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Reepithelialization in noise exposed skin wound

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ABSTRACT

Objective: To observe the effect of noise on reepithelialization in rat skin wound.

Methodology: A Quasi experimental study was conducted in Anatomy Department of Al-Nafees Medical College Isra University Islamabad & National Institute of Health (NIH) Islamabad from June 2015 to February 2016. Thirty male sprague Dawley rats were divided into control and experimental groups by convenient sampling. Each main group comprised of 15 rats. An incision of 2 cm was made on dorsal region of all rats. Control sub groups were left to heal with routine background noise exposure, while experimental sub groups underwent a 4 hour/day intermittent noise exposure of 85-95 db, five days a week for two consecutive weeks. Five rats from each group were sacrificed on day 3, 7 and 14. Wounds were excised, fixed and processed for haematoxilin and eosin stain to see the thickness of epithelium in rat skin wounds

Results: Process of reepithelialization was not as significant in noise induced group as noticed in control group (routine noise exposed)

Conclusion: Noise adversely affects the skin wound healing by interfering with epidermal regeneration

Key words: Reepithelialization, Noise, Skin Wound.

Introduction

The skin has an impressive quality to bear untoward circumstances but exceeding a certain level, all stimuli like physical, thermal and chemical can incite injury which is consequent in epidermal disruption.[1] Skin barrier properties depend heavily on its thickness and the recovery of skin integrity is affected greatly by the stresss.[2] Although skin provides a barrier for the pathogenic entrance but it is a fact that psychological stress increases the susceptibility to inflammation.[3]

Different researches provide the evidence that noise in the form of acute or chronic psychological stress significantly delay skin wound healing .[4] Noise exposure being a biological stressor disturbs the hearing sensation first through human ear then the brain and ultimately affects the gastrointestinal, cardiovascular and muscular system.[5] These alterations adversely affect the initial stages of wound healing by interfering with processes like lipid synthesis and cytokine expression.[6] Non healing or complications of skin wounds cause great suffering for the patients in the sense of physical and financial trouble and for the society in the sense of extensive health care costs as well.[7] No doubt extensive work has been done on

elusive process of wound healing but much knowledge must be gained yet to answer the many more questions about regenerative process affected by psychological stressors. Elucidating the effect of noise on process of reepithelialization and establishment of preventive measures may benefit both the patient and the society.[8]

So the objective of the study was to see the effect of noise stress on process of reepithelialization in rat skin wound.

Methodology

It was a quasi-experimental study from June 2015 to February 2016. Sprague Dawley male rats, 250-300 grams of weight and 3-5 month of age were included while rats with any skin disease before or during the study period were excluded. 30 sprague dawley rats were purchased from NIH Islamabad. The control and experimental groups were kept in separate cages. Each cage housed five rats. Standard setting of temperature, light and humidity was maintained for both groups. The animals were housed on a 12/12 hour light-dark cycle with lights on at 8 am and off at 8 pm, at 23-27^{0}c with 30-40% humidity and supplied with a standard pelleted diet and tap water ad libitum. In order to minimize all other stressors, such as handling and habitat etc, the animals were acclimatized to environment for one week before experiment.

Regeneration of injured epidermis was evaluated by counting number of epithelial layers and thickness (micrometers) at wound margins. Microscopic examination was done at 40X10.

Thirty rats were divided into two groups, control A (incised but not exposed to white noise) & experimental B (incised and exposed to white noise). Fifteen rats were included in each group Control and experimental groups were further divided into three subgroups (A1, A2, A3 and B1, B2, B3) containing five animals each. Grouping was done according to days of exposure (3, 7 & 14) of noise (routine noise in case of control and white noise in case of experimental groups).

Rats were anesthetized by giving ketamine and xylazine intramuscularly. Ketamine 5ml and xylazine 0.5 ml were mixed and rat dose was 0.1ml/100g body weight. A full thickness incision was made after shaving the skin, to create a single wound of about 2cm on the back parallel to the right side of vertebral column of all rats. Wounds were closed with metallic clips by using disposable skin stapler.

Rats of control sub groups (A1, A2 and A3) were housed as five rats per cage, kept in a quiet room and just exposed to routine background noise at 40-50 db (a). Rats of group A1 were sacrificed at third, A2 at seven and A3 at fourteenth days of noise exposure. All rats of experimental sub groups were exposed to white noise (85-95 db). Exposure was started in the morning of day o, from 8 am to 4 pm. The noise exposure was 4hrs/day with one hour interval, after each one hour exposure, 1st 5 days/week, for 2 consecutive weeks. Exposure of noise was intermittent to prevent the rats from becoming adapted to the noise. Recorded noise of pressure horns was produced by mp3 sound player and amplified by an amplifier which was connected to two loudspeakers (15 w) and installed 30cm from the cages. Sub group B1 was subjected to loud noise for three days, B2 for seven days and B3, for fourteen days. Rats of group B1 were sacrificed on third, B2 on seventh and B3 on fifteenth day of noise exposure (table I). Wounds were excised, fixed and processed for histological examinations. Slides were stained with haematoxillin and eosin to see the process of epithelial regeneration in all specimens of control and experimental rat skin wounds at day 3, 7 and 14. Epidermal thickness was measured manually with caliberated ocular micrometer scale introduced into microscope eye piece. Measurement was taken vertically from the basement membrane up to the end of granular layer. Student's t test was used to compare epithelial thickness between both groups. P-value of ≤ 0.05 was considered statistically significant

Results

Thickness of epithelium (18.24±2.18) (table II) was measured in group A1 at wound edges and found almost three layers (stratum basale, stratum spinosum, stratum granulosum) thick in control group A1. Keratinocytes were seen to be migrating at wound site and scabs were forming. Cells in stratum basale showed increased number of mitotic figures. It was noted that reepithelialization from the hair follicles was also taking place in control group A1 at day three. We observed insignificant difference of thickness (p-0.13) in control and experimental groups on third post incision day (table 2). We had observed scab formation at wound site in control group A1, because of the fact that immediately after a breech on skin surface, clotting factors are released to prevent loss of blood and to provide a hard fiberous matrix. All animals of experimental group B1showed two to three layers of epithelium and mean thickness was (16.32±1.30) (table 2). Basal layer of epithelium showed mitosis. Keratinocytes were migrating in the wound area, but gap was still present in experimental group B1. Using

light microscopy, difference in mean thickness of epithelium of group A1 and group B1 was found to be statistically insignificant (p=0.13) (table II). The basal epidermal cells possessed features of proliferation in both groups A1 & B1. Thickness of epithelium was measured in both groups A2 and B2 at day seven. All layers of skin (basale, spinosum, granulosum and corneum) were observed. Wound spaces of this group A2 were found to be completely bridged. Mean thickness of epithelium was (16.08 ± 01.81) (table 2) at day seven. Epithelial cells were completely regenerated after seven days at times of sacrifice. Anucleated layer of keratin had completely and evenly covered the wound surface and was densely packed.

Comparative value of thickness of epithelium, at wound margins of both A2 and B2 groups was significant (p=0.004) (table I) at day 7. Epithelium of control group A2 was fully regenerated as compared to experimental group by having all five layers and continuity of keratin experimental group B2 showed localized and patchy covering of keratin observed on 7th post incision day. Increased number of keratohyaline granules in stratum granulosum was found in this group B2. At day fourteen, thickness of epithelium of control group was measured (15.96 ± 1.77) (table I) and found to be fully regenerated in all rats. It was same as in normal unwounded skin in addition to formation of all skin appendages like, hair follicles and sebaceous glands in all rats of control group A3. Comparison of mean value of epithelial thickness of the two groups was done on 14th day and found to be statistically significant (p=0.004) (table I). Epithelium of wounds in experimental group was four to five layers thick (12.72 ± 0.50) (table I). Stratum corneum was not fully developed in wounds of all rats as in control group. Some rats of this group showed all the layers of epithelium including stratum corneum, but few of them had only four layers, same as in experimental group B3

Table no I: Quantitative analysis of thickness of epithelium (µm)

Groups	Days	Mean ± SD	p-value
A1	3	18.24 ± 2.18	0.13
B1	3	16.32 ± 1.30	
A2	7	16.08 ± 1.81	0.004*
B2	7	12.84 ± o.32	
A3	14	15.96 ± 1.77	0.004*
B3	14	12.72 ± 0.50	

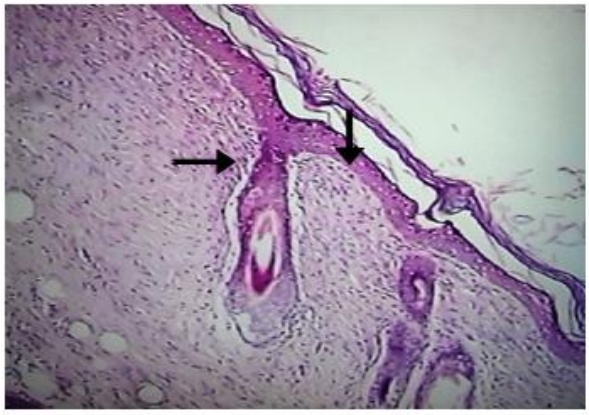

Figure 1. Photomicrograph showing skin wound at day 3 in specimen number 5 (Control Grougp 1). Arro shows thickness and regeneration of epithelium from hair follicle. H & E stain. 10X10

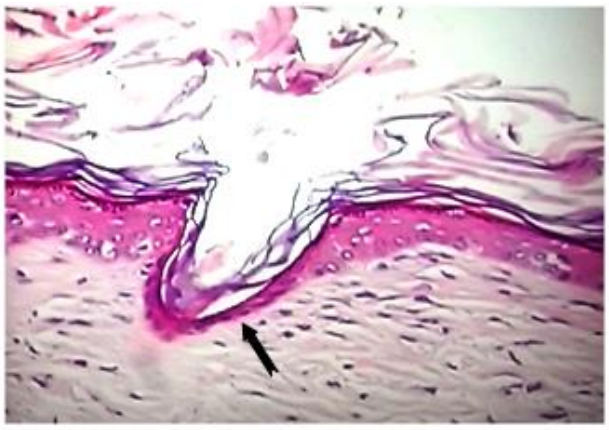

Figure 2. Photomicrograph showing Rat skin wound at day 3 in specimen number 1(Experimental groups B1). Arrow shows two layers of epithelium bridging the wound gap indicating the slow migration of keratinocytes

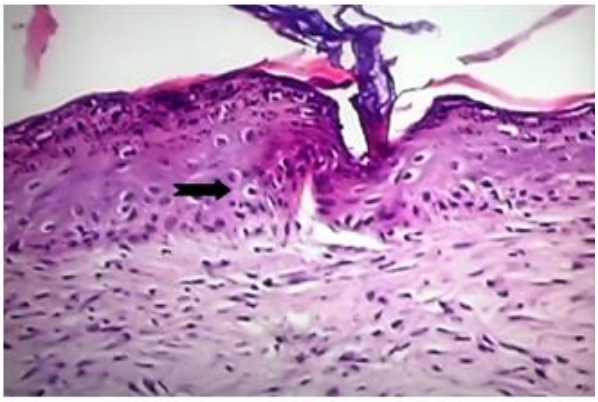

Figure 3. Photomicrograph showing skin wound at day 7 in specimen number 2 (control group A2). Arrow shows complete regeneration of all layers of epithelium with wound contraction. H & E stain. 10X10

Discussion

Healing and regeneration processes are characteristic of each part of human body, this study has focused the healing process of skin only and tried to highlight the classical wound healing stages with factors like noise that might impede it. Dermis and epidermis are two integral parts of skin.[9] The latter is composed of multilayered polarized epithelium (keratinocytes) overlying dermis.[10] Migration of epidermal cell was seen towards wound site from intact skin more in control group. Similar results have been observed by an author that epithelial cells regenerate from wound margins.[11] Hair follicles might migrate into the wound site by a process called "epiboly" the process of cell movement.[12] Similar results are reported by another study that most probably migration of cells was due to signals passed by absence of neighbouring cells at margins of wound. [13.]Animals of group B1 showed slow migration of keratinocytes at wound margin. We had exposed the animals of experimental group B1, B2 and B3 alternatively to noise stress and in this regard our results closely match with a previous study that alternate stress may negatively impact movement of keratinocyte and wound healing. Wound healing was assessed by observing the changes in stratum corneum as it was well developed in control group A2 and unevenly covered the wound surface of experimental group B2. The same findings were noticed by another researcher as well.[6] Reason might be the epinephrine released by stress caused increased formation of focal adhesions and stabilization of actin cytoskeleton leading to slow migration of cells at wound site.[14] An author has explained the underlying mechanism well by saying that glucocorticoid hormone inhabits repair functions, it has tendency to influence the immune system by suppressing the proliferation of Cellular differentiation, down regulating gene transcription and reducing expression of cell adhesion molecules that are essential for cell movement. [15,16] This fact was proved by another author that epinephrine antagonists have potential to accelerate the barrier recovery of skin and reduce epidermal hyperplasia induced by disrupted recovery of skin barrier in noise exposed group.[17] We also appreciated reepithelialization from hair follicles in control group A1. It has been observed by an author that hair follicle if placed in incision space might serve as center for reepithelialization.[12] It has been supported by another study that hair follicles infundibulum lodges stem cells responsible for epithelial regeneration.[12] Cells of stratum basale showed features of proliferation more in control group A1 (3.20±2.58) when compared with B1 (2.60±0.89) closely resemble with results of a study cells in the basal layer divide continuously within the epidermis, differentiate and stratify to restore the skin barrier.[18] Difference in thickness of epithelium of both control and experimental was significant on seventh day. Keratin has completely covered the wound surface in control group A3 while patchy distribution was noted in experimental group B3. Our findings were same as experiences of a researcher that wound healing was assessed by observing changes in stratum corneum and found to be delayed as compare to healing in non-stressed envoirnment.[19,20] It was comparable to human study conducted that complete reepithelialization took place till 5th day of wounding.[15] Most likely EGF (epidermal growth factor and TGF (transforming growth factor) produced by macrophages and keratinocytes were responsible for the process of reepithelialization.[6] We had selected rats as experimental model in our study. The fact that cannot be neglected ,as narrated by an author is that there must be some difference in duration required to complete the process of reepithelialization among both species because human skin is different from rat skin.[21] Another study done by Schmidt FP is also in accordance with us by postulating that noise stress triggers endothelial dysfunction by increasing stress hormone release.[22] Noise induced vascular oxidative stress is also described.[23] Studies done by charakida and Said presented the similar results.[24.25] The deteriorating effect of noise stress on thickness of germinal epithelium and epithelial barrier functions were proved by a study in past also supports the results of our study.[26]

Conclusion

It was concluded that noise in the form of psychological stressor adversely affects the skin wound healing by interfering with epidermal regeneration. Noise is the basic culprit in raising patients' discomfort, Insomnia, annoyance and anxiety, the factors responsible for delayed skin wound healing. Strategies to control the psychological stress created by noise should be adopted by clinicians and health care managements to ensure a quiet and calm environment for speedy recovery.

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