ORIGINAL ARTICLE

Loud Noise; Wound Angiogenic Stimulator or Inhibitor?

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ABSTRACT

Objective : To observe how loud noise affects the growth of new blood vessels in rat skin wounds.

Study Design :QUASI experimental study.

Place and Duration of Study: The study was conducted at National Institute of Health Islamabad from 28th June 2017 to 28thFebruary 2018.

Materials and Methods: Thirty male Sprague Dawley rats were split into control and experimental groups by convenient sampling, with 15 animals belonging to each leading group. On the dorsum of each rat, a 2 cm incision was created. While experimental subgroups endured 4h/d intermittent noise exposure of 85-95db five days a week for two consecutive weeks, control subgroup rats were left to heal with routine background noise exposure. On days 3, 7, and 14, five rats from each group were slaughtered. Wounds were excised, repaired, and prepared for H&E stain before being able to see and count the freshly grown blood vessels**.**

Results: Although difference in mean number of newly formed blood vessels among both control and experimental group was found with more numbers in control group but it was not statistically significant.

Conclusion: Intermittent noise exposure has tendency to impair the process of angiogenesis thus delaying wound healing.

Key Words: *Angiogenesis, Noise, Skin Wound, Stimulator, Inhibitor.*

Introduction

Restoration of skin integrity following injury is dependent on cell and ECM interaction and it can be hindered at any point during the process of wound healing.[1] Multiple research models concluded that psychological stress may affect wound healing. It is evident from experimental work done in the past that aircraft noise is linked to stress-induced vascular damage, which is mediated by inflammatory cells infiltrating the blood vessels, endothelial nitric oxide synthetase being uncoupled, and NADPH oxidase being oxidized.[2] Transcriptome research has also revealed alterations in the expression of genes involved in controlling vascular function, re modelling, and cell death in the aortic tissues of animals exposed to noise. This explains how noise-induced vascular damage works. White noise exposure between 70 and 100 dB is believed to cause structural alterations in the vasculature as well as morphological changes due to an increase in stress hormone and lipid peroxidation.[3] The sympathetic nervous system is activated as a result of elevated levels of epinephrine and norepinephrine, angiotensin II.[4] Angiotensin ii in turn activates NADPH oxidase, which is responsible for oxidation stress in vasculature. Angiogenesis is integral to successful wound repair involves, budding from capillaries of wound edge.[5] Soon after the invasion into damaged site newly formed vessels appear as a network to nourish the tissue and helps in formation of granulation tissue.[6] Formation of granulation tissue from the wound edge involves immigration of local fibroblast and lying down of fibrin network.[7] Sprouting of capillaries initiates neovascularization and angiogenesis.[8] Previously noise exposed subjects showed more pronounced association between noise and endothelial functions.[9] Although significant efforts have been made in the past to address the issue of delayed wound healing[10], further research on the angiogenesis process is still required to identify all the factors that contribute to the delay. Present study by demonstrating all stressors that harm the physical environment will aid in effective wound healing and, more specifically, by shedding light on noise-induced poor vascular regeneration.

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The objective of my study was to know how noise affects the growth of new blood vessels in rat skin wound.

Materials and Methods

Study period of this Quasi experiment was from 28th June 2017 to 28th February 2018. Institutional Ethical Review Committee approved the research proposal via registration no,F.2/IUIC-ANMC /EC 64/2025 on June 23,2015. 30 Sprague dawley male rats purchased from NIH Islamabad were included in the study aged between three and five months, they weighed between 250 and 300 grams, and rats with any skin conditions discovered during or after the trial were eliminated. The control and experimental groups of rats were housed in separate cages. Each cage had five rats. The normal settings for temperature, light, and humidity were given to both groups. The animals were kept in 12-hour cycles of light and darkness, with lights on at 8 a.m. and off at 8 p.m, at a temperature of 23–27 °C, 30–40% humidity, and a normal pelleted feed with unlimited access to tap water. The animals were acclimatized to the surroundings for a week prior to the experiment to reduce handling and habitat-like stressors. Two groups of thirty rats were created: control A (incised but not exposed to loud noise) .H&E stain was used to see the newly formed blood vessels.They were counted three times in incisional space in control A(incised but not exposed to white noise) and experimental B (incised and exposed to white noise). There were fifteen rats in each group (Table i). The control and experimental groups were then separated into three subgroups, each with five rats (A1, A2, A3, and B1, B2, and B3). Their days of exposure to noise (3, 7 and 14)—which consisted of white noise for the experimental groups and routine noise for the control groups—formed the basis for the grouping (Table-i). The rats were put to sleep using intramuscular injections of Xylazine and ketamine. The predicted dose for rats was 0.1ml/100g body weight and was created by mixing 5ml of ketamine and 0.5ml of xylazine. After shaving the back to produce a single 2 cm wound parallel to the right side of the vertebral column, a full-thickness incision was created. Metallic clips were used to close wounds with a disposable skin stapler. Five rats were housed in each cage, they were kept in a quiet environment, and they were only exposed to typical background noise at a volume of 40 to 50 dB. After three days of exposure to noise, seven days for group A2 and fourteen days for group A3, rats were slaughtered. All rats in the test groups were subjected to white noise (85-95db). The exposure period began in the morning and lasted from 8 am until 4 am. The noise exposure was 4 hours per day with hourly breaks, followed by 5 days per week for two weeks straight (sub-acute stress) occasionally to prevent adaption. A sound player amplified and recorded the sound of pressure horns by an amplifier that was mounted 30 cm from the cages and connected to two 15 w loudspeakers. B1for three days, B2 seven days, and B3 fourteen days, the subgroup was exposed to loud noise. Rats from group B1 were sacrificed on day 3, group B2 on day 7, and group B3 on day 14 after being exposed to noise (table - i). Wounds were repaired, histologically examined,in pre calibrated unit area at x100magnification.Mean value of these readings was calculated. Unit area was calculated by calibrating the stage and ocular micrometers. Statistical analysis was performed according to standard methods (SPSS version 20). Result was expressed as mean standard deviation (mean ± SD). Mean of the two groups was compared using independent student's t test. p-value of ≤ 0.05 was considered statistically significant.

Table I: Group Distribution of Rats

Groups	Sub Groups	Exposed to	Days of Excision
A n=15 Control	A1 n=5	Routine background noise	3
	A2 n=5		7
	A3 n=5		14
B n=15 Experimental	B1 n=5	85- 95 db of noise	3
s	B2 n=5		7
	B3 n=5		14

Results

New blood vessels were counted at wound site for all three days of noise exposure.

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Blood vessels count per unit area in incisional space in control group. Three days of white noise exposure to A1 resulted in (12%,1.20 ±0.44). (Table ii). In the experimental sub-group B1 exposed to white noise for three days, blood vessels were discovered to be present with a mean number (08%,0.80 ± 0.83). (Table ii). Process of angiogenesis was slower in noise exposed group than routine noise exposed group. Comparison of mean number of blood vessels present per unit area showed insignificant p value (p=0.37) (Table ii) on day three. Mean number of newly formed blood vessels counted in group A2 on day seven was (16%,1.60 ± 0.89) and blood vessels in experimental group B2 were seen in number (14%,1.40 ± 0.89) (Table ii). Difference in mean number of newly formed blood vessels among both groups on day seven was not significant (p value= 0.73) (Table ii). Newly formed blood vessels were counted in control group (A3) on fourteenth day showed mean number (20%,2.00 ± 0.00) (Table ii) while Mean number of blood vessel in experimental group B3 was counted as (16%,0.70 ±.18) (Table ii) per unit area, difference in mean number of blood vessels was not significant (p value= 0.14) (Table ii).

Table II: Number of Blood Vessels/Unit Area at Different Days of Sacrifice

Groups	Days	Blood vessels Mean± SD	% age	p-value
A1	3	1.20 ± 0.44	12	0.37
B1	3	0.80 ± 0.83	08	
A2	7	1.60 ± 0.99	16	0.73
B2	7	1.40 ± 0.89	14	
A3	14	2.00 ± 0.00	20	0.14
B3	14	1.60 ± 0.54	16	

Discussion

Non-healing of wounds drives patient morbidity and increases healthcare cost, became a major medical problem.[11] Reduced vascular growth by the process of angiogenesis is found to be one among the key factors of chronic non healing wounds.[12] We observed and counted newly formed blood vessels at wound site in both control and experimental groups and found the fact mentioned previously, to be true

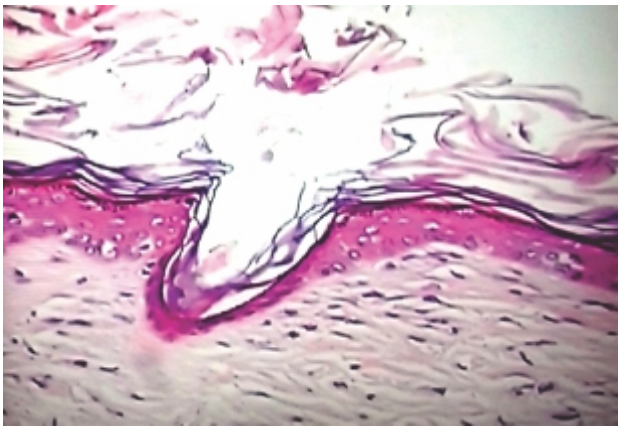

Figure 1: Photomicrograph of Specimen Number 1's Third Day Skin Wound(Group experiment B1). The arrow indicates how granulation tissue is growing and bridging the wound space. the H&E stain 10X10.

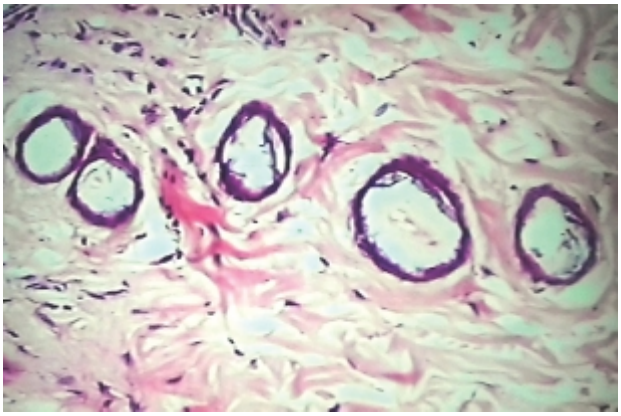

Figure 2: Photomicrograph showing newly formed blood vessels at base of wound in specimen number 5 (control group A2). H&E stain.40X10

that as a normal phenomenon in intact tissue delivery of oxygen and nutrients equalizes the removal of waste products , hence maintaining the vascular homeostasis.[13] While in injured tissue disrupted microvasculature leads to inflammation and hypoxia which in turn activates endothelial cells.[14,15]Thereafter; the new vessels differentiate into arteries and venules. The difference between capillaries and arterioles was made by the presence or absence of smooth muscles in their walls. When all control groups A1, A2 & A3 were compared with experimental groups B1, B2 & B3, on days three, seven, and fourteen. The difference was found to be insignificant (p >0.05) . These findings don't match with previous studies which said that most probable factor responsible for process of angiogenesis, were a protein called hypoxia inducible factor I (HIF-1),

thought to be inhibited by noise stress.[16] This has been shown by another researcher that ''HIF'' has a key role in wound healing.[17] Another study done in past has also supported the above-mentioned conclusion that at the site of wound, pro-inflammatory cytokines were affected by stress, that might be a possible mechanism of inadequately formed blood vessels, resulted in delayed wound healing in noise exposed group.[18] Another study showed not only difference in inflammatory cell count but significant inhibition of epidermal regeneration in experimental rats too.[19] In addition to count the proliferation and migration 0f fibroblast and number of new vessels was also decelerated.[20] Underlying mechanism is explained by another author in terms of correlation of reduced number of inflammatory cytokines and enzymes involved in tissue repair with inhibition of regeneration of endothelial cells consequent on delayed wound healing.[21] Any study in favor of our conclusion regarding formation of new blood vessels in experimental groups subjected to noise stress for fourteen days doesn't exist as per my knowledge. The matter of conflict might be the alternative exposure of noise, with (one hour exposure followed by one hour rest) as mentioned in ''subject and method'' in our study was quite different from previous above-mentioned studies. Present study adopted the said pattern of noise exposure based upon the fact that prolonged repeated stress can be extremely harmful to auditory apparatus of rats as they are more sensitive to auditory stimuli than humans.[22] It is also a fact that harmfulness of noise to hearing or even healing depends not only on the sound level only, but there are some other factors like sensitivity of the exposed person or experimental animal and the duration of exposure.[23]

Conclusion

Intermittent noise exposure has tendency to impair the process of angiogenesis thus delaying wound healing.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.