EFFECT OF CHRONIC NOISE STRESS ON MANTLE ZONE OF LYMPHOID FOLLICLE OF SPLENIC TISSUE IN ALBINO RATS

[1]SYED MEESAM IFTEKHAR-H-RIZVI, [2]MUSSARAT NAFEES, [3]MUHAMMED FAROOQ ALAM SIDDIQUI

[1]Dept. of Anatomy, Jinnah Medical & Dental College, Karachi. [2]Dept. of Anatomy, Karachi Medical & Dental College, Karachi.
[3]Dept. of Pharmacology, Dow International Medical College. Dow University of Health Sciences, Karachi

ABSTRACT

Background:

Noise is an ever growing problem in modern society. Apart from its effect on hearing, behavior, & mood it also has a potent effect on immune system. Therefore the present study was designed to asses effect of chronic noise stress on mantle zone (MnZ) of lymphoid follicle (LF) of spleen in albino rats.

Material & Methods:

Two groups of twenty rats, each group labeled A&B were used; serving as control (no noise) and noise stress (30 days) respectively. Group B was exposed to noise stress of 100 dB for 30 day (3-4hrs/day). Animals were sacrificed at the end of study, spleen was removed prepared, sectioned & stained with CD79a.

Result:

A highly significant decrease in mean no of cell in MnZ of LF was observed (15747±87.82/mm^2) in noise stressed group when compared with MnZ of control animals (16567±106/mm^2). There was also a highly significant increase in mean perimeter of nuclei observed in MnZ of stressed group (2.74±0.009µm) when compared with rats of control group (2.68±0.01µm).

Conclusion:

Our study concludes that noise stress induced release of glucocorticoid can significantly decrease number of B lymphocytes in MnZ of LF of spleen in albino rats.

Keywords:

Lymphoid follicle (LF), Mantle zone (MnZ), Noise stress, B lymphocytes, CD79a

INTRODUCTION

Stress is simply a fact of nature and an unavoidable consequence of life. Stress can be provoked by any stimulus called stressor, which produces a demand for adaptation. These stressors could be social, emotional, institutional, psychological, traumatic or environmental. Among these innumerable stressors, environmental stress is the most commonly encountered stress to which almost all individuals are exposed to a variable extent. Noise is a significant environmental problem[1] and has potential to cause stress reaction [2].

Noise is a class of sound that is considered unwanted and can be produced by different sources. Noise level exceeding maximum permissible limits (60db day & 50dB night) causes several physical ailments as well as social and emotional problems[3].

Noise is the greatest threat to the people of large metropolitan cities in Pakistan. Several researches conducted in Pakistan reveals that many of the areas in metropolitan cities of Pakistan were identified where noise levels were greater than maximum permissible level[4].

Noise acts as a physical stressor on animals that can lead to behavioral, physiological and anatomical responses. Exposure to intense noise can trigger a cascade of neuro-endocrine events reminiscent of a stress response including activation of Hypothalamic-Pituitary-Adrenal (HPA) axis[5].Thus noise

causes the release of different stress hormones (e.g. Corticotrophin releasing hormone: CRH; Adrenocorticotrophin hormone: ACTH) even while person is sleeping[6].

Stress is a potent modulator of immune system[7]. Recent studies indicates that neuroendocrine-immune interaction can cause sufficient immunosuppression which adversely affect human health[8] and had described the relationships between stress related hormones or neurotransmitter and immune functions[9]. Receptors for these molecules are present on immune cells[10]. The majority of the well characterized effects of adrenal steroids on immune system are produced by the type II receptor agonist, RU28362[11]. RU28362 decreases the number of all lymphocyte subsets (T-cells, B-cells and natural killer cells) to very low absolute levels. The largest relative decrease was seen in B-cells[11]. In stress, glucocorticoids type II receptor is a major mediator of the stress induced changes in the blood lymphocytes and monocytes distribution[12], both type I and type II receptors are expressed in spleen[11].

This study was designed to evaluate effect of noise stress on B lymphocytes in mantle zone of lymphoid follicle of splenic tissue in albino rats.

MATERIAL & METHOD

This study was conducted in department of anatomy BMSI, JPMC Karachi and microscopy was done in department of anatomy KMDC, Karachi. Twenty normal, adult and healthy male albino rats of ages (90 - 120 days) weighing 220-350 grams were selected. The animals were kept under observation for one week prior to the commencement of the experiment for assessment of their general state of health on the basis of weight gain or loss. Furthermore, this was also essential to acclimatize the animal to the environment of experimental room. All the animals were kept on standard laboratory diet and under closely controlled environmental conditions of 12 hour light / 12 hour dark cycle at room temperature.

The animals were divided into two groups A&B. The group A served as control and group B was exposed to 100 dB noise stress 4 hrs / day for 30 days. The animals were numbered, weighed and kept in proper cages, under natural environment. Diet and water were supplied ad libitum. All the experimental procedures were performed between 8:00 am to 12:00 pm in order to avoid circadian variations.

General appearance, i.e. activity, behavior and their food intake were checked daily. The intensity of the sound was measured by precision sound level meter (TES -1351) Noise level was set at 100 dB uniformly through out the cages.

All the animals were weighed at the commencement of the study and at the time of sacrifice. The animals exposed to chronic noise were sacrificed at the end of day 30th by prolong ether anesthesia in a glass container. After dissection blood samples were collected by intra-cardiac puncture to detect the hormonal levels of Corticosterone and ACTH in the plasma. Spleen was removed and cleared from fatty tissues. It was weighed by Sartorius electronic balance, washed by normal saline and then fixed in buffered neutral formalin (with pH 7.0) for 24 hours.

After fixation of the sections on the slides, they were stained with routine Haematoxylin and Eosin (H&E) staining technique to observe the general morphology of the tissue under the light microscope.

For immunohistochemistry 4-µm thick sections were cut and placed on poly-L-lysin coated glass slides. All the slides were routinely dewaxed in xylene and hydrated in graded alcohol. Slides then kept in tap water till the procedure of antigen retrieval.

Later slides were incubated with primary antibody CD 79a (Genetex, USA) in 1:600 dilution, diluted in standard primary antibody diluent (Genetex, USA) for one hour in a humidity chamber. The slides were then mounted with resin based mount-

ing medium after dehydration and clearing sequences.

Plasma ACTH was measured by ACTH, RIA kit purchased from Immunotech, France. Standard acylated sample was added to ACTH antibody coated tube and then was incubated for 1 hour at room temperature with shaking. The assay sensitivity was 1.2pg/ml.

Plasma corticosterone was measured by rat corticosterone EIA kit purchased from Diagnostic System Laboratory, USA.

All the results were analyzed statistically by using SPSS version 12, by applying student t test.

RESULT

Microscopic Examination:

The spleens of the two groups were observed under light microscope, stained by routine haematoxylin & eosin and immunostain (CD79a).

Control Animals:

The section of spleen in control animals was observed under light microscope stain by CD79a shows normal architecture (Photomicrograph 1). The white pulp showed splenic nodule with eccentrically located central artery surrounded by periarteriolar lymphoid sheath (PALS). The nodule showed central pale germinal center and outer darker mantel zone separated from each other by rim of large follicular dendritic cells (FDC). The cells in central part of germinal center showed weak positivity to CD79a and are of different sizes, while the cells in peripheral part of germinal center germinal center showed relatively strong positivity to CD79a with cells of relatively uniform diameter, with large proportion of darkly stained nuclei. Large number of pale stain nuclei was observed in mantle zone with few undifferentiated cells with darkly stain nuclei.

Animals Exposed To Noise Stress (30 Days):

In section of spleen in animals exposed to chronic noise stress (100dB) for 30 days the general architecture was found intact with apparently decrease in area of white pulp. A few large histiocytes were observed in mantel zone (Photomicrograph 2). A large number of polymorphnuclear Neutrophil were observed in mantel zone and in marginal zone (Photomicrograph 3). A large number of apoptotic cells were also observed in mantle zone (Photomicrograph 4).

B Lymphocyte Count In Mantle Zone:

CD79a positive cells (B lymphocytes) were counted in mantle zone of splenic nodule in animals of the two groups with the help of a counting reticule. Cells were counted in randomly selected 10 different fields in mantle zone. Their results and comparisons are summarized in table 1 and figure 1.

A highly significant decrease in B lymphocytes count was observed in animals exposed to 30days noise stress (15747±87.82/mm^2) when compared with control animals 16567±106/mm^2 ($P<0.0001$).

Perimeter Of Nuclei In Mantle Zone:

The perimeter of nuclei was measured with the help of eye piece micrometer in 10 different fields in mantle zone of each splenic nodule. Perimeter of twenty randomly selected nuclei was measured in each field. The result and their comparison are mentioned in figure 2 and table 2.

The mean perimeter of nuclei in mantle zone in animals exposed to chronic noise stress of 30 days was 2.74±0.009µm. This showed highly significant increase when compared with mean perimeter of nuclei in mantle zone of control animals 2.68±0.01µm ($P <0.0001$).

Plasma acth concentration:

Plasma ACTH concentration in the two groups was analyzed by using radioimmunoassay kit. Their results and comparison of results are summarized in table 3 figure 3.

A highly significant increase was observed in mean plasma concentration of ACTH in animals exposed to chronic noise stress for 30days (131.50±9.07pg/ml) in comparison to mean plasma

ACTH concentration of control animals 53.80±5.75pg/ml (P <0.0001).

Plasma Corticosterone Concentration:

Plasma corticosterone concentration was analyzed in animals of the two groups by enzyme immunoassay kit and their observation with comparisons are summarized in table 4, figure 4.

The mean plasma corticosterone concentration in animals exposed to 30days (chronic) noise stress was found to be 4.21±0.22µg/dl, which shows highly significant increase (P <0.0001) when compared with mean plasma corticosterone concentration of control animals (1.75±0.25µg/dl).

TABLE 1
MEAN NO OF CELLS IN MANTEL LAYER /MM²

Animal No	Control (A)	Chronic Noise Stress (B)
1	16938	15292
2	17037	16198
3	16823	16230
4	16510	15473
5	17235	15802
6	16165	15770
7	15685	15951
8	16185	15588
9	16796	16241
10	16130	14889
Mean	16567	15747
SEM	106	88

TABLE 2
MEAN PERIMETER OF NUCLEI IN MANTLE ZONE (µM)

Animal No	Control (A)	Chronic Noise Stress (B)
1	2.63	2.62
2	2.67	2.67
3	2.75	2.74
4	2.72	2.77
5	2.62	2.79
6	2.73	2.82
7	2.69	2.74
8	2.68	2.73
9	2.68	2.76
10	2.69	2.78
Mean	**2.684**	**2.742**
SEM	**0.01**	**0.01**

TABLE 3
MEAN PLASMA ACTH CONCENTRATION (PG/ML)

Animal No	Control (A)	Chronic Noise Stress (B)
1	65	162
2	76	112
3	82	145
4	34	108
5	44	194
6	48	118
7	53	122
8	29	132
9	68	98
10	39	124
Mean	**53.8**	**131.5**
SEM	**5.75**	**9.07**

TABLE 4
MEAN PLASMA CORTICOSTERONE CONCENTRATION µG/DL

Animal No	Control (A)	Chronic Noise Stress (B)
1	1.3	4.2
2	1.6	4.25
3	1.4	2.8
4	1.26	3.8
5	1.6	3.9
6	3.75	5.3
7	2.25	3.85
8	0.8	4.45
9	1.8	4.65
10	1.7	4.9
Mean	**1.746**	**4.21**
SEM	**0.25**	**0.22**

DISCUSSION

Noise is increasingly being recognized as physical factors in the environment that is injurious to health and it affects the body system at various levels. According to the environmental expert council of Germany, severe annoyance persistent over prolonged periods of time is to be regarded as causing distress[13].

In the present study we have observed the effect of high intensity noise level (100 dB) applied for longer duration 30 days (chronic) to observe

changes in the level of stress hormones and changes in the morphology of white pulp of spleen in albino rats.

It is now accepted that there are complex interactions among the central nervous system, the endocrine system, and the immune system. Studies in the field of psychoneuroimmunology have shown that stress, through hypothalamic pituitary adrenal (HPA) and sympathetic adrenal medullary (SAM) axis, can induce modulation of the immune system[14]. Recent studies indicate that neuroendocrine immune interactions can cause sufficient immunosuppression to adversely effect human health[8].

Increase in plasma glucocorticoid concentration secondary to stress induced by injury causes splenic atrophy, splenic leucopenia & suppressed B lymphocyte functions[15]. Marked changes were observed in our study in white pulp of splenic tissue in albino rats when exposed to noise stress. A marked decrease in B-lymphocyte in mantle zone was observed. Our finding is in conformity with Kainuma et al.,[16] who observed decrease in number of plaque forming cells on exposure to glucocorticoids and with Zimecki et al.,[17] who found stress induced decrease in antibody forming cells. Exposure of 4 week noise stress has shown to cause a reduction of splenic lymphoproliferation and decrease serum IgG concentration[18]. The study reported here demonstrates that 30 days noise stress significantly decrease the number of B lymphocyte in mantle zone of lymphoid follicle.

Exposure of different concentration of glucocorticoid analogous to those produced during stress, had rapid but varying effects on the major classes of cells of immune system. Immature B-lymphocytes are more susceptible to stress induced rise in glucocorticoid level as glucocorticoids are potent inducer of apoptosis in B-cell line[19]. A large amount of cell loss occurs at the intermediate stages of B-lymphopoiesis[20]. Dexamethasone at physiologic induce rapid apoptosis of most B-lineage precursor[21].

Exposure of noise stress provokes cascade of reactions resulting in activation of HPA axis. Activation of HPA axis is the consequence of the convergence of stimulatory inputs from different region of brain into paraventricular nucleus of hypothalamus where the most important ACTH secreatougogues, Corticotrophin releasing hormone and Arginine vasopressin are formed. Plasma levels of ACTH and corticosterone are considered as good marker of stress.

In our study we have observed the highly significant rise in plasma ACTH and corticosterone concentration when animals were exposed to chronic noise stress. Our finding was found in agreement with the observation of Goshen et al[22]. Glucocorticoids are the final hormone of HPA axis secreted from zona fasiculata of adrenal cortex. The hormones bind with intracellular glucocorticoid receptor present on almost all cells. Glucocorticoid has important anti inflammatory and immunosuppressive properties[23]. In addition exposure of restrain stress increases the binding of glucocorticoid to cytosol of spleen cells[24]. This increase in stress hormone has shown to modulate immune function as blocade of glucocorticoid by pretreatment of glucocorticoid antagonist has shown to reduce effects of stress[25].

Our study concludes that noise is a potent modulator of immune system by activating HPA axis.

REFRENCES

1. Bluhm G, Nordling E, Berglind N. Road traffic noise and annoyance—an increasing environmental health problem. Noise Health. 2004 Jul-Sep;6(24):43-9.
2. Ising H, Babisch W, Kruppa B. Noise-Induced Endocrine Effects and Cardiovascular Risk. Noise Health. 1999;1(4):37-48.
3. EPA Identifies Noise Levels Affecting Health and Welfare EPA press release - April 2, 1974.
4. Khan MW, Memon MA, Khan MN, Khan MM. Traffic noise pollution in Karachi, Pakistan. JLUMHS September-December 2010; 09 (03): 114-120.
5. Schell LM, Gallo MV, Denham M, Ravenscroft J. Effects of pollution on human growth and development: an introduction. Physiol Anthropol. 2006 Jan;25(1):103-12.

6. Spreng M. Possible health effects of noise induced cortisol increase. Noise Health. 2000;2(7):59-64.

7. Butcher SK, Lord JM. Stress responses and innate immunity: aging as a contributory factor. Aging Cell. 2004 Aug;3(4):151-60.

8. Pruett SB. Quantitative aspects of stress-induced immunomodulation. Int Immunopharmacol. 2001 Mar;1(3):507-20.

9. Edgar VA, Silberman DM, Cremaschi GA, Zieher LM, Genaro AM. Altered lymphocyte catecholamine reactivity in mice subjected to chronic mild stress .Biochem Pharmacol. 2003 Jan 1;65(1):15-23.

10. Correa SG, Rodríguez-Galán MC, Sotomayor CE. [Basic aspects of neuroendocrinoimmunology] Rev Fac Cien Med Univ Nac Cordoba. 1999;56(2):9-20.

11. Miller AH et al., Effects of selective type I and II adrenal steroid agonists on immune cell distribution. Endocrinology. 1994 Nov;135(5):1934-44.

12. Dhabhar FS, Miller AH, McEwen BS, Spencer RL Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. J Immunol. 1996 Aug 15;157(4):1638-44.

13. Ising H, Kruppa B. Health effects caused by noise: evidence in the literature from the past 25 years. Noise Health. 2004 Jan-Mar;6(22):5-13.

14. Yang EV, Glaser R. Stress-induced immunomodulation: impact on immune defenses against infectious disease. Biomed Pharmacother. 2000 Jun;54(5):245-50.

15. Lucin KM, Sanders VM, Popovich PG. Stress hormones collaborate to induce lymphocyte apoptosis after high level spinal cord injury. J Neurochem. 2009 Sep;110(5):1409-21. Epub 2009 Jun 22.

16. Kainuma E, Watanabe M, Tomiyama-Miyaji C, Inoue M, Kuwano Y, Ren H, Abo T. Association of glucocorticoid with stress-induced modulation of body temperature, blood glucose and innate immunity. Psychoneuroendocrinology. 2009 Nov;34(10):1459-68. Epub 2009 Jun 2.

17. Zimecki M, Artym J, Chodaczek G, Kocieba M, Kruzel M. Effects of lactoferrin on the immune response modified by the immobilization stress. Pharmacol Rep. 2005 Nov-Dec;57(6):811-7.

18. Zheng KC, Ariizumi M. Modulations of immune functions and oxidative status induced by noise stress. Occup Health. 2007 Jan;49(1):32-8.

19. Laakko T, Fraker P. Rapid changes in the lymphopoietic and granulopoietic compartments of the marrow caused by stress levels of corticosterone._Immunology. 2002 Jan;105(1):111-9.

20. Osmond DG. Proliferation kinetics and the lifespan of B cells in central and peripheral lymphoid organs.__Curr Opin Immunol. 1991 Apr;3(2):179-85.

21. Pifer J, Stephan RP, Lill-Elghanian DA, Le PT, Witte PL. Role of stromal cells and their products in protecting young and aged B-lineage precursors from dexamethasone-induced apoptosis. Mech Ageing Dev. 2003 Feb;124(2):207-18.

22. Goshen I, Yirmiya R, Iverfeldt K, Weidenfeld J. The role of endogenous interleukin-1 in stress-induced adrenal activation and adrenalectomy-induced adrenocorticotropic hormone hypersecretion. Endocrinology. 2003 Oct;144(10): 4453-8. Epub 2003 Jul 10.

23. Fushimi T, Okayama H, Seki T, Shimura S, Shirato K, 1997. Dexamethasone suppressed gene expression and production of interleukin-10 by human peripheral blood mononuclear cells and monocytes. Int. Arch. Allergy Immunol; 112: 13–18.

24. Warner A, Ovadia H, Tarcic N, Weidenfeld J. The effect of restraint stress on glucocorticoid receptors in mouse spleen lymphocytes: involvement of the sympathetic nervous system. Neuroimmunomodulation. 2010;17(5):298-304. Epub 2010 Apr 17.

25. Fukuzuka K, Edwards CK, Clare-Salzer M, Copeland EM, Moldawer LL, Mozingo DW. Glucocorticoid and Fas ligand induced mucosal lymphocyte apoptosis after burn injury. J Trauma. 2000 Oct;49(4):710-6.