Effect of Noise on Quality of Collagen Formed in Rat Skin Wound

Khalida Moeed¹, Khadija Iqbal², Anum Usman³, Azam Qureshi², Waseem Kausar⁴

ABSTRACT

OBJECTIVE: To observe the effect of noise on quality of collagen formed in rat skin wounds.

STUDY DESIGN: A Quasi experimental study.

PLACE AND DURATION: At Anatomy Department of Al-Nafees Medical College & Hospital Isra University Islamabad Campus Pakistan and National Institute of Health (NIH) Islamabad from 25th June 2015 to 15th February 2016.

METHODOLOGY: 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 4h/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 Masson’s Trichrome stain to see the maturation of collagen content.

RESULTS: Mature collagen formed in rat skin wounds was significantly decreased in experimental groups as compared to control groups.

CONCLUSION: Results of present study showed that noise adversely affects the skin wound healing by interfering with maturation of collagen content.

KEY WORDS: Collagen, Noise, Rat skin, Healing, Wound

INTRODUCTION

Although skin is being considered as largest body organ¹ but still has tendency to be affected by environmental hazards like noise stress.² Skin and nervous system, both are derived from same primary germ layer (ectoderm).³ Both express the same type of protein (acetylcholine) which serves as neurotransmitter in central and autonomic nervous system and is involved in modulation of skin immune system.⁴ World Health Organization (WHO) recommends average noise levels below 35 dB in OPDs where patients are under observation and no more than 30 dB in wards or rooms of admitted patients.⁵ Hospital noise in this age is exceeding WHO’s acceptable standards so, creating restlessness.⁶ Reduction in noise stress can leads to improvement in stress and anxiety, better sleep and wound healing in patients.⁷ A researcher has stated that exposure to 85 dB of noise every hour for fifteen minutes over nineteen days influences healing process of surgical wounds.⁸ Other than loud noise, low noise if exposed for a long time can suppress the immune response in rats.⁹ Excessive noise when exposed intermittently also delayed wound healing in experimental than routine noise exposed group.¹⁰ Noise stress not only affects cells involved in, but phases of wound healing too.¹¹ Although in our country work has been done on effect of noise on cutaneous wound healing at inflammatory phase but quantitative and qualitative analysis of other parameters of wound healing like maturity of collagen content in granulation tissue need to be explored. Moreover most of the studies have been done on effect of noise on wound healing by assessment of collagen content formed during first seven days (acute wounds) or more than fifteen days (chronic wounds), while standard text books of surgery and pathology state that maximum amount of collagen and wound strength reaches its peak during or at the end of second week. Noise is among those factors often observed to impede the healing process. This affects health status through mechanism including disturbed sleep, poor wound healing, rehospitalization and discomfort. By improving acoustic environment we can reduce these health hazards to some extent. Objective of my study was to observe the effect of noise on quality of collagen formed in rat skin wounds.
METHODOLOGY

This Quasi experimental study was done from 25th June 2015 to 15th 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°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. 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 (Table1). 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) (Table-I). 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 not 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 (sub acute stress). Exposure of noise was used intermittently 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 Masson’s trichrome to see the content of collagen in granulation tissue. Criteria for grading the content of collagen was based upon staining characteristics of collagen and cross linking of fascicles. Light bluish staining of the section was labeled as mild, dark bluish staining as moderate and appearance of fascicles was labeled as intense. While unstained sections were recognized as absent. Maturation of collagen was assessed by cross linking of collagen fascicles with other structural proteins. 

RESULTS

Connective tissue containing collagen was observed, graded and compared under 40X10 in both groups. To see the amount and maturity of collagen, staining intensity and cross linking of fascicles was observed. Content of collagen was mild in all rats of control group (A1) whereas microscopic examination of granulation tissue of noise exposed group (B1) exhibited variation in density among individual bands of collagen. 20% rats of experimental group showed moderate intensity of collagen fibers, while 80% rats presented with mild content of collagen (Table-I). Difference in quality of collagen among two groups was found to be insignificant p-value (0.31) at day three. Collagen content of both control and experimental groups were also observed at seventh day of experiment. Wounds of control group showed mature collagen bundles with 20% moderate and 80% intense content. Organization of fascicles of collagen was quite fine and homogenous in granulation tissue of group A2. While in experimental sub group B2, collagen bundles were trying to be organized. Collagen fascicles in bluish stained sections showed 40% moderate and 60% intense collagen in noise exposed group of rats. Difference of collagen among two groups was found to be insignificant as (p=1.00) Collagen formed at the end of second week (14th day) was observed to see the wound healing. Content of collagen in wounds of group (A3) was 20% mild and 80% intense and finely cross matched with other proteins. While it was 10% mild, 30% moderate and 60% intense in wounds of experimental group B3. Insignificant p-value (0.65) was also computed in this group. More collagen was formed in noise exposed group B3 but was less organized or cross linked with other proteins as compared to control group A3. Parallel arrangement of collagen fascicles was also noticed in the same group (Table-II).

DISCUSSION

Wounds of present study were healed by primary intention. The mechanism of action during such a type of healing is deposition of connective tissue matrix in the form of collagen. Fascicles of collagen in control group were more mature regarding density and cross linking with other proteins, while those of experimental group were less organized or cross linked with other proteins present in granulation tissue at the same duration. The above mentioned findings had revealed the characteristic features of remodeling phase that upon completion of the epidermal barrier restoration, the repair process enters the late stage, which is characterized by tissue maturation. In this context, our results were in accordance with a previous study that perhaps the difference in maturation of collagen fascicles among both groups was due to activation of interleukin 8, an inflammatory α chemokine, which influenced other cells like fibroblast and caused rapid maturation of granulation tissue in control group, on the other hand, made inactivated by noise.
stress in experimental group. Another researcher in past had correlated these findings with probable causative mechanism that glucocorticoid might inhibit that protein (interleukin-8) so, consequent in immaturities of collagen tissue. Another author has experienced the same difference and stressed on causative factor, by saying that glucocorticoids are known to suppress cellular response of wound in terms of decreased proliferation of fibroblast synthesis and even maturation of collagen. An animal study showed that glucocorticoides when administered systemically, had prevented the maturation of collagen regardless of presence or absence of pathology. The increase in collagen fibers deposition under different stresses was also observed by an author in past. Another study is in accordance with us saying that noise stress induced decreased synthesis of collagenolytic enzymes (responsible for optimum amount of collagen) might contribute to further accumulation of collagen in experimental group. Our findings has denied the findings made by an investigator that psychological stress reduced collagen deposition, but increased myofibril density and TGF-β expression in experimental group when compared with non-stressed control mice. So, we had to accept the fact that to meet the increased tissue demand induced by hypoxia, collagen being the active component, supports other extracellular cells and has strong correlation with induction of fibroblast and capillaries.

**CONCLUSION**

Results of present study showed that noise adversely affects the skin wound healing by interfering with maturation of collagen content.

**RECOMMENDATIONS**

It is recommended that following steps must be taken on both community and national level.
1. Vital alarms should be masked in hospitals.
2. Establishment of quiet zones.
3. Adjustment of low volume level for loudspeakers at places near hospitals.
4. Reducing verbal communications in ICUs and post operative wards

**CONTRIBUTION OF AUTHORS**

Moeed K: Manuscript Writing. Data Collection, Data Interpretation. Literature Search
Iqbal K: Conceived Idea, Designed Research Methodology
Usman A: Histological analysis of all specimen. Data Interpretation
Qureshi A: Manuscript final reading and approval
Kausar W: Manuscript Writing

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