

Wound healing processes in the rat (*Rattus Norvegicus*) and the agouti (*Dasyprocta Leporina*) – A comparative study

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Abstract

Aims: This study attempts to investigate variations in skin and wound healing, by comparatively examining the rate of healing between Sprague Dawley rats (*Rattus Norvegicus*) and Brazilian agouti (*Dasyprocta Leporina*), reflective of two skin types.

Materials and Methods: Following the excision wound model, wound healing times and dimensions between the two species, were compared. Each species was divided into two groups of 3. The standard/experimental group was treated with mupirocin ointment and the control group was left untreated.

Results: In comparing both species, on day 15 the rats exhibited a greater reduction in wound area (92%), as compared to the agouti (67%). The histological study of the granulation tissue from rats showed well developed collagen than the granulation tissue obtained from agouti.

Conclusions: The results of our study demonstrated that the thick and hard skin takes more time to heal than the skin with smooth/thinner texture.

KEYWORDS: agouti, wound healing, rats

Introduction

This study, delves into wound healing with regard to healing time variations while also investigating the application of such information as it applies to a worldwide populous. For this to be applicable, examination of its effect upon different skin types will help in its practical application towards being effective as possible alternatives to current treatment regimes.

Human skin is composed of three basic layers: epidermis, dermis and hypodermis. The epidermis consists of stratified squamous epithelium and epidermal ridges, and the papillary and reticular layers constitute the dermis. The papillary layer contains small blood and lymph vessels, nerves and fine collagen and elastic fibres. The reticular layer has vascular plexus, lymph, nerves and appendages, with compact collagen fibres and thick elastic fibres. The

innermost layer, the hypodermis, is predominantly composed of adipose tissue (2).

The dermal-epidermal junction (DEJ) is a three-component layer, comprising of the outermost layer, the hemidesmosome, next, the basal lamina consisting of anchoring proteins and cross-linking fibers, and the innermost layer, subjacent connective tissue, comprising collagen and elastic fibers (3,4). Comparing African skin to Caucasian, there is no difference in the elastic fiber organization, but in African skin, immune stainings of type IV and VII collagens, laminin 5 and nidogen proteins at the DEJ, were lower (5). Significantly higher levels of monocyte chemotactic peptide-1 (MCP-1) protein are found in papillary fibroblast cultures from African donors. Also, with regard to keratinocyte growth factor (KGF), matrix metalloproteinase-1 (MMP-1) and tissue inhibitor metalloproteinase protein 1 (TIMP-1), there is a two-fold increase in the ratio of papillary to reticular fibroblast expression in African skin compared to Caucasian (6). Also, impaired wound healing often experienced in diabetic patients is synonymous with persistently high levels of MMP, leading to delayed wound closure (7).

Wound healing, is a four part sequence of events. The initial phase of hemostasis involves the initiation of the intrinsic and extrinsic clotting mechanisms and vasoconstriction. The second phase inflammation is characterized by the migration of polymorphonuclear leukocytes (PMN's) and macrophages to the wound site. Of specific importance, are the factors secreted by macrophages, such as cytokines and interleukin-1, which play

a role in proliferation, migration and matrix synthesis and regulation of inflammation. Failure to resolve inflammation results in chronic wounds with delayed healing time (8). Granulation and proliferation characterizes the third phase. It involves the sequence of fibroplasia, matrix deposition, angiogenesis and re-epithelization. The final phase of remodeling and maturation involves the degradation and deposition of collagen and contraction of the wound (9).

Acknowledgement of different skin types correspondent to ethnicity is based upon evidence that in comparison to Caucasian skin, African skin has no significant difference in epidermis thickness or superficial dermal thickness, but the dermal-epidermal junction (DEJ) has threefold the thickness and a greater convoluted appearance (6). Therefore we have undertaken this study to determine whether the healing depends on the skin texture.

Materials and methods

Animals

Approval for this study was granted by the Ethics Committee for animal experimentation (AHC06/07/1), The Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad. Healthy inbred Sprague Dawley male rats weighing 180-200 g and Brazilian male agoutis weighing 3-3.5 kg were individually housed and maintained on normal food and water *ad libitum*. Animals were periodically weighed before and after the experiment. Evaluation of wound healing activity was done adhering to the excision wound model. Both species were randomly distributed into two groups of 3 each.

Excision Wound Model

Excision wounds were created according to the method of Morton and Malone for all animals involved (10). Using 1ml of intravenous ketamine hydrochloride (120mg/kg body weight), the animals were anesthetized and then shaved using an electric hair clipper. The outline of the area of wound to be created on the back was made using a circular stainless steel stencil and methylene blue. A 200mm² circular area, 2mm in depth full excision wound was made and left open. The animals were carefully monitored for infection and in instances of such, they were separated, excluded from the study and replaced. Animals were divided into experimental/standard and control groups. The experimental/standard group was treated with mupirocin ointment and the control group was left untreated.

The investigation lasted 15 days. Wound areas were measured on 1, 5, 10 and 15 using a transparency sheet and a permanent marker. Recordings of the wound areas were done using a graph paper. The period of epithelialization was considered as the day of eschar falling, after wounding, without any residual raw wound. On day 15 all the animals were sacrificed using ketamine and pieces of wet granulation tissue were excised from the healed area for histological studies.

Histological study

The granulation tissue obtained on day 15 from both the groups were fixed in 10% buffered formal saline and processed for routine histological evaluation. The tissues were sectioned at 7 microns thickness and subsequently stained with van Gieson's stain

Results

In the excision wound model, by day 15, the rats showed 92% wound contraction compared to 67% by the agoutis (Figures 1 and 2).

Between species, the rats showed greater healing rate than the agouti with the same period of treatment. The wounds of both groups appeared to be hard and crusty with undermined margins and were generally unclean with a biofilm glaze on the surface. The histological evaluation of the granulation tissue obtained from the rats (Figure 3 and 4), demonstrated wavy strands of sparse collagen deposition, inflammatory cells and more macrophages than the granulation tissue obtained from agouti.

Discussion

The results of our investigations are indicative of a faster rate of healing in the rats as compared to the agoutis. The microscopic findings revealed greater deposition of well-organized collagen band. There was also minimal presence of inflammatory cells.

With inflammation being the 2nd phase of wound healing and remodeling and maturation being the final phase, the microscopic results are suggestive that the rats, progressed further along the wound healing sequence than agoutis.

Fibroblast expression in skin types is a critical determinant towards evaluation of the healing capacity of skin (9). For instance, African skin has a twice the ratio of papillary to reticular fibroblast expression than Caucasian, and given its role in synthesis of type III collagen, it can be expected that decreased levels of collagen production and thus a slower rate of healing may be the result of this underlying difference (11). Fibrillar

collagen, type III collagen is mainly found in wounds, granulation tissue and the skin (12). In all four phases of wound healing, collagen plays a crucial role, from guiding fibroblasts to the wound site, to initiating the hemostatic plug by blood platelets to the collagen. They also aid in healing by attracting fibrogenic cells and serve as a framework for new capillary growth and collagen deposition (13).

For practical application of information attained in this study, close research into the specific morphology of the skin of all ethnicities should be done to attain effectiveness of treatment options for wound healing especially with the increasing applications of collagen scaffolds (14). Formulation of dosages based on these results should then yield optimal treatments to suit each patient thereby reducing recovery periods.

Acknowledgements:

Authors sincerely thank the staff of School of Veterinary sciences for helping us to maintain the experimental animals during this study period

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Table 1: Wound healing between two different species, within a specified time period.

Day	Rats (mm ²) (%)	Agouti (mm ²) (%)
1	198	200
5	150	185
10	62	160
15	16 (92)	65 (67)

Legends

Figure 1:– Progression of wound healing of rats on day 1 and 15.



Figure 2: Progression of wound healing of agouti on day 1 (A) to day 15 (B).



Figure 3: Histology of rat skin after 15 days, with H& E (A) and Van Gieson (B).

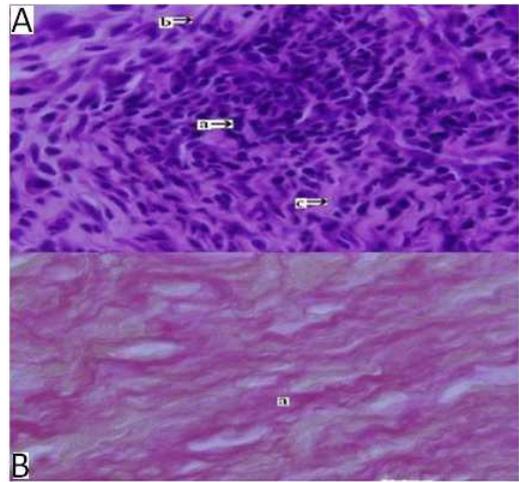


Figure 4: Histology of agouti skin after 15 days in H & E stain (A) and Van Gieson stain (B)

