by planimetry. The measurements were carried out every day. The area of the wound was repeatedly measured and the percent of its shrinkage over 24 h was evaluated by the formula:

$$(S\text{-}Sn)\times 100/St,$$

where S is wound area during previous measurement, Sn wound area during the present measurement, and *t* is number of days between measurements.

RESULTS

The dynamics of local changes in burn injury is the same as in any other injury: initial anatomic and functional changes, reactive inflammatory events, and regenerative processes. In controls, the area of thermal injury had a clear-cut borderline, with starting formation of burn bleb (Fig. 1) and erythema around the wound. Later, the damaged area increased 1.2-1.3 times; signs of inflammation and necrosis were seen (Fig. 1, *b*). In the experimental group, the area of involved skin and paranecrotic area did not increase with time (Fig. 1, *c*, *d*). The wound crust was smooth, with even healing under its surface, without signs of inflammation; the burn wound area shrank by 15±2% after 3 days.

For detailed study of the effects of Flamena, histological sections of the skin were made at equal time intervals after thermal burn (Fig. 2). Destruction of the epidermis and destructive processes in the derma were seen in the control group 5 days after thermal burn (Fig. 2, *a*). In animals treated with Flamena, a destroyed fragment of the epidermis was clearly seen under remaining necrotic tissue, while the adjacent area regenerated and all layers constituting it were seen (Fig. 2, *d*). Chaotically scattered fibroblast-like cells were seen under the epidermis in the dermal layer. The number of proliferating cells increased in the basal layer of the epidermis and in the papillary and reticular layers of the derma close to the injury. After 10 days, destroyed skin sites were still seen in the thermal burn focus in the control group: the epidermis was destroyed, hair follicles, sebaceous glands, and the adjacent connective tissues were damaged (Fig. 2, *b*). In experimental animals regularly treated with Flamena, the demarcation line between the destroyed

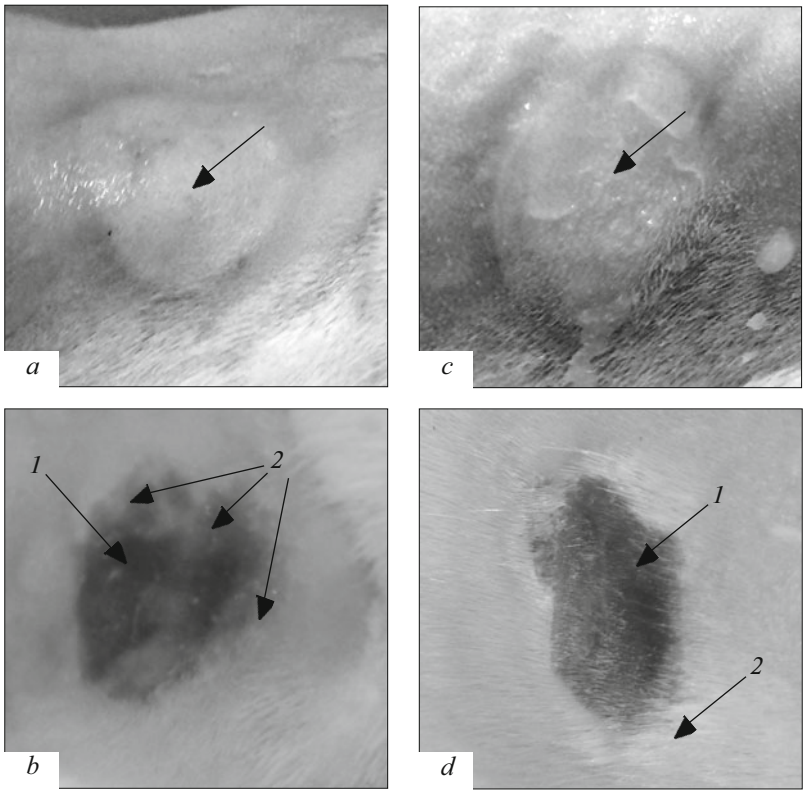

Fig. 1. Wound status after thermal burn in control (*a*, *b*) and experimental (*c*, *d*) groups. *a*) wound directly after burn (arrow shows the center of the wound; S=1.16 cm^2); *b*) 72 h after burn (*1*: inflammation focus; *2*: paranecrosis; S=1.5 cm^2); *c*) wound directly after burn infliction (arrow shows the center of the wound, S=1.32 cm^2); *d*) 72 h after treatment (*1*: center of the wound; *2*: restored hair; S=1.05 cm^2).

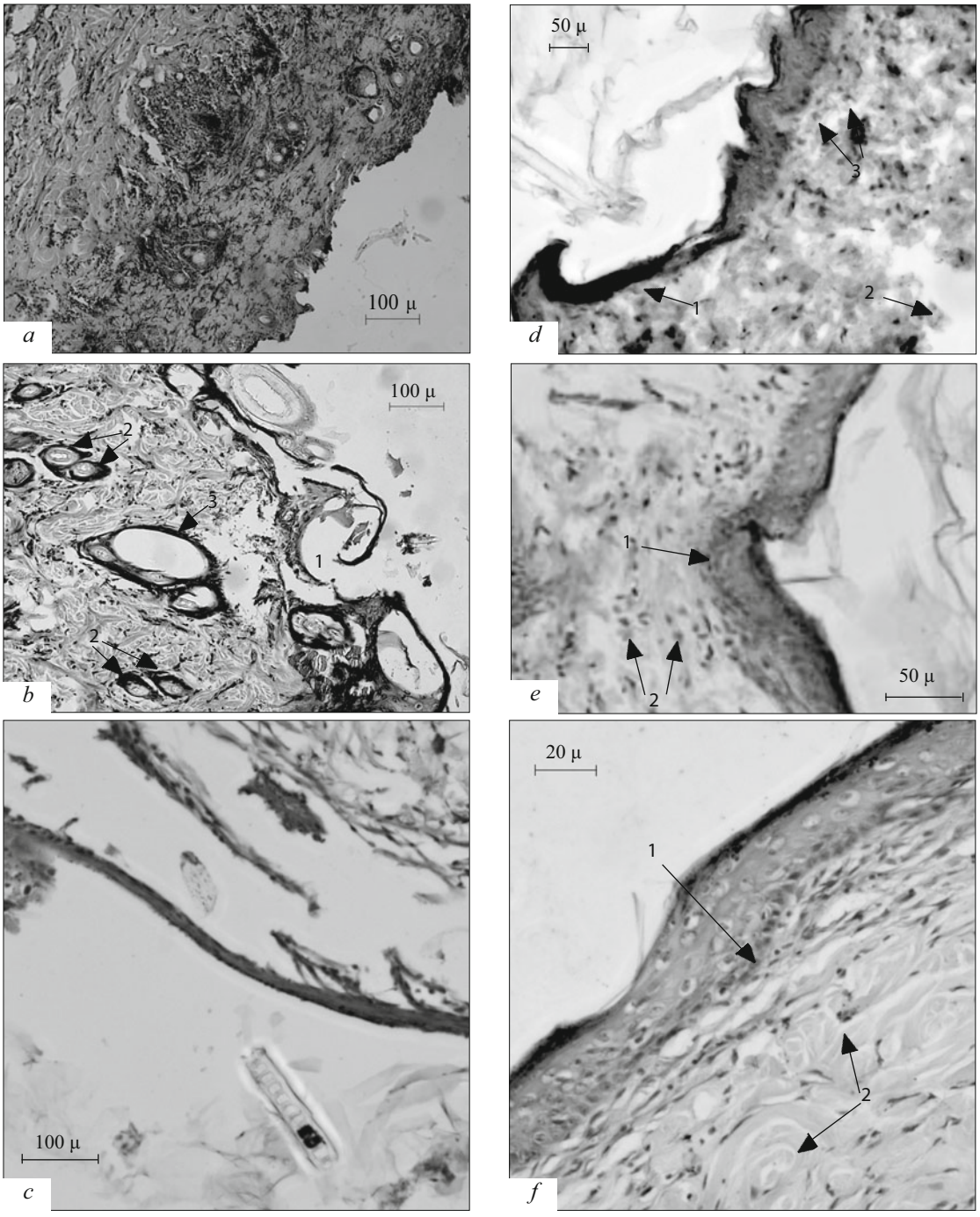

Fig. 2. Time course of skin regeneration in rat 5, 10, and 15 days after thermal burn in the scapular area in control (*a*-*c*) and experimental (*d*-*f*) groups. Hematoxylin and eosin staining. *a*) destroyed epidermis and destructive process in the derma as a result of thermal burn; *b*) skin destruction in burned area. The epidermis (*1*), hairy follicles (*2*), sebaceous glands (*3*), and adjacent connective tissues are destroyed; *c*) clearly seen homogeneously stained epidermis, which lost its cellular structure. Empty spaces under it formed at the site of destroyed derma; *d*) destroyed fragment of the epidermis (*1*) under the rudiments of necrotic tissue. Derma with destroyed structure (*2*) and chaotically scattered fibroblast-like cells under destroyed epidermis (*3*); *e*) activation of basal layer cells under necrotic epidermal layers (*1*). Islets of cells in different mitotic phases (*2*) in the derma; *f*) well-formed epidermal layer and cell multiplication in the basal layer (*1*). Fibrous connective tissue in the underlying derma. Potent bundles of collagen fibers (*2*) in the deeper reticular layer. The structure approaches the structure of intact skin.

and regenerating layers was clearly seen 10 and 15 days after burn; cell activation in the basal layer was observed (Fig. 2, *e*, *f*). Cells in different mitotic phases were clearly seen in the dermal layer. Analysis of specimens from controls showed destruction of the epidermis, accumulation of undifferentiated dermal cells,

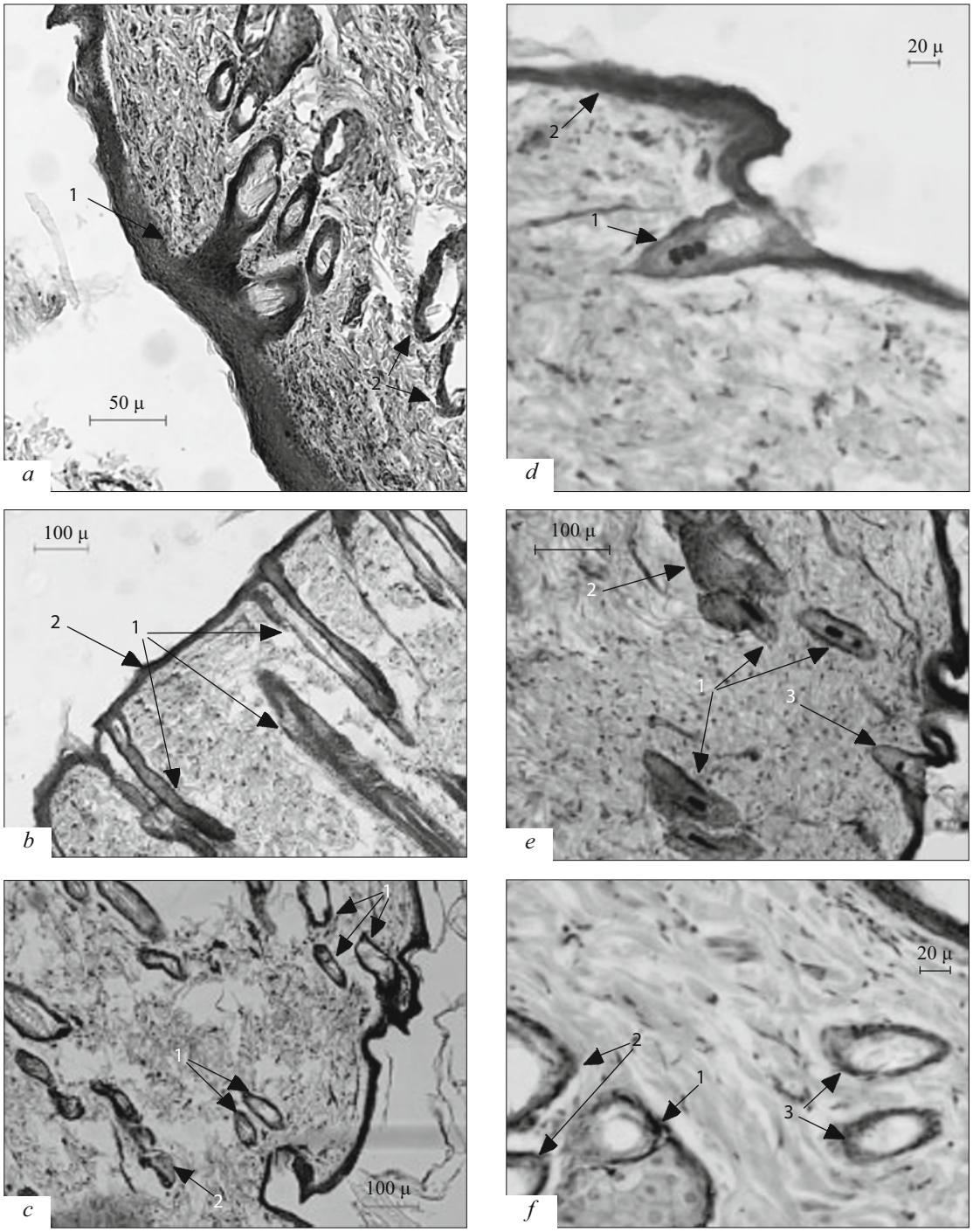

Fig. 3. Formation of hairy follicles during skin regeneration in control (*a-c*) and experimental (*d-f*) groups. Hematoxylin and eosin staining. *a*) destroyed epidermis (*1*), destroyed hairy follicles and accumulation of undifferentiated cells in the derma (*2*); *b*) destroyed hairy follicles (*1*), destroyed epidermis (*2*), impaired structure of underlying derma; *c*) destruction of the surface layer and hairy follicles (*1*). No integral structures of sebaceous glands are seen (*2*); *d*) formation of a hairy follicle (*1*) in regenerating epidermis (*2*); on the top of this fragment from the left to right: dark thick fragment of regenerating epidermis, restored hairy follicle, and fragment of destroyed epidermis; *e*) group of hairy follicles (*1*) and sebaceous gland (*2*) at the site of burn and hair growth above skin surface (*3*); *f*) sebaceous gland (*1*) in the surface layer of the derma; poorly differentiated cells, capable of mitosis (*2*), in the terminal compartments of sebaceous gland at the interface between the papillary and reticular layers. Sites of hairy follicles nearby (*3*).

indicating destructive processes in this zone (Fig. 3). Destruction of hair follicles and impaired structure of the lower dermal layers were seen 15 days after burn. In the experimental group, new hairy follicles were forming in the regenerating epidermis (Fig. 3, *d*, *e*), the structure of sebaceous glands was much better

retained and capable of regeneration (Fig. 3, *f*). These data suggest that Flamena is an effective drug for local treatment of wounds after chemical [1] and thermal burns. This dosage form reduced the effects of factors promoting the progress of necrosis, development of tissue ischemia, and wound infection. All this created optimal conditions for tissue regeneration. For many years, antioxidants were widely used for prevention and therapy of many diseases, including burn injuries. Dihydroquercetin, the basic component of Flamena, is a highly effective antioxidant binding free radicals and protecting from LPO processes. The use of antioxidants in burn injuries stabilizes the endogenous antioxidant system and limits the zone of secondary necrosis in the wounds by inhibiting the development of free radical LPO in biomembranes and other cell structures.

In addition, this drug exhibited anti-inflammatory effects. This was seen from the absence of wound suppuration and minimization of damaged surface. The absence of inflammatory reaction after thermal and chemical burns can be due to the flavonoid capacity to form complexes in the presence of metals of alternating valence, for example, complexes with iron. In addition to antioxidant effects, these complexes can exhibit prooxidant activity [9] preventing bacterial development on the wound surface. Another possible cause of inhibition of the inflammatory reaction is reduced histamine secretion by mast cells [7] in the presence of DHQ.

In addition, the composition of liposomal Flamena preparation supports the mitochondrial respiratory function [6], inhibits apoptosis, and due to its components glycin and lecithin promotes cell membrane regeneration. All components of Flamena normalize and support tissue homeostasis and hence, stimulate skin regeneration with restoration of skin derivatives after II degree chemical [1] and thermal burns.

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