

ORIGINAL ARTICLE

QUANTITATIVE ANALYSIS OF MACROPHAGES IN WOUND HEALING OF RAT SKIN SUBJECTED TO LOUD NOISE STRESS

Aisha Rafi, Muhammad Yunus Khan*, Liaqat Ali Minhas**

Department of Anatomy, Shifa College of Medicine, Islamabad, *Department of Anatomy, College of Physicians & Surgeons Pakistan, Regional Centre, Islamabad, Pakistan, **Department of Anatomy, Northern Borders University, College of Medicine, Arar, Kingdom of Saudi Arabia

Background: Factors affecting skin wound healing have always been a central consideration in medical practice. Loud noise is biological stressor affecting the body systems at various levels. The present study was taken to study the effect of loud noise stress on the macrophages during wound healing process in male rat skin. **Method:** One hundred and eighty male Sprague Dawley rats were randomly divided into control group-A and experimental group-B. Each group comprised 90 animals. Control and experimental groups were further subdivided into three subgroups of 30 animals each, corresponding to the day of sacrifice of animals, i.e., day 3, 5 and 7 after surgery. After induction of local anaesthesia a linear full thickness incision paravertebral to thoracic spine was made on the dorsum of rat. The experimental group B was exposed to loud noise stimulus (recorded noise of aero planes and gun fire) set at 97dBA to 102 dBA with a sound level meter. The animals were decapitated on day 3, 5 and 7 after surgery. Tissue was processed for paraffin embedding and stained by Hematoxylin and Eosin and Mallory's trichrome stain. Data was collected for the incisional space of the wound. Quantitative data of number of macrophages was analysed by Student's 't' test for the detection of any significant differences between the mean number in the experimental and control groups. All the quantitative data was expressed as means \pm SE. A *p*-value of ≤ 0.05 was considered statistically significant. **Results:** In this study macrophages were decreased statistically significantly at day 3 after surgery and thereafter increased significantly on day 5 and 7 after surgery in the experimental subgroups as compared to their match control subgroups. **Conclusion:** These results show that loud noise stress affects the cells (macrophages) involved in the healing of the wound therefore it is expected to have impact on the stages of wound healing.

Keywords: Macrophages, wound, healing, stress, noise

J Ayub Med Coll Abbottabad 2014;26(2):115-7

INTRODUCTION

Macrophages play an important role in cellular proliferation and functional tissue regeneration within wounds. These cells produce many specific proteins needed for regeneration at the site of injury.¹ Numerous growth factors that regulate mesenchymal cell proliferation, migration, and synthesis of extracellular matrix proteins are produced by macrophages.²

Various studies have shown that stress affects the immune cells. Macrophages of stressed mice have an altered mode of function more complex than a simple general suppression or activation.³ Acute cold stress suppresses the function in macrophages.⁴ During any type of stress elevated plasma levels of adenocorticosterone, corticosterone and adrenaline are found. These hormones, through interactions with specific receptors, are known to affect the function of cells of the immune system.⁵ During the early phase of wound healing depletion of macrophages results in significant reduction in the formation of vascularized granulation tissue, impaired epithelialization, and minimized scar formation. Whereas, depletion of macrophages at

mid-stage of the repair response resulted in severe hemorrhage in the wound tissue. This affects the subsequent phase of tissue maturation and wound closure.⁶ It has been shown that loud noise causes loss of hearing,⁷ loss of sleep,⁸ raised blood pressure,⁹ prenatal sound stress produced age-dependent and mitogen specific alterations in lympho-proliferative activity and reduced immunoglobulin G levels in post natal life.¹⁰ Loud noise is a biological stressor. So far it has not been acknowledged as a factor affecting the wound healing process. The effect of stress on wound healing may have important implications in the context of surgical and naturally occurring wounds, especially among at risk population. Factors affecting the wound healing need to be understood to improve the treatment plans.

MATERIAL AND METHODS

This laboratory based randomized control trial was carried out at the department of Anatomy, College of Physicians & Surgeons Pakistan (CPSP) Regional Centre, Islamabad, from September 2007 to September 2008. Animals used in the study were obtained from National Institute of Health (NIH), Islamabad. A sample of 180 male Sprague Dawley

rats weighing between 250-300g were randomized in to two main groups, control group-A and experimental group-B. Each main group comprises 90 animals. Macrophages appear in the wound after two days of injury, i.e.; 48 hours, so wound healing was studied at 3, 5 and 7 days after surgery. Therefore main groups were divided according to day of sacrifice of animals in to three subgroups i.e., A₁ to A₃ and B₁ to B₃. Each subgroup comprises 30 animals. The animals were sacrificed at 3, 5 and 7 days after surgery. The animals were housed in cages in a temperature-controlled room (28°C–31°C). Twelve hours light-dark cycle was maintained with lights on at 6:00 am and off at 6:00 pm.

After an intramuscular infection (Ketamine 5 ml (50 mg) + Xylazine 0.5 ml (50 mg), rat dose is 0.1ml/100g body weight)¹¹, a linear 2 cm full-thickness skin incision para vertebral to thoracic spine was made. The incision was closed with metallic clips with disposable skin stapler. Loud noise was produced by two loud speakers (50 watts each) placed one on each side, at distance of 40 cm from the cages. A precision sound level meter was used to set the intensity of sound ranging between 97dB (A) to 102 dB (A) in cages. Experimental group-B was exposed to loud noise after surgical procedure. Control group-A was not exposed.

The number of macrophages was determined in an incisional space in a pre-calibrated unit area in eye piece graticule. Statistical analysis of the whole data was done with statistical package for social sciences (SPSS) computer software program, version 10. Data was analyzed by Student's t-test for the detection of any significant differences. Data was expressed as mean±SE and $p \leq 0.05$ was considered statistically significant.

RESULTS

In subgroups A₁ and B₁, 3 days after surgery macrophages were seen both in control and experimental subgroup. In the control animals they were mostly found in the incisional space of the wound whereas in experimental subgroup they are mostly observed within the lumen of the blood vessels and some were found along the vessel wall, they were not included in the count. There was a significant decrease in number of macrophages in the experimental subgroup B₁, ($p < 0.01$) (Table-1). Five days after surgery macrophages were found in a significant increased number in the experimental subgroup-B₂, ($p \leq 0.05$) compared to their matched control subgroup-A₂ (Table). Similarly Quantitative analysis of the macrophages in subgroups-A₃ and B₃, 7 days after surgery exhibited macrophages present in a significantly increased number in the experimental subgroup-B₃ ($p < 0.05$) (Table-1).

Initially macrophages were decreased in experimental subgroup (B₁) 3 days after surgery. Macrophages number increases after five days onwards in experimental subgroups (B₂, B₃) (Figure-1).

Table-1: Mean number of macrophages in the incisional space of the wound in control (a) and experimental (b) group per unit area

DOS	Sub-groups (n=30)	Macrophages Mean±SE	p-Value
3	A ₁	2.186±0.153	$p < .01^{**}$
	B ₁	1.618±0.147	
5	A ₂	1.706±0.092	$p < 0.05^*$
	B ₂	1.950±0.06	
7	A ₃	0.696±0.08	$p < 0.05^*$
	B ₃	0.996±0.109	

Key: SE=Standard error of the mean=Number of animals, DOS=Day of sacrifice of animals, *=Significant $p < 0.05$

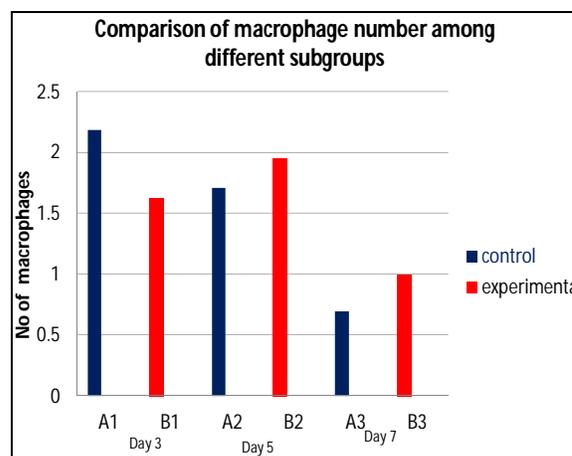


Figure-1: Subgroups-Number of macrophages decreased in experimental subgroup 3 days after surgery, after that their number increases in the experimental subgroups

DISCUSSION

The initial depression of macrophage number in stressed subgroup can be explained on account of the studies that showed that the innate non-specific immune response as well as the specific immune response is delayed by stress.¹² The differentiation and function of mono-cytosis attenuated by raised glucocorticoids levels in acute stress conditions.¹³ In one of the studies mice exposed to restraint stress showed reduced population of cells involved in the early phase of healing at the margins of the punch biopsy wounds.¹⁴ Our results are in line with this earlier study. The effect of stress is manifested early in the inflammatory phase, Singer and Clark in their study on cutaneous wound healing stated that the key function of inflammatory phase is the recruitment of cells which clear bacteria and other foreign substances from wound. If the monocytes/macrophages do not accumulate in wound, enzymes and growth factors essential to new

tissue deposition may not be produced.¹⁵ This link can be further solidified by the data that showed that stress reduces the expression of genes for IL-1 and TNF that initiate the inflammatory phase of repair.¹⁶ Macrophages from noise-exposed animals secreted significantly less superoxide anion and interleukin-1 than cells from control animals.¹⁷ As a result of low expression of genes the inflammatory cytokines within the wound may not be sufficient to recruit cells during the early phase of healing.

One of the previous studies has shown that macrophage number in the peritoneal lavage of stressed mice was significantly reduced in comparison to macrophages isolated from non-stressed animals.¹⁸ So by affecting the inflammatory phase of wound repair, noise stress induced glucocorticoid hormones reduce the recruitment of macrophages to the wound margin in the same way. This also impairs the anti-bacterial function and slowing of wound healing. But in this study none of the wound was infected which may be attributed to the high resistance of the animal species (rat), proper wound care and sterile method of wounding.

Macrophages were seen in increased number in the stressed subgroups 5 and 7 days after surgery. It might be due to the delayed migration of the macrophages in the incisional space of experimental wounds.

CONCLUSION

Loud noise stress affects the inflammatory phase of wound healing process by decreasing the number of macrophages, thus affecting the subsequent stages of healing.

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Address for Correspondence:

Dr Aisha Rafi, Department of Anatomy, Shifa College of Medicine, Islamabad, Pakistan. **Cell:** +92-332-516 7381
Email: rafi.aisha@gmail.com