METHODS

Modeling of Thermal Skin Injury for the Development of Local Treatment Drugs

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The paper describes simple and reliably reproducible methods of inducing superficial and deep skin burns (both infected and non-infected) in rats, which model IIIa and IIIb degree burns in humans. These methods reduce the costs and accelerate the development of local preparations.

Key Words: *skin; thermal burn; infected burn; animal burn model; reproducibility*

Experimental modeling of thermal injury on animals helps to study pathogenesis of thermal injury and develop therapeutic strategy and is a necessary element of preclinical trials [1]. The choice of specific burn model determines the generality of experimental results and duration and the cost of the development of drugs.

The degree of experimental burn (the depth of tissue damage) is determined by the tasks of the study. Superficial skin burn is an adequate model for preclinical testing of local wound healing preparations, while deep burns are used in the studies of pathogenesis of burn disease and for development the therapeutic strategy [5].

In large laboratory animals (pigs and dogs) thermal burns develops through the same stages and approximately in the same periods as in humans: in both species thermal injury is aggravated by pronounced suppurative inflammation and intensive exudation [5, 6]. The use of small animals (rodents) helps to save time and money and allows performing parallel experiments on large groups, but has a number of disadvantages due to species-specific peculiarities of these animals [2,3,6,8].

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Mouse and rat skin considerably differs from human skin: it is characterized by poorly developed subcutaneous fat and easy folding and retraction. In rodents, cleansing of thermal and suppurative wounds from necrotic detritus goes on predominantly via sequestration and contraction, while suppuration and enzymatic cleansing, the basic mechanisms in primates, are poorly expressed [2,6,8].

Modeling of superficial skin burns (guarantied absence of deep burns) with high-temperature agents is associated with some difficulties. Since the thickness of the skin depends on its localization and animal weight, age, and gender, it is necessary to control the depth of tissue damage in each series with every species both immediately after burn and during follow-up in order to develop a reproducible model. The severity of the trauma (depth of the burn) depends on the time of exposure. It should be noted that during the first 2-3 days after modeling superficial and deep burns skin surface in small animals looks similarly.

In the study of local drugs, burns are produced predominantly on the back and sides of the body. Burn of the extremities should be avoided, because the formation of dense circular crust can lead to ischemia of their distal parts. In addition, this trauma disturbs animal mobility and self-service. By the same reason, burns of the snout and perineum should also be avoided.

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The effect of thermal agents on the skin is determined by their physical nature (flame, hot water, steam, hot or molten metal, molten plastic, bitumen, *etc*.). In each case, heating of the tissues has specific peculiarities, which determine the size of paranecrotic area (and primary and secondary necrosis), the dynamics of eschar development and rejection, type of necrosis (coagulation or colliquative), the degree of thermal damage to visceral organs or general overheating, *etc*. Modeling of burns with the above agents has some peculiarities determining the advantages and shortcomings of the corresponding model [5].

Since the inflammation in rat skin are less expressed than that in human skin, additional infection of the thermal wound is required. To this end, a method providing certain degree of infection of the modeled thermal wounds has to be developed.

Here we describe simple and reliably reproducible methods of inducing superficial and deep burns (infected burns included) in rats. These models were used for studying the efficiency of ointments containing pine resin [7] and enzymatic preparations (the enzyme preparations Hydrobiont-Derived Collagenase, Polycollagenase-K, Fermenkol, Highly Purified Collagenolytic Protease, and the wound drainage sorbents Collasorb and Colladiosorb are currently produced) with collagenolytic proteases from invertebrates [4] for the treatment of IIIa and IIIb degree burns.

MATERIALS AND METHODS

Experiments were carried out on male and female random-bred rats (180-200 g) from Rappolovo Animal Breeding Department (Russian Academy of Sciences). The animals were maintained under vivarium conditions on a standard ration [4,7]. The skin (4×4 cm) was shaved with a safety razor or treated with a depilatory agent 2 days before burning.

In experiments with superficial burns the skin was shaved; 0.9% NaCl (5-10 ml) was injected subcutaneously before shaving (this procedure stretches the skin and prevent damage. In experiments with deep

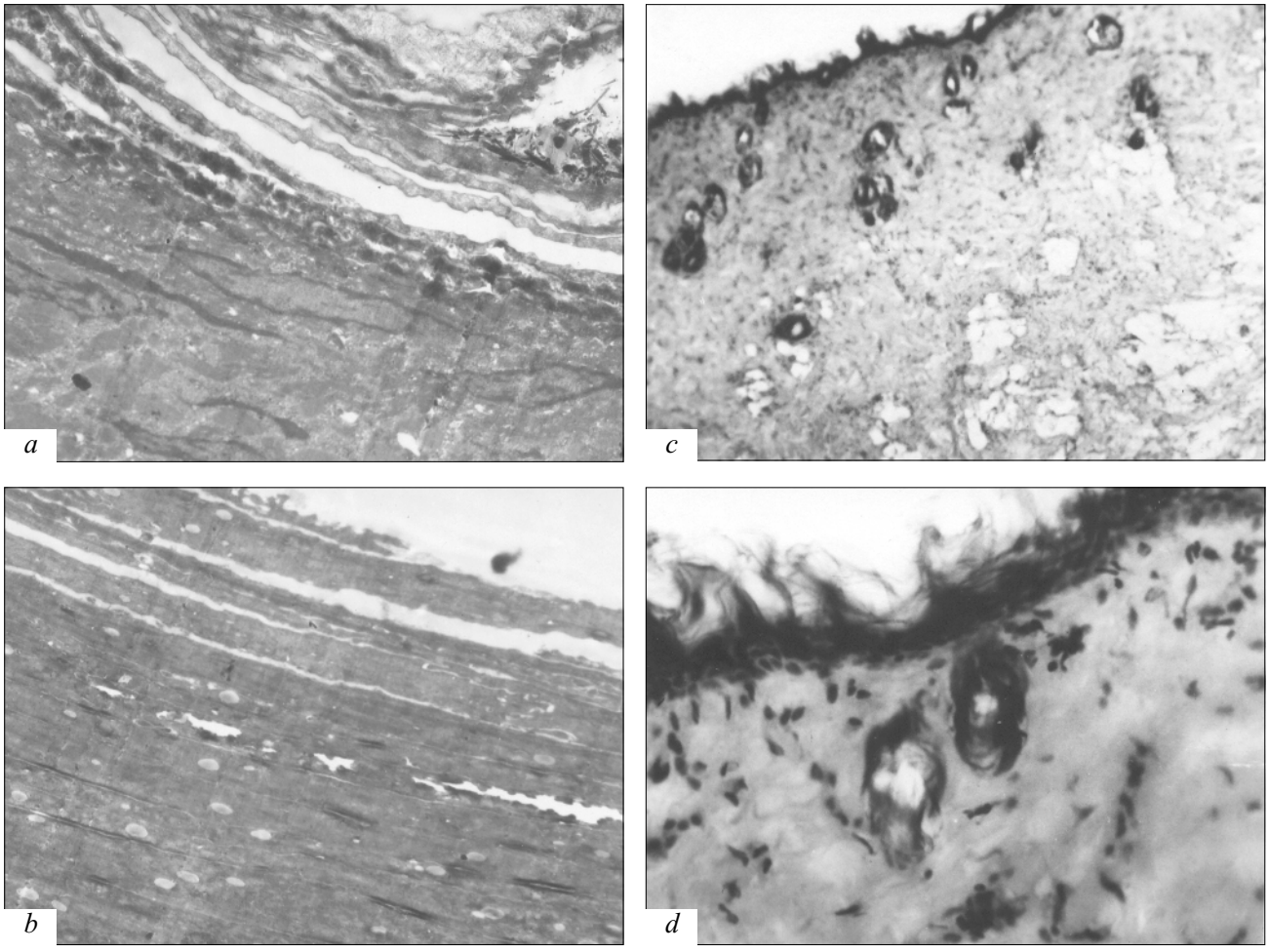

Fig. 1. Structure of the upper layers of rat epidermis 10 min after exposure to light (*a, b*) and hot water (*c, d*) for 10 sec (*a*), 30 sec (*b*), and 15 sec (*c, d*). ×7000 (*a, b*), ×120 (*c*), ×280 (*d*).

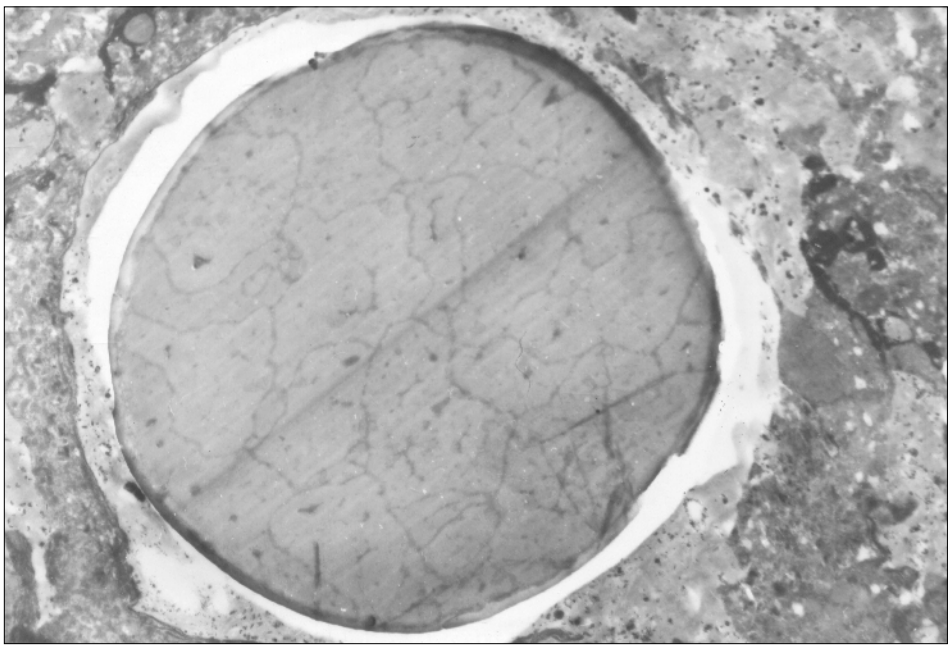

Fig. 2. Structure of a hair in rat skin 10 min after light irradiation (30 sec) producing deep burn, ×5000.

burns the skin was depilated [5]. The depilatory agent (10% Na_2SO_3 at 40°C) was applied with a tampon for 5 sec until the hair became off-yellow, washed with heated water (40°C), dried, and treated with talcum powder. No irritation was observed after accurate treatment.

The rats were narcotized intramuscularly with calipsol and droperidol (70 and 1 mg/kg, respectively). Burns (4 cm^2) were produced on the center of the back or on both sides. The later procedure was performed for precise comparison of two drugs or a drug and control agent. Extensive burns (more than 5% body surface) never used in such experiments because they aggravate general state of the animal, which notably affects the healing process in local wound.

For modeling flash burn, a pasteboard mask (2×2 cm) was tightly pressed to the skin and the animal was placed in front of a KG220-1000-4 halogen lamp (1000 W) shielded with a stainless steel shutter. The distance between the skin and the lamp was 3 cm (along the line connecting the center of irradiated skin part perpendicular to its surface and the center of glower filament situated in parallel to the plane of skin part). The shutter was removed for 10 or 30 sec, which modeled superficial and deep burns, correspondingly.

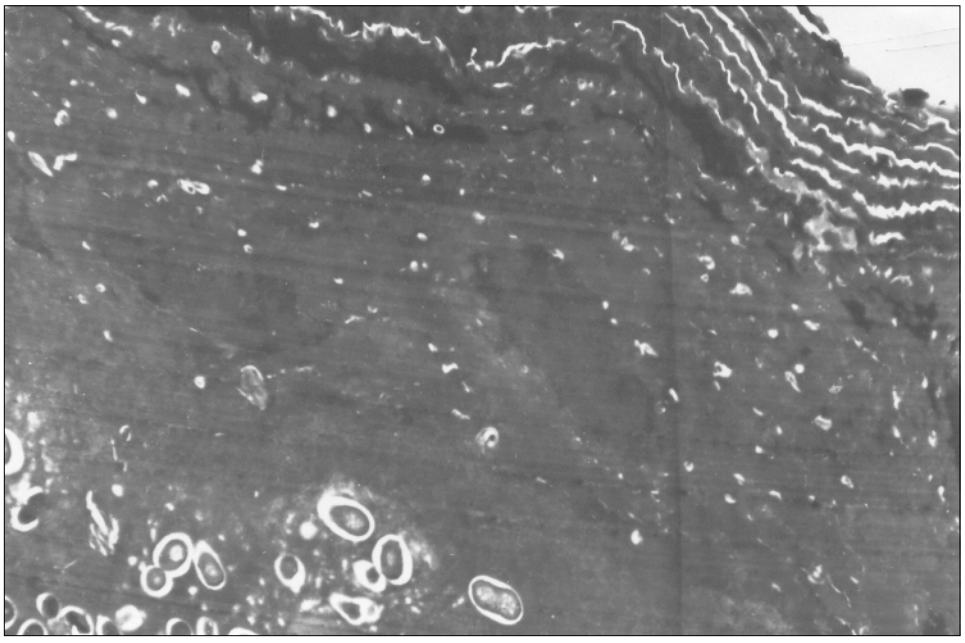

Fig. 3. Colonization of the scab with coccus microflora (*S. aureus*), ×10,000.

For modeling scald, an empty glass test-tube with internal diameter of 22 mm and the length of 20-25 cm was placed vertically in boiling water (100°C) by $^2/_3$ its length and warmed for 1 min. Then the tube was filled with water to $^2/_3$ its length and tightly applied to the skin. The rat (in contact with the tube) was turned by 180° and exposed to hot water for 15 or 30 sec, which modeled superficial and deep burns, correspondingly. After exposure, the rat was turned into normal position, and the tube was removed from the skin.

Infected burns were modeled in two stages. First, deep burn was produced by one of the described methods. Then 10^9 cells of standard Gram-positive (*S. aureus*) or Gram-negative (*E. coli, Ps. aerugenosa*) strains were suspended in 0.9% NaCl (0.5 ml) and injected under the developed scab (4 cm^2) on day 2 or 3 after burn. Both collected and hospital strains (obtained from patients' wounds) were used. Concentration of microbes was controlled by a direct count of the cells in a fixed volume at specified dilution.

For ultramicroscopy, skin biopsy specimens were fixed for 2 h in 2.5% glutaraldehyde in Hank's solution (4°C). Then the specimens were washed and fixed in 2% OsO_4 (12 h, 4°C), dehydrated with ethanol in increasing concentrations (from 40 to 100%), and embedded in Epon-812 and Araldite mixture. For electron microscopy, ultrathin sections were cut in an LKB ultramicrotome, contrasted with uranyl acetate and lead salts by the method of Reynolds, and examined under a JEM-100c emission electron microscope. Specimens for light microscopy, were fixed in 10% neutral formalin for 24 h at 24°C, dehydrated with ethanol in increasing concentrations, and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and examined under an Opton microscope.

RESULTS

Irradiation of the skin with a 10-sec light flash produced damage similar to IIIa degree burn in humans with the development of white thin surface crust, which tended to make folds (Fig. 1, *a*). Epidermal cells died, but the basal lamina and deep skin appendages were preserved, and then the epithelium regenerated from these structures. The 30-sec flash produced deep damage of the skin accompanied by elimination of subcutaneous muscle layer, which is similar to IIIb degree burn in humans with the development of dense white crust clearly seen against the intact skin (Fig. 1, *b*). It is accompanied by coagulation necrosis involving all structures and layers of the derma.

Hot water applied for 15 and 30 sec induced similar superficial and deep damages to the skin, respectively (Fig. 1, *c*). However, the histological structure of the affected skin differed from that observed after flash burn. Peculiarity of this model is the development of specific dermal ridges from dead epithelium (Fig. 1, *d*).

The degree and specificity of the deep burns can be assessed with the "hair test" widely used to diagnose burns: in deep burn, skin hairs can be easily pulled out because of weakening of their bonds with the surrounding tissues (Fig. 2). When modeling the infected burns (Fig. 3), injection of a large dose of microbes under the crust induced the development of typical inflammation specific of the applied strain.

The described simple and reliably reproducible methods of inducing superficial and deep skin burns in rats model IIIa and IIIb degree burns of human skin can be used in the large-scale modeling during the development of local drugs.

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