Animal Model of Thermal Injuries

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Abstract

Experimental studies of burns require the use of different animal models with the aim to imitate and reproduce pathophysiological conditions. The aim of this work was to establish experimental model of thermal injury.

New Zealand rabbits, weighted from 1.8 kg to 2.3 kg, were utilised during our study. Another, also utilized, animal types were laboratory Rattus rats, species Wistar, albino type, females with body weight of about 232 g. All animals were from our own litter (Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine in Sarajevo). During the experiment, animal were properly situated in adequate cages and rooms, at the controlled temperature (22 ± 2^0C), and in the air with normal humidity level. All animals took food and water ad libitum.
Rabbits received anesthesia - intravenous pentobarbital sodium in a dose of 60 mg/kg, and then, hair from the upper side of the each rabbit ear was removed and burns were caused by a metal seal in the same manner as in rats. Rats were primarily anesthesied by intraperitoneal pentobarbital sodium in a dose of 35 mg/kg, and then, their hair was removed from the scapula zone (5 cm x 5 cm). Burns were caused by contact with a round metal seal, heated at 80^0C in a water bath, during the period of 14 seconds together with contact thermometer control. Round metal seal (radius: 2.5 cm; weight: 100 g; surface: 5 cm^2) was just placed on the rat skin without any additional pressure. In order to maintain the microcirculation in the burn wound and to reduce the conversion of partial-thickness skin burns to the burns of the full-thickness skin, all burn wounds were immediately sunk in the 4^0C water. Subsequent to that procedure, all animals were individually situated in the proper cages, and left to rest for 4 hours with a constant cautious monitoring of the wound development and animal general state.

Key words: animal model, thermal injury, rabbit, rat, skin.

Introduction

Skin burn causes a complex damage of the skin. Differently from the simple incised wounds, skin burns cause gradual damages of the wound margins and make degradation biochemical complexes in the wound, what represents two additional complicating factors for the process of healing. Animal models for the thermal injury simulation, developed in the first half of the twentieth century, were crucial for the improvement of scientific acknowledgements in the field of treatment of cutaneous wounds. In addition, they were very important for the revealing of systemic effects caused by skin burns, including immunosuppression, vasodilatation, shock, sepsis and multiple-organ damages. Studies, evaluating systemic changes subsequent to the burn injury, had general use on the models of injuries of the full-thickness skin, in which epidermis and dermis were destroyed while inducing wound contraction and tissue granulation or skin grafting during the treatment. On the contrary, thermal injury treatment studies, conducted in the second half of the twentieth century, examined burns of the partial-thickness skin. Such burns do not affect all of the epithelial elements within dermis, allowing the spontaneous epidermal regeneration (re-epithelization) throughout the proliferation and migration of the epithelial cells from dermal appendages beneath the injury and from epidermis of the wound margins (1).

Experimental studies of burns require the utilisation of different animal models with aim to imitate or reproduce pathophysiological states. Objectives of animal experiments, in the field of burn investigations, were significantly changed in the last decade of the previous century. Experimental variables might be better controlled in animal models highly reflecting situation in humans (2). Skin burs are still medical problem of significance. For this reason, investigators continually use animals in their studies while making effort to develop reliable experimental models that fulfil all requirements for the scientific investigation. The objectivity of experimental model is in the reproduction of parhophysiological state of the burn with aim to analyse local or systemic changes. Main goal of the animal model development is removal or reduction of these local or systemic burn consequences (3). Different animals have been used in the animal thermal wound models. Importance of the water temperature and duration of the heat exposition regarding the profundity of thermal wound was described on the pig model (4), while another model investigated the influence of thermal wounds of the partial-thickness skin on the regional blood circulation changes (5). Matsumura at al. (1997) noticed and recorded that contact burns of the skin of partial thickness were completely re-epithelialized in

18 to 30 days depending on the depth of cutaneous damage (6). These results closely correspond to those observed in the research data on human burns of the partial-thickness skin. A white rabbit animal model of thermal injuries has been described in New Zealand, as well (7, 8).

Wound healing processes are similar in all mammals. Animal wound models utilising rodents are prevailing because of the easier animal handling and possibility of the utilisation of larger number of the experimental animals with aim to obtain improvement in the result validity. Isler et al. (1991) referred that untreated deep contact burns in rats completely healed within the period of 33 days following the injury (2). Hull and Cool (1990) proved, in their study on mice, that epithelial cell proliferation under the burn wound of the partial-thickness skin reached its maximum on the day 6th after the injury detection throughout the incorporation process of H isotope (H3-thymidine) in the burn wound microscopic sections (9). A burn wound model, developed for the monitoring of bacteria translocation from the gastrointestinal tract to the extra-intestinal areas, has also been described. The mechanism that indicates that type of translocation includes the following components: rapid increase in the number of intestinal bacteria, decreased immune response of the host, and increased intestinal barrier permeability (10).

Material and methods

Study design

Our study was designed as prospective, cross-sectional, interventional animal study with the primary goal to adopt an experimental burn wound model. Study was carried out at the Institute of Pharmacology, Clinical Pharmacology and Toxicology of the Faculty of Medicine in Sarajevo. Animals were raised and cared according to European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (11).

Experimental animals and preparation

New Zealand rabbits, weighted from 1.8 kg to 2.3 kg, were utilised during our study. Another, also utilized, animal types were laboratory Rattus rats, species Wistar, albino type, females with body weight of about 232 g.
All animals were from our own litter (Institute of Pharmacology, Clinical Pharmacology and
Toxicology, Faculty of Medicine in Sarajevo). During the experiment, animal were properly situated in adequate cages and rooms, at the controlled temperature (22 ± 2^0C), and in the air with normal humidity level. All animals took food and water ad libitum.

Study protocol and experimental model

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Rats were primarily anesthesied by intraperitoneal pentobarbital sodium in a dose of 35 mg/kg, and then, their hair was removed from the scapula zone (5 cm x 5 cm). Burns were caused by contact with a round metal seal, heated at 800C in a water bath, during the period of 14 seconds together with contact thermometer control. Round metal seal (radius: 2.5 cm; weight: 100 g; surface: 5 cm2) was just placed on the rat skin without any additional pressure. In order to maintain the microcirculation in the burn wound and to reduce the conversion from partial-thickness skin burns to the full-thickness skin burns, all burn wounds were immediately sunk in the 4^0C water. Subsequent to that procedure, all animals were individually situated in the proper cages, and left to rest for 4 hours with a constant cautious monitoring of the wound development and animal general state.

Equipment and instruments

Standard equipment and instrument were utilised during the experiment carrying out.

Macroscopic and histological sample elaboration

Histological examinations of the skin tissue samples, isolated from the animal burn wounds in different stages of the wound development, were carried out at the Institute of Pathology of the Faculty of Veterinary Medicine in Sarajevo. Macroscopic and microscopic examinations used during our study are classic methods of morpho-functional examinations.
Material for the histological examination was taken immediately after the animal sacrifices performed by pentobarbital sodium overdose (250 mg/kg, intraperitoneally). Samples of the burned skin, sized 2 cm x 2 cm, were taken from the wound zones bordering upon the healthy skin. Samples were fixed in 10% buffered formalin and embedded in paraffin. Sections of 5 μm were serially cut on a LITZ microtom and stained with haematoxylin and eosin (haematoxylin and eosin staining method - HE). Characteristic histological changes we documented by digital photos taken through an Olympus camera (12).

Macroscopic examination of rabbits

In all cases, preliminary burn on the rabbit ears resulted

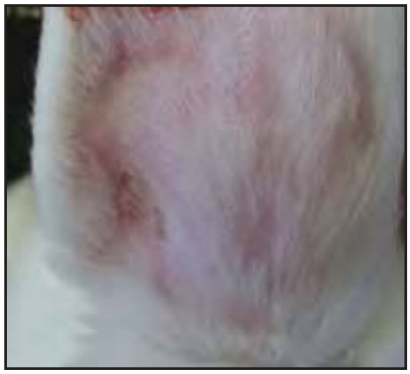
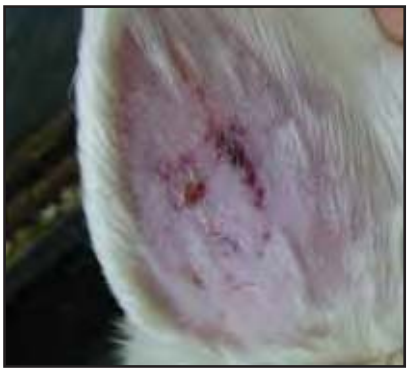


Photo 1.



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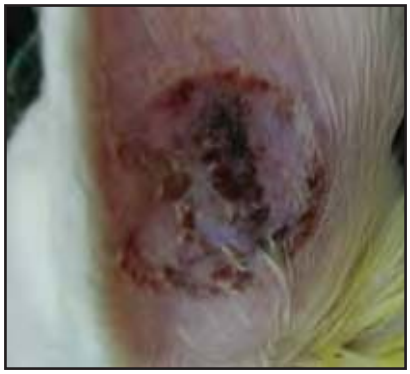

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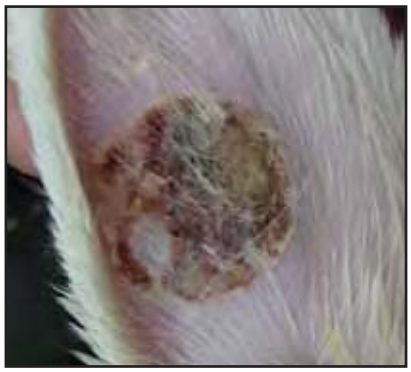


Photo 4.

in uniformed burns wounds of the full-thickness skin. All burn wounds were manifested as complete dermal necroses three to five days following the burn inducements.

Four hours after the burn inducement, burn wound is evidently separated from the surrounding tissue, dry, and with visible blister on the surface (Photo 1). On the first day after the burn inducement, burn wound is still red, while epidermis comprising visible oedema and blisters is disintegrated (Photo 2). On the third day after the burn inducement, the crust is still not formed, while inflammation signs are very expressed, accompanied with oedema, haemorrhage and surrounding tissue inflammation (Photo 3). After seven days, skin burn is covered with the coagulated necrotic mass. Inflammation is significantly expressed, damage of the cartilage is evident, and burn wound spreads through all layers of the skin (Photo 4).

Microscopic examination of rabbits

Epidermis shows signs of coagulation necrosis at the burn wound area. The forms of cell nuclei are flattened and half-mooned with huge perinuclear white spaces. Blood vessels are hyperaemic and dermis is oedematous (Photo 5).

Injury margins are covered with the inflammation infiltration containing numerous erythrocytes out of the hyperaemic vessels by the hair follicles. Epithelial cells are completely necrosed with numerous eosinophils within the necrotic mass. Eosinophil infiltration is beneath the damaged epidermis allowing stratum disjunctum to become visible. Necrosed epithelial cells of the hair follicles, thrombosed blood vessels, and few eosinophils in epidermis are visible under the burn wound. Coagulation necrosis expands to the cartilage, while degenerative changes with the vesicle formation in epidermis and haemorrhage around the blood vessels are visible on the other side of the cartilage (Photo 6).
Photo 7 illustrates the coagulation necrosis of epidermis and deeper layers of the dermis together with inflammatory cell infiltration containing eosinophils predominantly; hyperaemia and haemorrhage with the beginning fibroblast activation under the inflammatory infiltration; oedema, necrosis and haemorrhage reach the ear cartilage.
Coagulation necrosis of epidermal and deeper dermal layers reaches the ear cartilage. Inflammatory cell infiltration is particularly expressed and some damages of the ear cartilage are visible (Photo 8).

Macroscopic examination of the rats

Photo 9 shows a spherical whitish zone, partially shriv-

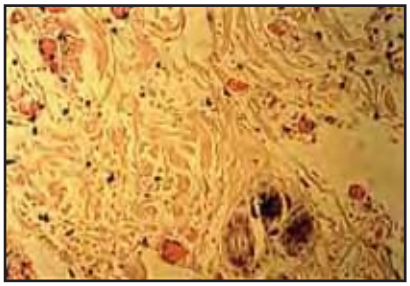
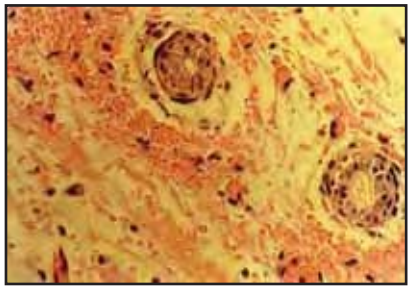


Photo 5.



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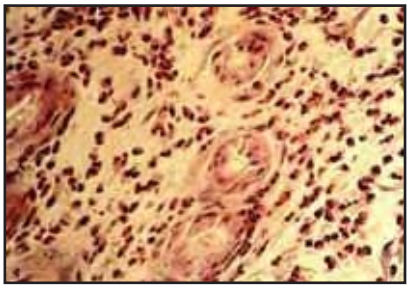

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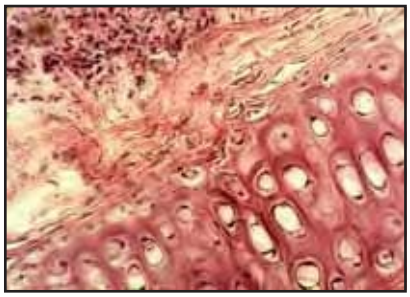

Photo 8.

elled, dry, and with faintly red margins in comparison to the surrounding skin (Photo 9). A day after the burn inducement, burn wound is red and faintly cracked. Photo 10 shows oedema and inflammation signs. Burn wound is dark red, with whitish plagues and minor inflammation reduction (Photo 11).

Burn wound is dry, shrivelled, and covered with the formed crust. Inflammation reaction is almost disappeared and cicatrisation process is improved and visible (Photo 12).

Microscopic examination of the rats

Big and bright, multiplied stratum spinosum epidermidis cells are visible at the burned skin transition zone (Photo 13). Stratum germinativum and stratum granulosum epidermidis are wide. Numerous hyperkeratic cells in the thickened stratum corneum epidermidis are visible. Massive granulocyte inflammatory infiltration leans on the stratum corneum in the form of demarcation line. Necrotic coagulated mass with rich cellular detritus is above it. Under the area of burn, some blood vessels of the stratum corneum epidermidis are thrombosed, while granulation tissue replenish almost entire stratum corneum epidermidis.

Discussion

Numerous *in vitro* and *in vivo* models of the wound healing monitoring have been described in the literature. The choice of animal model depends on many factors, including animal availability, costs, animal handling and anatomical and functional similarity to the humans. Little animals are by far more frequently used in the animal wound healing models. However, there are many anatomical and physiological differences between frequently used little mammals and humans. Pigskin, from the anatomical and physiological point of view, is most similar to the human skin. Nevertheless, the size of pigs limits their more frequent usage as animal models (13).

In our animal model, we used available animal type that was simple for handling and that fulfilled all necessary criteria and requirements. We succeeded to obtain very simple and reproducible animal model of the thermal injury. Equipment and instruments, used for the model introduction, are present in all pharmacological-toxicological laboratories. In adopted animal model, we induced burns overtaking about 20% of the animal body surface. Scientific literature reporting the evaluation of efficacy of the topical preparation in the treatment of burns mostly describes burns overtaking 10-20% of the body surface, so our results become comparable from that point of view (14, 15, 16).

Depth of the burn wound is determined by heat source, skin thickness, duration of the heat source exposition and blood circulation. One of the oldest techniques for the burn depth determination is a clinical wound observation. There are burns that heal within the three-week period, and those that heal after the several-week period, which are called burns of the full-thickness skin. Burns that heal within the three-week period do not leave hypertrophic scars or functional defects, while the changes in pigmen-

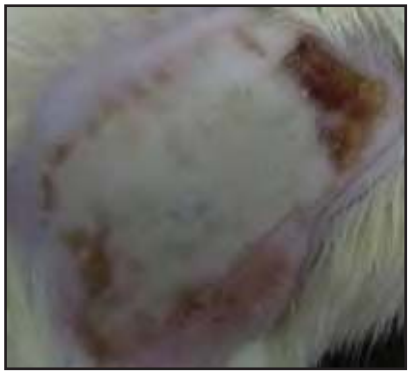

Photo 9.

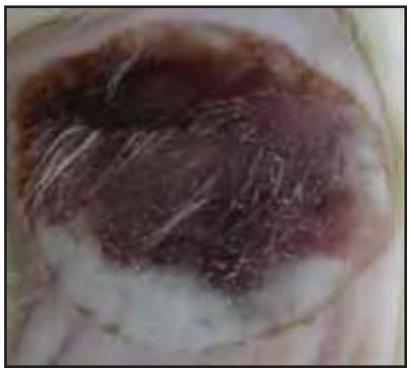

Photo 10.

Photo 11.

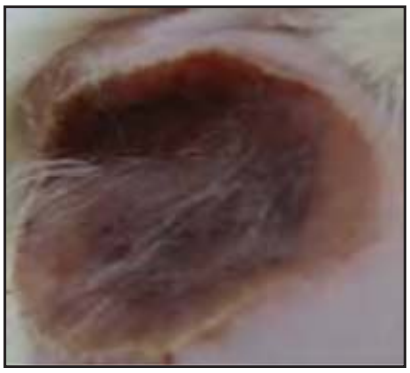

Photo 12.

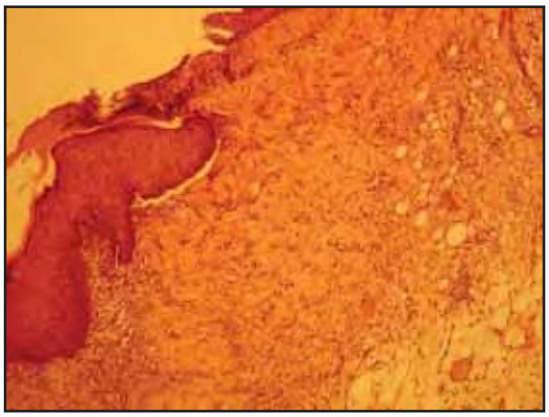

Photo 13.

tation of the burn area are possible. Burns, which heal in a longer than the three-week period, frequently cause functional damages and leave hypetrophic cicatrices with tenuous, fragile layer of the epithelium. Burn depth might be defined by the healing methods (17). Our study results are also comparable from this point of the view.

Boykin at al. (1979) developed model for the in vivo study of cutaneous microcirculation after the thermal injury of the mouse ear (18). Immediately after the thermal injury, it was possible to determine three zones of the abnormal capillary circulation: zone of the complete capillary occlusion, zone of the incomplete capillary occlusion and zone of hyperaemia. Beside that, there was a significant oedema within the burned ear of the non-reanimated animals. Six hours after the burn inducement, water content in the burned ear was increased for 20%. Formed oedema might be caused by few factors: increased capillary permeability, increased hydrostatic pressure (for the reason of post-capillary obstruction of the erythrocyte aggregation and lymphocyte conglutination), and increased oncotic pressure.

Sinking in the water at of the 4°C reduces oedema formation and support the burn wound microcirculation, that is, a conversion from the second-degree burns to the third-degree burns.

Conclusion

A very simple, reproducible, inexpensive and recommendable model for research of the thermal skin injuries was adopted. In our experimental conditions, no adverse effects in the each animal general state were noticed, so we can conclude that utilized model guarantees low morbidity and mortality rate in all experimental animals.

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