MEDICAL CHANNEL

Original Article

A MORPHOMETRIC STUDY OF AMELIORATING ROLE OF CYANOCOBALMIN (VITAMIN B-12) ON TESTUCULAR THERMAL INJURY IN ALBINO RATS

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ABSTRACT

Objective: The purpose of this study was to evaluate the possible effects of Cyanocobalamin (vitamin B-12) in ameliorating the harmful effects of heat on seminiferous tubules of testes in albino rats.

Methods: Thirty adult albino rats of 200-220 grams of weight were taken for this study. They were divided into three groups A (control), B (heat treated), and C (heat plus Cyanocobalamin treated). At the end of study period i.e., 6 weeks, blood samples were taken for serum ACTH and serum Testosterone. Morphometric study of seminiferous tubules of testes was done by staining with Periodic Acid Schiff Iron Hematoxylin stain.

Results: There was increase in the serum levels of ACTH whereas serum testosterone was markedly decreased in heat (37^0C) treated group. The diameter of seminiferous tubules of testes decreased along with decreased thickness of germinal epithelium. This damage was well protected with concomitant use of Cyanocobalamin (vitamin B-12). There was restoration of serum ACTH and testosterone levels, and germinal epithelium and diameter of seminiferous tubules also returned to normal.

Conclusion: This study showed ameliorating effect of Cyanocobalamin on heat induced damage in testes of albino rats.

Key Words: Thermal injury, Cyanocobalamin, Testes.

INTRODUCTION

All tissues are susceptible to damage by heat[1]. Dada[2] et al (2001) had mentioned that exposure to high temperature at the workplace is an occupational hazard and leads to deterioration of sperm morphology and impaired motility which may result in infertility. Studies in cell lines and animal models suggest that heat directly causes tissue injury[3,4,5]. Experimental studies have suggested that a mild increase in testicular temperature could be a potential contraceptive method for men[6], and that nocturnal scrotal cooling could improve sperm count but there is compelling evidence that there is a correlation between male infertility and testicular heat stress[7].

A physiologically relevant increase in body temperature is sufficient to activate the cellular stress response [8,9]. Stress induced activation of the adrenocortical axis could cause an inhibitory effect on reproduction. Administration of ACTH has been shown to cause a reduction of basal LH secretion and suppression of the LH response to LHRH. An inverse relationship between stress and normal reproductive efficiency has frequently been recognized in human and veterinary medicine [10, 11].

Antioxidants such as vitamin C[11], vitamin A[13], methionine[14] and minerals such as zinc and chromium have been shown to ameliorate the effects of environmental stresses [15, 16,17,18,19].

Vitamin B-12 has been used in the therapy of a number of conditions, including

trigeminal neuralgia, multiple sclerosis and other neuropathies, various psychiatric disorders, poor growth, and as a tonic for patients complaining of tiredness or easy fatigability[2]. It leads to the synthesis of important antioxidant, glutathione[21]. The effects of vitamin B-12 are particularly pronounced on rapidly dividing cells such as bone marrow and intestines[22].

Various studies have been carried out to observe the protective effects of agents like vitamin A, Vitamin C, Vitamin E, selenium etc, but so far scanty work has been done to observe morphometric changes in testicular tissue due to thermal injury with Cyanocobalamin protection. With this back ground, this study was planned to observe the protective role of Cyanacobalamin on thermally injured testicular tissue.

MATERIAL AND METHODS

This experimental study was conducted in the department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. Thirty young male albino rats about 90-120 days of age and 200-220 gm of weight were obtained from animal house of BMSI, JPMC.

Animals were divided into three groups A, B, and C. The animals were kept under observation for one week, prior to the commencement of study for assessment of their health status. The animals were weighed at the beginning of the study. The animals were kept in plastic cages and were maintained on 12 hours light and 12 hours dark cycle. The standard laboratory chow and tap water were available ad libitum.

- Group-A animals served as control.
- Group-B animals had received heat at 37ºC for six hours daily with electric room heater and maintained with room thermometer for 6 weeks.
- Group-C animals received heat as given in group B and injection Cyanacobalamin (UCB Pharma Limited, 2005), at dose of 500 mcg/ kg body weight[23] intraperitoneally for 6 weeks.

The animals were sacrificed under deep ether anesthesia at the end of their treatment period. A mid line incision was made up to scrotum and extended upwards to the thoracic region. Blood sample was taken from each animal by intra-cardiac puncture with the help of disposable syringe, for the detection of serum ACTH and Testosterone levels.

Both the testes were exposed and then removed. They were fixed in Bouin's fluid for 24 hours. After 24 hours each testis was cut longitudinally into two equal halves and again post fixed in fresh Bouin's fluid for next 24 hours. The tissues were kept in capsules and then washed in running water for 3 to 4 hours to remove excess fixative. After fixation, tissues were processed in ascending strengths of alcohol, infiltrated and embedded in paraffin. 4µm thick longitudinal sections were cut on rotatory microtome. Sections of tissues were floated on hot water bath at 42ºC and transferred on albumenized glass slides. The slides were placed on hot plate at 37ºC for 24 hours for fixation of tissues and stained with Periodic Acid Schiff Iron Haematoxylin Technique[24]. The morphometric examination was done under light microscope. From each animal, ten sections were selected randomly. In each section, five fields were observed and in each field, three randomly circular or nearly circular tubules were selected. In this way, fifteen tubules were observed from each section for measurement of diameter and thickness of germinal epithelium of seminiferous tubules.

The statistical significance of differences of various quantitative changes between different groups of animals was evaluated by the paired sample't' test. The differences were noted as statistically significant if p-value was equal to or less than 0.05. All calculations were done utilizing computer software, Microsoft Excel in Windows 2000 XP[25].

RESULTS

There was highly significant increase ($P<0.001$) in the mean ACTH level in group B, when compared with control group A (Table-1). The level of ACTH was insignificantly increased ($P>0.05$) in group C animals when compared with control animals, whereas there was moderately significant decrease ($P<0.01$) in ACTH

Table-1
***MEAN SERUM LEVELS OF ACTH (PG/ML) IN DIFFERENT GROUPS OF ALBINO RATS**

Groups (n=10)	Treatment given	Serum enzyme level (pg/ml)
A	Control	62.31 ± 7.60
B	Heat treated	111.84 ± 2.18
C	Heat + Cyanocobalamin (vitamin B 12)	65.01 ± 7.43

*** Mean ±SEM**

STATISTICAL ANALYSIS OF SERUM LEVELS OF ACTH (PG/ML) IN DIFFERENT GROUPS OF ALBINO RATS

Statistical Comparison	P- Value
B VS A	P<0.001****
C VS A	P>0.05*
C VS B	P<0.01***

Key: *Insignificant **Significant ***Moderately significant ****Highly significant

Table-2

*MEAN SERUM LEVELS OF TESTOSTERONE (NG/ML) IN DIFFERENT GROUPS OF ALBINO RATS AT VARIABLE TIME INTERVALS

Groups (n=10)	Treatment given	Serum enzyme levels (ng/ml0
A	Control	6.92 ± 0.30
B	Heat treated	0.60 ± 0.04
C	Heat + Cyanocobalamin (vitamin B 12)	4.01 ± 0.30

*** Mean ±SEM**

Statistical Analysis of Serum levels of Testosterone (ng/ml) in different groups of Albino rats

Statistical Comparison	P- Value	
B VS A	P<0.001****	
C VS A	P<0.001****	
C VS B	P<0.001****	

Key: *Insignificant **Significant
*****Moderately significant ****Highly significant**

Table-3

*MEAN DIAMETER (µM) OF SEMINIFEROUS TUBULES OF ALBINO RATS IN DIFFERENT GROUPS

Groups(n=10)	Treatment given	Mean diameter of seminiferous tubules (ìm)
A	Control	284.60 ± 1.36
B	Heat treated	196.20 ± 1.15
C	Heat + cyanocobalamin treated (vitamin B 12)	281.80 ± 0.37

***Mean ±SEM**

STATISTICAL ANALYSIS OF MEAN DIAMETER OF SEMINIFEROUS TUBULES BETWEEN GROUPS

Statistical Comparison	P-Value	
B VS A	P < 0.001****	
C VS A	P > 0.05*	
C VS B	P < 0.001****	

Key: *Insignificant **Significant
*****Moderately significant****Highly significant**

Table-4

*MEAN THICKNESS OF GERMINAL EPITHELIUM (µM) OF SEMINIFEROUS TUBULES IN DIFFERENT GROUPS OF ALBINO RATS

Groups(n=10)	Treatment given	Mean thickness of Germinal epithelium (ìm)
A	Control	76.60 ± 0.92
B	Heat treated	34.40 ± 0.92
C	Heat + Cyanocobalamin treated (vitamin B 12)	73.40 ± 0.92

*** Mean ±SEM**

STATISTICAL ANALYSIS OF MEAN THICKNESS OF GERMINAL EPITHELIUM OF SEMINIFEROUS TUBULES BETWEEN GROUPS

Statistical Comparison	P-Value	
B VS A	P < 0.001****	
C VS A	P > 0.05*	
C VS B	P < 0.001****	

Key: *Insignificant **Significant
*****Moderately significant ****Highly significant**

levels when group C animals were compared with heat treated group B animals (Table-1).

There was highly significant decrease (P<0.001) in mean testosterone level in group B, when compared with control group A (Table-2), whereas highly significant decrease (P>0.001) in group C when compared with control group A (Table-2). The data also showed highly significant increase (P>0.001) in serum testosterone level in group C when compared with heat treated group B (Table-2).

There was highly significant decrease (P<0.001) in diameter of seminiferous tubules in group B when compared with control group A, whereas insignificant decrease (P>0.05) in group C when compared with control group A (Table-3). The data also showed highly significant increase (P<0.001) in diameter of seminiferous tubules in group C when compared with heat treated group (Table-3).

There was highly significant decrease (P<0.001) in the thickness of germinal epithelium of seminiferous tubules in group B when compared with control group A (Table-4).There was insignificant decrease (P>0.05) in germinal epithelium in group C when compared with group A. The data also showed highly significant increase (P<0.001) in thickness of germinal epithelium in group C when compared with heat treated group B (Table-4).

DISCUSSION

High ambient temperature, local heat stress or any other factors that cause an increase in testicular temperature are able to damage spermatogenesis[18]. Recent observations suggest that cobalamin may modulate the oxidative stress responses[21].

. The observations on ACTH in the present study showed vitamin B12 ameliorates the negative effects of heat stress and decreased the level of ACTH.

In the present study group B animals showed significantly decreased levels of serum testosterone. This might be during the stress; HP axis is activated leading to increased ACTH secretion which in turn causes increased glucocorticoid secretion and consequently circulating testosterone levels are decreased via glucocorticoid receptors in Leydig cells.

Norman[26] observed in an experimental study, the effects of CRH on LH, testosterone and cortisol secretion in intact male Macaque, and reported that CRH is involved in the inhibition of hypothalamic-pituitary- testicular-axis in response to stress. CRH inhibits the release of GnRH, resulting in suppression of LH. Setchell[1] in his study exposed male rats to temperature of 33-35°C for 3-5 weeks and found decreased testosterone concentration. Yazawa[27] observed the effect of immobilization stress on testicular germ cells and also found transient decrease in serum testosterone levels. Gonadotrophin (LH) production is under control of GnRH. The decreased secretion of GnRH causes down regulation of LH, which in turn inhibits the secretion of testosterone from Leydig cells[28].

The present study showed inverse ratio between ACTH and testosterone levels. In heat treated animals increased levels of ACTH were associated with decreased testosterone levels.

In group C animals the levels of testosterone were restored because co-administration of vitamin B12 decreased the oxidative stress, resulting in removing the inhibitory effect on GnRH and subsequently LH, which was responsible for testosterone release. Amara[19] in their study on preventive effect of zinc against cadmium-induced oxidative stress in the rat testis noted that zinc supplementation attenuated DNA oxidation induced by cadmium in the gonad and restored the testosterone level.

The present study showed significant decrease in thickness of germinal epithelium and diameter of seminiferous tubules, and the damaged germinal epithelium was leading to apoptosis.

Kumar[5] describes that apoptosis is a pathway of cell death that is induced by a tightly regulated intracellular program in which cell destined to die activates enzymes that degrades the cell's own nuclear DNA and nuclear cytoplasmic proteins.

This was due to the direct effects of heat on germ cells of seminiferous tubules of testes. This may also be due to reduced secretion of testosterone in heat treated animals leading to increased release of ACTH via hypothalamic-pituitary-adrenal axis as also suggested by Faldikova[29]. Lue[6] reported that short exposure of the testes to heat causes degeneration of germ cells, and apoptosis is the underlying mechanism of heat-induced (43ºC for 15 minutes) germ cell death in the adult rat. A single exposure (43ºC for 15 minutes) of the rat testis to heat resulted in selective, but reversible, damage to the seminiferous epithelium through increased germ cell apoptosis[6].

The animals in group C showed restoration of germinal epithelium, decrease in vacuolation and improved tubular diameter. This could be the role of vitamin B12 in restoring serum testosterone levels and in reducing the oxidative stress. As B12 reduces the stress, ACTH levels were decreased to near normal levels, resulting in might be near normal levels of LH which restored normal morphology of germinal epithelium. Courten and Ploen[30] in their study on adult cryptorchid testis observed that intra-testicular infusion of Lactate had improvement in various stages of spermatogenesis. Yang[13] observed restoration of germinal epithelium by á-Tocopherol in Cadmium-induced testicular damage, by reduction of free radical damage and oxidative stress. The results of present study are in complete agreement with Watanabe[31] who mentioned that vitamin B12 supplementation increased the diameter of seminiferous tubules and thickness of germinal epithelium in rats.

CONCLUSION

It is concluded that thermal injury leads to severe damage of the testicular tissue in albino rats, which increases with the duration of exposure. It is further concluded that Cyanocobalamin plays a definitive role in protecting testicular tissue from this damage. It is therefore suggested that, humans who are working at high ambient temperature in factories or under direct sun, can be protected by concomitant use of therapeutic doses of Cyanocobalamin from heat stress, heat stroke, nervous irritability and infertility.

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