Methodology Report

Development of Animal Model for Studying Deep Second-Degree Thermal Burns

Danielle dos Santos Tavares Pereira,1 Maria Helena Madruga Lima-Ribeiro,2 Nicodemos Teles de Pontes-Filho,3 Ana Maria dos Anjos Carneiro-Leão,2 and Maria Tereza dos Santos Correia1,4

1 Programa de Pós-Graduação em Ciências Biológicas, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brazil
2 Programa de Pós-Graduação em Biociência Animal, Universidade Federal Rural de Pernambuco, 52171-900 Recife, PE, Brazil
3 Departamento de Patologia, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brazil
4 Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brazil

Correspondence should be addressed to Danielle dos Santos Tavares Pereira, dstpereira@yahoo.com.br

Received 9 January 2012; Revised 9 March 2012; Accepted 13 March 2012

Academic Editor: Monica Fedele

Copyright © 2012 Danielle dos Santos Tavares Pereira et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Thermal lesions were produced in 12 male Wistar rats, positioning a massive aluminum bar 10 mm in diameter (51 g), preheated to 99°C ± 2°C/10 min. on the back of each animal for 15 sec. After 7, 14, 21, and 28 days, animals were euthanized. The edema intensity was mild, with no bubble and formation of a thick and dry crust from the 3rd day. The percentage of tissue shrinkage at 28 days was 66.67 ± 1.66%. There was no sign of infection, bleeding, or secretion. Within 28 days reepithelialization was incomplete, with fibroblastic proliferation and moderate fibrosis and presence of modeled dense collagen fibers. It is concluded that the model established is applicable in obtaining deep second-degree thermal burns in order to evaluate the healing action of therapeutic agents of topical use.

1. Introduction

Burns are tissue lesions from thermal origin for exposure to flames, hot surfaces and liquids, extreme cold, chemicals, radiation, or friction [1]. Even with improved prognosis [2] and progress in the use of biological skin substitutes [3], burns are an important cause of mortality [4].

Burns are classified depending on the lesion severity into superficial or first degree, when lesion is restricted to the epidermis or skin causing redness; partial thickness or second degree that can be superficial when reaching the epidermis and superficial dermis, showing hypersensitivity and pain, or deep when it extends to the deepest layer of the dermis and may have reduced sensitivity with red and/or white coloration of the tissue; full-thickness or third degree when lesion involves the subcutaneous layer, with no sensitivity and white coloring [5].

The use of animals as experimental models in different areas of biological research was encouraged by Claude Bernard [6], who around 1865, described in his paper entitled “Introduction to the Study of Experimental Medicine” the use of animals as a model for study and transposition into human physiology. Experimental models are essential in mammals when studying on burns. There are literature reports on the use of rabbits [7], pigs [8], dogs [9], rats [10], and mice [11] as models in the study of burns.

The healing of skin lesions induces the burn-injured tissue inflammation, edema, and hypertrophic and unsightly scars [12]. Thus, the choice of a topical agent or the type of coverage to be used in treating burns should be conducted based on the assessment of lesion characteristics and evidence reported in the specific literature. These products must have features such as antimicrobial or bacteriostatic activity, absence of toxicity and hypersensitivity, compliance, reduced healing time, and cost/benefit [13]. However, many of the methods used in healing injuries caused by burns are controversial [14].
In this context, the objective of this study was to establish an experimental protocol for induction of deep second-degree thermal lesions in Wistar male rats to obtain clinical and histopathologic data that will facilitate understanding of results concerning the evolution of the healing action of topical therapeutic agents.

2. Materials and Methods

2.1. Animals. The experiment was conducted at the Department of Experimental Surgery, Federal University of Pernambuco, using albino Wistar male rats (Rattus norvegicus) weighing 250 ± 50 g, kept in individual cages of polypropylene measuring 30 × 20 × 19 cm and controlled lighting conditions (12 h light/dark photoperiod), temperature (24 ± 2°C), receiving water, and food (Labina) ad libitum. The experimental procedure was approved by the ethics committee on animal experimentation of the Federal University of Pernambuco (Case no. 23076.015015/2009-31).

2.2. Thermal Burn Experimental Model. Initially, 12 animals were weighed and intramuscularly preanesthetized with atropine sulfate (0.04 mg/kg) and 10 minutes after subjected to anesthetic combination of 10% ketamine (90 mg/kg) and 2% xylazine (10 mg/kg) intramuscularly [15, 16]. With the animal properly anesthetized trichotomy of back was performed and antiseptic with 1% polyvinylpyrrolidone iodine. Thermal injuries were made with a solid aluminum bar 10 mm in diameter (Figure 1(a)), previously heated in boiling water and so that the temperature reached 100°C measured with a thermometer. The bar is maintained in contact with the animal skin on the dorsal proximal region for 15 sec (Figure 1(b)). The pressure exerted on the animal skin corresponded to the mass of 51 g of aluminum bar used in the burn induction. Immediately after the procedure, analgesia with dipyridone sodium (40 mg/kg) was performed intramuscularly, being maintained for three consecutive days sodium dipyridone at 200 mg/kg orally administered in the drinking water supplied to animals.

2.3. Clinical Evaluation. The clinical course of skin lesions by burns was evaluated for 28 consecutive days based on the following aspects: blistering, swelling, redness, crust, bleeding, secretion, granulation tissue, and scar tissue. The wound retraction was evaluated using a caliper in 7, 14, 21, and 28 days after burn induction. Wound contraction was expressed as reduction in percentage of original wound size. % wound contraction on day X = [(area on day 0 – open area on day X)/area on day 0] × 100 [17].

2.4. Microbiological Evaluation. Microbiological evaluation was carried out using “swabs” in the injury area at the moment of surgery and respective days of biopsies. This sample was transferred to a Petri dish of 20 × 150 mm containing nutrient agar medium in a laminar flow chamber. After 24 h incubation, plates inoculated in triplicate for each sample were evaluated. This routine evaluation was performed to evaluate the degree of contamination of injuries.

2.5. Histological Analysis. At the preestablished times for biopsy (7, 14, 21, and 28 days after burn induction), three animals randomly selected underwent anesthesia combination of 10% ketamine (90 mg/kg) and 2% xylazine (10 mg/kg), intramuscularly [15, 16] for tissue samples collection. Euthanasia was performed by excessive doses of sodium pentobarbital intraperitoneally (100 mg/kg) [18]. Tissue samples were immediately fixed by immersion in 4% formaldehyde (v/v) prepared in PBS (0.01 M, pH 7.2), followed by routine histological processing paraffin embedding, microtomy with 4 µm cuts, and Masson’s trichrome staining. Histological study was performed by comparative descriptive analysis of the experimental groups in binocular optical microscope (Zeiss-Axiostar model) where were evaluated the evolution of skin healing after thermal trauma.

The histological analysis was performed by independent pathologist who was experienced in the examination of burn wound specimens, in the following ways: (1) inflammatory response, characterized by the presence of polymorphonuclear leukocytes (PNM), (2) granular tissue, characterized by the presence of fibroblasts, myofibroblasts, and neovascularization, (3) fibrosis, characterized by the density of collagen fibers identified by the intensity of blue color observed under optical microscopy due to staining by Masson’s trichrome. A score was made for all parameters evaluated: − = absent, + = mild presence, ++ = moderate presence, and +++ = strong presence.
2.6. Statistical Analysis. Data were analyzed using nonparametric tests. To detect differences between groups, the Kruskal-Wallis was used. All results were expressed as mean values for group ± standard deviation and analyzed considering $P < 0.05$ as statistically significant.

3. Results and Discussion

3.1. Study Design. This experimental model was established to standardize thermal burn injuries in order to obtain injuries with the same size and depth degree. The choice of Wistar rats due to these animals shows a great ease of handling, accommodation and resistance to surgical aggressions, and infectious processes, with low mortality [19, 20]. However, the choice of male rats is due to variations in hormonal cycles in females that could intervene in the process of tissue repair [21]. The result of clinical evaluation showed no signs of infection, secretion, bleeding, or death in both groups. If wounds are not well treated, they can be infected. Infected wounds heal more slowly, reepithelisation is more prolonged, and there is also the risk of systemic infection [22].

Shaving the back of the animals was performed by manual traction of hair (Figure 2(a)) thus preventing secondary skin lesions that often occurs by the use of laminated devices [23]. The option to induce only one burning
The standardization of procedures, systematization, and organization of knowledge about the interrelationships of models is necessary to provide more reliable knowledge about the interrelationships of physiological phenomena aiming at ensuring tissue restoration. For this reason, only the clinical evaluation of a burn injury does not provide information on the evolution degree of tissue healing, being of fundamental importance of the histopathologic evaluation of these lesions.

3.3. Microscopic Evaluation. The histopathological findings confirmed the acquisition of deep second-degree burns based on the histopathological examination of these lesions.
Figure 4: Histopathological aspects of deep second-degree thermal burns. Masson’s trichrome staining. 100x Magnification. (a) Animal showing thin crust and epithelial tissue with complete destruction of dermis and epidermis and hypodermis maintenance at the 7th day after the thermal lesion induction. (b) Animal at day 14, with crust and tissue reepithelialization, showing collagen, not modeled and slight fibrosis. (c) Animal at day 21, tissue reepithelialization showing intense fibroblastic proliferation with the presence of dense collagen, not modeled and moderate fibrosis. (d) Animal at day 28, with incomplete tissue reepithelialization, moderate fibroblastic proliferation, presence of modeled dense collagen mesh, and moderate fibrosis.

Tissue still presented a complete destruction of the dermis and epidermis (Figure 4(a)) on the 7th day after lesion induction. Histopathology section of the burned skin of control animals on 5th day showed denuded epidermis, diffuse infiltration of plasma cells, lymphocytes, and polymorphs [37]. After 14 days the histopathological evaluation revealed moderate autolysis of the tissue, with discrete neovascularization and fibroblast proliferation, with loose collagen fibers, not modeled with mild fibrosis and crust absence (Figure 4(b)). Yaman and colleagues [38] confirm the presence of crust formed by remnants of necrotic tissue and infiltration of mononuclear cells on the 4th day of experimentation in the control group. The crust detachment was only observed by these authors on the 14th day of study. By day 21 we observed the absence of autolysis, discrete neovascularization and intense fibroblastic proliferation, with dense collagen, not modeled and moderate fibrosis (Figure 4(c)). By the end of the experiment at 28 days, histological observations showed incomplete reepithelialization of the injured tissue with autolysis and absent neovascularization, showing moderate fibroblastic proliferation and...
Wound healing includes number of stages like clotting, inflammation, granulation, fibrosis, arrangement of collagen with spasm of wound, and epithelization. The time required for complete healing of deep second-degree burns, without the application of specific therapeutic agents, can be three to six weeks or more, and these burns will leave a scar tissue that may hypertrophy and contract itself [29, 30].

4. Conclusion

In this new model of second-degree thermal burns, injuries are easy to create and easily reproducible. There are similarities with the human second-degree burns in clinical and pathologic aspects. Thus, the animal model presented in this study is applicable in evaluating the use of therapeutic agents in the healing evolution of deep second-degree burns.

References


