Original Article

Anatomy

Cytoprotection of Heat – Induced Splenic Tissue by Cyanocobalamin in Albino Rats

1. Abdul Hafeez 2. Ashok Kumar 3. Abdul Lateef Jokhio

1. Assoc. Prof. of Anatomy, PUM&HSW, Nawabshah 2. Asstt. Prof. of Medicine, Unit-I, PUM&HSW, Nawabshah 3. Assoc. Prof. of Anatomy, CMC, Larkana

ABSTRACT

Objective: To evaluate the cytoprotective role of the Cyanacobalamin on the detrimental effects of heat –induced stress on splenic tissue.

Study Design: An Experimental study

Place and Duration of the Study: Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from January 2010 to April 2010.

Materials and Methods: Forty five Albino rats (180-200 grams) were selected and divided into group A (Control), group B (Heat - induced) and group C (Protective). Each group was further subdivided into three subgroups, based on the period of the study. The animals of the subgroups B and C received heat and the temperature was set at 42 C for six hours daily. Group C (C1, C2 and C3) animals were protected with Cyanocobalamin (BETOLVEX) at the dosage of 0.8 mg/ kg of body weight intraperitonealy, two hours before heat induction. Then animals were sacrificed according to their time duration, spleens were removed and fixed in alcoholic formalin for 24 hours .Then tissue were processed in ascending strengths of alcohol from 70 to 100%,cleared in xylene, infiltrated and embedded in paraffin.5 micron thick sections were stained with haemotoxylin and eosin and e. Blood samples were collected in EDTA K3 containing vacutainers for the hormonal assay of plasma ACTH level.

Results: in group B animal's heat-stress alters the immunoarchitecture of the spleen; size of all comportments of the white pulp reduces with the appearance of the tingitble body macsophages and individual apoptotic cells and free apoptotic bodies. This group also shows significant increase in plasma ACTH levels. Whereas the splenic architecture and plasma ACTH level in group C animals return to normal by the cytoprotective effects of the Cyanocobalamin.

Conclusion: The current study demonstrates the beneficial effects of the Cyanocobalamin to alleviate the detrimental effects of the heat-stress on the immune organs.

Key Words: Albino rats, Spleen, Heat- stress, ACTH, Cyanocobalamin

INTRODUCTION

It is predicted that global warming will cause an increase in the frequency and severity of heat waves with an associated rise in mortality, unless proactive measures are taken [1]. Studies in cell lines and animal models suggest that heat directly induces tissue injury and cell death. Extreme temperatures (49ºC to 50ºC) cause damage to most cellular structures and their functions, resulting cell death by necrosis in less than 5 minutes, whereas rat models showed apoptosis at moderate temperature [1]. Heat stress causes the release of corticosterone and catecholamine and initiates lipid peroxidation in cells membranes [2]. Cells of immune system express the receptors for glucocorticoids and catecholamine. These signals alter the several aspects of immune cell function [3]. According to recent studies heat- stress had more effect on pathophysiology of white blood cells, lymphoid organs and immune responses [4, 5]. The splenic architecture is similar across species; therefore it is one of the recommended organs to evaluate for enhanced histopathology of the immune system [6].

High ambient temperature depletes such antioxidants and induces oxidative stress [7]. Oxidative stability has been improved by antioxidant supplementations for foods of animal origin [4]. Stress doubles the requirement for the Cyanocobalamin [8].

Nutrition has important role in immune function [9]. The concentration of antioxidant vitamins decrease with heat stress [10, 11]. Antioxidant vitamins counteract the free radicals and decrease the ACTH and cortisol levels, protecting the metabolism from the effects of stress [12] .Rapidly growing cells show on increased demand for nutrients and vitamins. All living cells require Cyanocobalamin (Vitamin B12) for survival [13]. Cyanocobalamin plays an important role in immune system regulation [14] and modulates the oxidative stress responses [15].

MATERIALS AND METHODS

This experimental study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. In this experimental study 45 male albino rats of sprauge-dawely variety, 90-120 days of age, weighing180-200 grams were used. They were obtained from the Animal house of the Jinnah Postgraduate Medical Center, Karachi. These animals were housed in the experimental room of the animal

house for a week prior to the commencement of the study and maintained on the balanced diet and water was provided ad libitum. Study design: The animals were subdivided into three groups A, B and C. Each group was further subdivided into three subgroups, A1, A2, A3, B1, B2, B3, C1, C2 and C3 based on the period of the treatment, that was two four and six weeks respectively, whereas each subgroup comprised of five animals. Group A- (A1, A2 and A3) served as (Control). Group B - (B1, B2 and B3) received heat only (Heat-induced). Group C- (C1, C2 and C3) received heat and Cyanocobalamin (Protected). Group C animals were protected with Cyanocobalamin (BETOLVEX) manufactured by Alpharma Aps, Denmark at the dosage of 0.8 mg/ kg of body weight intraperitonealy, two hours before heat induction. Then animals of group B and C were shifted in another experimental room for heat induction provided by double rod electric room heater of 2000 WATT. The temperature was set at 42 C for six hours daily [16], according to their time duration. Then the animals were sacrificed at the end of their respective treatment period by the overdose of Ether anesthesia in a glass jar, spleens were removed and fixed in alcoholic formalin for 24 hours .Then tissue were processed in ascending strengths of alcohol from 70 to 100%,cleared in xylene, infiltrated and embedded in paraffin.5 micron thick sections were stained with haemotoxylin and eosin for detailed microscopy.While the animals were still breathing, blood samples about 2 ml were collected from each animal by cardiac puncture in the plastic vacutainers containing EDTA-K2 as an anticoagulant(BD- Franklin NJ ,USA) for the hormonal assay of plasma ACTH level by using Mouse /Rat adrenocorticotropic hormone (ACTH) ELISA antibody test Kit (Catalog#40 - 109 – 325002;Genway Biotech ,INC ,CA).

RESULTS

Microscopic Observations: The Haemotoxylin and Eosin stained sections in heat-treated animals of subgroup B-1, the histological picture of spleen do not differ significantly from that found in the control group except few differences.In subgroup B-2, splenic architecture was moderately altered. The capsule showed scalloped appearance indicative of decreased splenic size due to the marked loss of lymphocytes. Germinal center contained a moderate number of tingible body macrophages laden with cytoplasmic engulfed apoptotic fragmentations of dead cells giving characteristic “moth eaten” appearance was observed in all compartments of the white pulp (Fig-01). The periarteriolar lymphoid sheath (PALS) showed a moderate number of tingible body macrophages laden with apoptotic fragmance and also showed a reduction in the number of T-cells. In subgroup B-3, splenic architecture was markedly altered. The capsule showed prominent scalloped appearance (Fig-02). The marked “moth eaten” appearance was observed in the PALS region, the follicle and the mantle zone.

Red pulp showed a marked hypocellularity in parenchyma and venous sinuses (Fig-03).

The Haematoxylin and Eosin stained sections in subgroup C-1 showed splenic architecture comparable to control. The capsule showed normal thickness comparable to control. The size and cellularity of white pulp and its compartments returned near to control. Few tingible body macrophages and apoptotic foci were observed (Fig-04).The subgroups C-2 and C-3 showed less distortion. The notches of the capsule disappeared. The size and cellularity of white pulp improved compare to the heat induced subgroups B-2 and B-3. The number of tingible body macrophages decreased. The cellularity of red pulp was returned close to the control group animals.

Table No.1 *Mean Plasma Level of ACTH (pg/ml) in Different Groups of Albino Rats at Variable Time Intervals

Group	Sub-groups	Treatment Given	Plasma level of ACTH		
			2nd week	4th week	6th week
A (n=15)	A1 (n=5)	Control	153.20 ± 2.31		
	A2 (n=5)			158.401± 1.93	
	A3 (n=5)				162.80 ± 1.77
B (n=15)	B1 (n=5)	Heat	355.60 ± 8.22		
	B2 (n=5)			359.20 ± 2.08	
	B3 (n=5)				361.40 ± 3.01
C (n=15)	C1 (n=5)	Heat+Cyano-cobalamin	165.0 ± 4.04		
	C2 (n=5)			166.60 ± 6.45	
	C3 (n=5)				171.80 ± 3.59

Statistical Analysis of Mean Levels of ACTH in Different Groups of Albino Rats

Statistical comparison	P-value	Statistical comparison	P-value
B1 vs A1	P<0.001****	C2 vs B2	P<0.001****
C1 vs B1	P<0.001****	C2 vs A2	P>0.05*
C1 vs A1	P>0.05*	B3 vs A3	P<0.001****
B2 vs A2	P<0.001****	C3 vs B3	P<0.001****
		C3 vs A3	P>0.05*

Key: Insignificant*, Significant**, Moderately Significant***, Highly Significant****

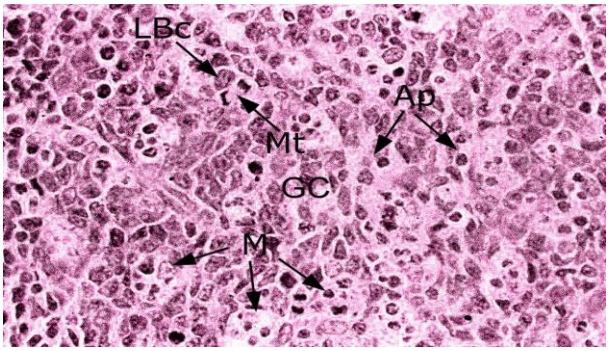

Figure No.1: H&E stained 5 µm thick section of spleen, after 6 weeks of heat treatment, showing (LBc) large B-lymphocytes in the (GC) germinal center, a large number of (M) tingible body macrophages with cytoplasmic engulfed apoptotic bodies , (Ap) apoptotic cells and few (Mt) mitotic cells (Photomicrograph x 400)

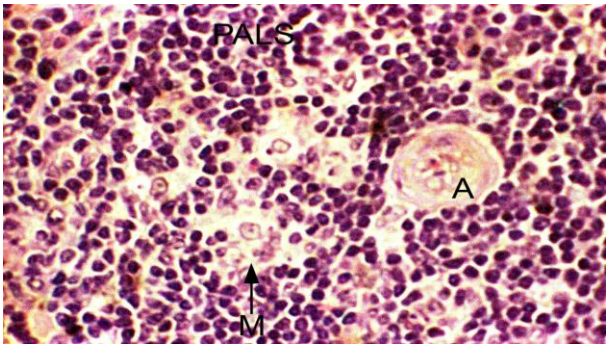

Figure No.2: H&E stained 5 µm thick section of spleen, after 6 weeks of heat treatment, showing (Tc) T-lymphocytes in the (PALS) periarteriolar lymphoid sheath around (A) central artery, (M) tingible body macrophages with cytoplasmic engulfed apoptotic bodies and (Ap) apoptotic cells (Photomicrograph x 1000).

Analysis of Plasma Acth Level: The mean values of the plasma ACTH levels (table-1) show a highly significant increase (p<0.001) in subgroups B-1, B-2 and B-3 compared to control subgroups A-1, A-2 and A-3. The data (table-1) of group C animals showed and insignificant increase (p>0.05) in subgroups C-1 C-2 and C-3 compared to control subgroups A-1, A-2 and A-3 the data (table-1) also showed a highly significant decrease (p>0.001) in subgroups C-1,C -2 and C-3 compared to subgroups B1, B2 and B3 respectively.

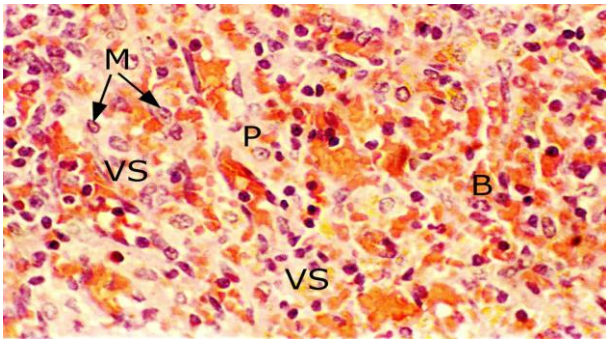

Figure No.3: H&E stained 5 µm thick section of splenic red pulp, after 6 weeks of heat treatment showing hypocellularity in (P) parenchyma and (VS) venous sinuses, a large number of (M) macrophages and (B) band cells cells (Photomicrograph x1000)

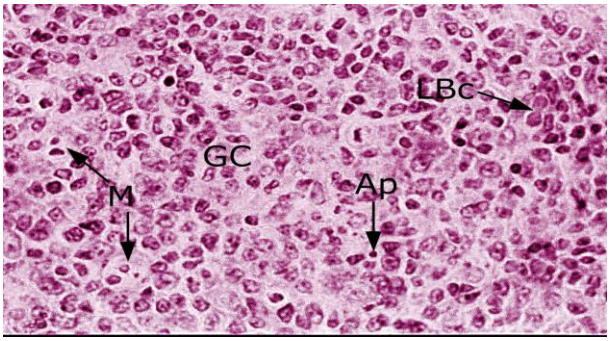

Figure No.4: H&E stained 5 µm thick section of splenic white pulp, after 6 weeks of Cyanocobalamin therapy, showing (LBc) large B-lymphoctyes in the (GC) germinal center with few (M) tingible body macrophages and (Ap) apoptotic cells (Photomicrograph x 1000).

DISCUSSION

In the present study microscopy of heat-induced group B animals show hypocellularity and decrease in size of all white pulp compartments with increased number of pyknotic nuclei of apoptotic and necrotic cells were observed, indicative of marked degenerative changes. Most of the splenic lymphatic nodules, marginal zones and periarteriolar lymphoid sheaths contain large sized macrophages laden with nuclear and cytoplasmic fragments of apoptotic cells. Free apoptotic bodies found scattered among the intact cells. All compartments of the white pulp were equally affected.

These extensive changes might be due to apoptosis induced by heat-stress. Lymphocytes and other blood cells are classified as high turnover and heat labile cells and our study is in conformity with Khan and Brown who suggested that cells which are in high turnover state, are programmed for apoptosis and thus easily activate this mode of cell death in response to lethal stimuli. The heat-stress activate protein kinase-C Jun and –Terminal Kinase pathway. This in turns triggers activation of the caspases cascade, which target several proteins, to bring about apoptotic cell death [17]. Animals show atrophy of spleen after exposure to heat-stress [18,] and simillar findings were observed at the time of sacrifice in our animals. The findings of the present study were also similar to Sakaguchi et al, who observed apoptosis in spleen of rat models in his study in tumor and normal tissues induced by whole body hyperthermia [19]. Elmore reported apoptosis in splenic tissues of Sprague Dawley rat treated with dexamethasone. He explained that apoptosis was marked in the B-cell rich zone and around central artery in periarteriolar lymphoid sheath (T-cell rich zone), a large number of tingible body macrophages laden with cytoplasmic engulfed apoptotic debris and free apoptotic bodies found between intact cells. These results are in confirmation with the present study [6].

Group B animals show a moderately significant increase in plasma ACTH level This findings is also in accordance with koko et al, who observed a significant rise in plasma ACTH in Wistar rats exposed to heat for 60 minutes [20].

Group C animal's morphology showed that cellularity and size of the different compartments of white and red pulp of spleen returns near to control because of the substantial protection provided by Cyanocobalamin through its growth promoting effects against the apoptosis and induction of lymphocyte proliferation as described by as described by some other researchers [21,22]. Findings of present study were also similar with the study of Tamura et al, who observed immunomodulatory effects of Cyanocobalamin by restoring the proportion of lymphocytes and functions of the natural killer (NK) cells [23]. The observations in present study were also in agreement with the study of Brich et al suggesting that Cyanocobalamin modulate the oxidative stress responses by contributing in the synthesis of important intracellular antioxidant glutathione to prevent hydrogen peroxide mediated stress and also protects against apoptosis by reducing the caspase-3 cleavage [15].

Plasma ACTH sections returns near to normal values, it might be due to direct or indirect inhibitory effect of the Cyanocobalamin on the ACTH session and this finding is similar to a study which describe the inhibitory effects of vitamin B-12 on ACTH and costicosterone [24].

CONCLUSION

Based on the present study it is concluded that heat-stress severely damages the immune organs and causes depletions of immunocytes Cyanocobalamin has expressed itself as an immunopotentiating agent under heat stress by restoring the architecture and cell count of immune organs.

REFERENCES

1. Roberts GT, Ghebeh H, Chishti MA, Al-Mohanna F, Sayed RE, Al-Mohanna Fet al. Microvascular Injury, Thrombosis, Inflammation, and Apoptosis in the Pathogenesis of Heatstroke, A study in Baboon Model. Arterioscler Thromb Vasc Biol 2008;28:1130-1136.
2. Sahin N, Orhan C, Tuzcu M, Sahin K, Kucuk O. The Effects of Tomato Powder Supplementation on Performance and Lipid Peroxidation in Quail. Poultry Science 2008;87:276- 283.
3. Sood AK, Bhatty R, Kamat AA, Landen CN, Han L, Thaker PH, et al. Stress Hormone-Mediated Invasion of Ovarian Cancer cells. Clin cancer Res 2006;12 (2): 369-375.
4. Altan O, Altan A, Cabuk M, Bayraktar H. Effects of Heat Stress on Some Blood Parameters in Broilers. Turk J Vet Anim Sci 2000;24: 145-148.
5. Al- Ghamdi ZH. Effects of Commutative Heat Stress on Immunoresponses in Broiler Chickens Reared in Closed System. Int J Poul Sci 2008;7 (10):964-968.
6. Elmore SA. Enhanced Histopathology of Spleen. Toxicol Pathol 2006; 34 (5): 648-655.
7. Sujatha V, Korde JP, Rastogi SK, Maini S, Ravikanth K, Rekhe BS. Amelioration of heat stress induced disturbances of the antioxidant defense system in broilers. J Vet Med Anim Health 2010;2(3):18-28.
8. Dubeski PL, Owens FN, Song WO, Coburn SP, Mahuren JD. Effects of B Vitamin Injections on plasma B vitamin concentration of feed-restricted Beef calves Infected with Bovine Herpes-1[1]. J Anim Sci 1996;74: 1358-1366.
9. Field CJ, Aerde AV, Drager KL, Goruk S, Basu T. Dietary folate improves age-related decreases in lymphocyte function. J Nutritional Biochem 2006; 17:37-44.
10. Sahin K, Onderci M. Sahin N, Gursu MF, Kucuk O. Dietary Vitamin C and Folic Acid Supplementation Ameliorates the Detrimental Effects of Heat Stress in Japanese Quail. J Nutr 2003; 133: 1882-1886.
11. Aengwanich W. Pathological Changes and the Effects of Ascorbic Acid on Lesion Scores of Bursa of Fabricius in Broilers Under Chronic Heat Stress. Res J Veterinary Sci 2008;1(1):62 – 66.

12. Imik H, Ozkanlar S, Kaynar O, and Koc M. Effects of vitamin E,C, and α-lipoic acid supplementation on the serum glucose, lipid profile, and proteins in quails under heat stress. Bull Vet Inst Pulawy 2009; 53: 521-526.
13. Waibel R, Treichler H, Schaefer NG, Staveren DRV, Mundwiler S, Kunze S, et al. New Derivatives of Vitamin B12 Show Preferential Targeting of Tumors. Cancer Res 2008;68(8): 2904-2911.
14. Tamura J, Kubota K, Murakami H, Sawamursa M, Matsushma T, Tamura T, et al. Immunomodulation by vitamin B-12: augmentation of $CD8^+$ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. Clin Exp Immunol 1999; 116: 28-32.
15. Brich CS, Brasch NE, McCaddon A, Williams JHH. A novel role for vitamin B12: Cobalamins are intracellular antioxidants in vitro. Free Radical Biol and Med 2009;47:184-188.
16. Al- Ghamdi ZH. Effects of Commutative Heat Stress on Immunoresponses in Broiler Chickens Reared in Closed System. Int J Poultry Sci 2008;7 (10): 964 – 968.
17. Khan VR, Brown IR. The effect of hyperthermia on the induction of cell death in brain, testis, and thymus of the adult and developing rat. Cell Stress and Chaperones 2002;7(1):73 – 90.
18. Naseem S, Younus M, Anwar B, Ghafoor A, Aslam A, Akhter S. Effect of Ascorbic Acid and Acetylsalicylic Acid Supplementation on Performance of Broiler Chicks Exposed to Heat Stress. Int J Poultry Sci 2005; 4(11): 900-904.
19. Sakaguchi Y, Stephens LC, Makino M, Kaneko T, Strebel FR, Danhauser LL. Apoptosis in Tumors and Normal Tissues Induced by Whole Body Hyperthermia in Rats. Cancer Res 1995;55:5459 - 5464.
20. Koko V, Djordjeviae J, Cvijiae G, Davidoviae V. Effects of acute heat stress on rat adrenal glands: a morphological and stereological study. The J Exp Biol 2004; 207: 4225-4230.
21. Guyton AC, Hall JE. Text Book of Medical Physiology. 11th ed. Philadelphia: Elsevier Saunders; 2006.p.889,953,955-956.
22. Guerra-Shinohara EM, Morita OE, Peres S, Pagliusi RA, Neto LFS, Almeida VD, et al. Low ratio of S-adenosylmethionie to S-adenosyl homocysteine is associated with vitamin deficiency in Brazilian pregnant women and newborns. Am J Clin Nutr 2004; 80 : 1312- 1321.
23. Tamura J, Kubota K, Murakami H, Sawamursa M, Matsushma T, Tamura T, et al. Immunomodulation by vitamin B-12: augmentation of $CD8^+$ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. Clin Exp Immunol 1999; 116: 28-32.
24. Lovgren O, Norman A, Winqvist G. Inhibitory effect of vitamin B12 on cortisone and ACTH. Acta Rheumatol Scand 1955;1(2):106-12.

Address for Corresponding Author:
Dr. Abdul Hafeez,
Associate Professor, Anatomy Department
Peoples University of Medical & Health Sciences for Women Nawabshah
E-mail: drabdulhafizdall@yahoo.com
Postal Address: Flat M-82 Doctors Colony
Nawabshah.