P: ISSN No. 0976 - 8602 RNI No.UPENG/2012/42622 VOL.-IV, ISSUE-III, July-2015
E: ISSN No. 2349 - 9443
Asian Resonance

Effect of Splenectomy on Testicular Activities in Immature Male Albino Rats

Madhumita Debnath
Assistant Professor,
Deptt.of Physiology,
Dr. K.L. Bhattacharyya College,
Ramrajatala, West Bengal

Moulik Debnath
Assistant Professor,
Deptt.of Anatomy,
Murshidabad Medical College &
Hospital, Berhampore,
West Bengal

Abstract

Immune system in mammals has intimate relationship with reproductive system. Steroids in both sexes have been shown to influence activities of primary and secondary lymphoid organs. Various immune effector cells and molecules too, on the contrary, modulate functional activities of sex organ. This present study has been designed to explore the functional relationship between male reproductive system and immunity. The specific objective is to study the influence of a major lymphoid organ (spleen) on male gonads in immature system of rats. The study was conducted on 12 immature male albino rats of Wister Strain. Animals were divided into two groups. One group was subjected to splenectomy and the other was considered as control, not being exposed to splenectomy. Both groups of animals were kept for a period of 56 days in aseptic laboratory conditions. The animals were sacrificed and tissues were collected to perform biochemical and histometric assay. It was found that testicular Δ^5, 3β-HSD and 17β-HSD activities were marginally reduced in splenectomised group. Plasma testosterone level decreased significantly in splenectomised group in comparison to control. Histometric studies indicated that seminiferous tubular diameter and epididymal sperm count were also reduced in splenectomised group. Activities of two important anti-oxidant enzymes- catalase and peroxidase were found to decrease in experimental group in comparison to control. Estimation of lipid peroxidation from quantification of TBARS and CD was performed in both groups which showed that there was increase in the level of these products of free radicals in splenectomised group. Overall the study showed an inhibition of testicular activities in male albino rats following splenectomy.

Keywords: Splenectomy, Plasma Testosterone, Testicular-Δ^5,3β And 17β-HSD, Oxidative Stress

Introduction

This research work has its background lying on some interesting observations related to the sexual dimorphism in various aspects of immune system in human population. Women, in general, mount a more vigorous immune response than male. The prevalence of autoimmune diseases among women has long being reported. On the contrary, male has found to suffer from a different degree of immune suppression that is now being associated with higher level of androgens. Greater occurrences of HIV infection and prostate cancer in male are now extensively correlated to the immune suppressive action of testosterone. It is now an established fact that immune system has an intimate relationship with reproductive system in mammals, and, it is the ‘neuro-immuno-endocrine’ interaction in body that maintains the overall homeostasis. Importance of such interactions between immune and reproductive system in mammals can not be overlooked and needs to be explored.

Review of literature

A large number of experimental and clinical studies indicate a strong relationship between immune and reproductive system in mammals today. It is true for both sexes. Female steroids influence the immune susceptibility as indicated by a greater occurrence of autoimmune diseases in women (1). Splenectomy has been reported to cause a delay in the ovulation in rats (2), and a rise in the level of serum progesterone, which get normalized after injection of splenocytes (3). Ovariectomy increases the weight of thymus, a primary lymphoid organ, while estrogen replacement causes a decrease of the same along with a reduction of numbers of thymocytes (4).

In male, testosterone is considered to be an immune suppressive agent (5). Prostate cancers in male have been correlated to high levels of

androgen. Seminal fluid can interfere with the functions of T- lymphocytes (6), B- lymphocytes (7), natural Killer cells (8), and antibody (9) and complement proteins (10). Castration of normal male mice leads to splenic enlargement and expansion of bone marrow B-cell population. These changes were reversible following androgen replacement (11). Castration in mice resulted in enlarged thymus with a predominance of thymocytes positive for helper phenotype (CD4+, CD8-), where as testosterone replacement resulted into thymic regression with a shift towards suppressor /cytotoxic phenotype (CD4-,CD8+) (12).

Present research work has been planned to explore this area of interrelationship between male reproductive system and immunity.

Materials and Methods

Selection of Animal and Care

The study was conducted on twelve healthy, immature (25 days of age) male albino rats of Wister strain. These rats had an average body weight of 58 ± 10 g. The animals were acclimatized to laboratory condition prior to experimentation. Animals were housed two per cage in a temperature controlled room (22 ± 2° C) with 12-12 h dark light cycle at a humidity of 50 ± 10 %. They were provided with standard food and water *ad libitum.* Animal care was provided according to the guideline for the care and use of animals. The experimental protocol was approved by University ethical committee.

Experimental Design

Twelve healthy immature male albino rats of Wister strain were divided into the following groups.

Group I (Control Group): Control rats were kept in animal cage for a total of 56 days of experimental period.

Group II (Splenectomised group): Rats of this group were subjected to splenectomy. Splenectomised rats were kept in animal cage for a period of 56 days. After completion of 56 days of experimentation, all the animals were sacrificed. Tissues were collected from each animal and were processed for biochemical and histological assay.

Assay of Testicular Δ^5, 3β-Hydroxysteroid Dehydrogenase (Δ^5, 3β-HSD) And 17β-Hydroxysteroid Dehydrogenase (17β-HSD) Activity:

Testicular Δ^5, *3β*-hydroxysteroid dehydrogenase activity was measured biochemically (13). The right testis of each animal was homogenized. It was centrifuged at 10,000 rpm for 30 minutes at 4° C. The supernatant at a volume of 1 ml was mixed with 1 ml sodium pyrophosphate buffer (pH=8.9), 40 µl of dehydroepiendosterone and 0.9 ml of redistilled water. Enzyme activity was measured after addition of 100 µl of 0.5 µm of nicotinamide adenine dinucleotide (NAD) to the tissue supernatant mixture in a spectrophotometric cuvette at 340 nm against a blank (without NAD). The same supernatant prepared for the assay of Δ^5,*3β-HSD* was used for assaying 17 *β-HSD* activity (14). The supernatant was mixed with 1 ml of sodium pyrophosphate buffer, 40 µl ethanol containing 0.3 µm testosterone and 0.9ml of redistilled water. Enzyme activity was assessed after the addition of 100 µl of 0.5 µm NAD to the incubation mixture in a spectrophotometric cuvette at 340 nm against a blank without NAD.

Assay of Plasma Testosterone

The collected blood was centrifuged and plasma fraction was separated for the study. Plasma level of testosterone was measured following the immuno-enzymatic method by ELISA reader (Merck, Japan) according to the standard protocol of National Institute of Health & Family Welfare (15). Here we followed commercially available competitive solid phase enzyme immuno assay, where horse radish peroxidase is used as enzyme–labeled antigen (supplied by IBL, USA).

Assay of Catalase and Peroxidase

The activity of the enzyme catalase was measured biochemically (16). For the assay of catalase activity, tissue samples were homogenized separately in 0.05 M Tris-Hcl buffer solution (pH= 7.0) at the tissue concentration of 50 mg/ml and then centrifuged. This tissue homogenate was then used for assay. The peroxidase activity of tissues was determined biochemically using the modified procedure described by Sadisivam and Manickam (17).

Estimation of lipid peroxidation from the concentration of TBARS and CD

The tissues were homogenized at the tissue concentration of 50 mg/ml in 0.1 ml ice-cold phosphate buffer solution (pH=7.4). Homogenates were centrifuged at 10,000 g 4^0 C for 15 minutes. Each supernatant was used for the estimation of TBARS and CD. TBARS, the product formed due to the peroxidation of lipid, was determined by the reaction of thiobarbituric acid with malondialdehyde (18). Quantification of CD was performed by a standard method (19).

Histometric Study and Epididymal Sperm Count

The prepared histological slide of testis, stained with hematoxyline and eosin, were placed under high power objective of microscope, and with the help of stage & ocular micrometer, the seminiferous tubular diameters (STD) were measured. The epididymal sperm count was measured by standard method using hemocytometer.

Statistical Analysis

Student's two-tail t-test was used for statistical analysis of the collected data. Differences were considered significant when $p<0.05$.

Results

Body weight was found to increase in both the groups at the end of 56 days of study in respect to their initial body weight (Table-1). The percentage of weight gain was higher in the control group as compared to splenectomised group. Testicular Δ^5, 3β-HSD and 17β-HSD activities were decreased marginally in splenectomised group in comparison to control. Plasma testosterone level was decreased significantly in splenectomised group in comparison to control (Table-2). Activities of catalase and peroxidase in testis were decreased in splenectomised group in comparison to control. The

P: ISSN No. 0976 - 8602 RNI No.UPENG/2012/42622 VOL.-IV, ISSUE-III, July-2015
E: ISSN No. 2349 - 9443 Asian Resonance

quantity of CD in testis increased significantly in splenectomised group in respect to control. The quantity of MDA (TBARS) in testis also increased in splenectomised group (Table-3). Histometric studies indicated that seminiferous tubular diameter was decreased in splenectomised group in comparison to control. Epididymal sperm count was found to decrease significantly in splenectomised group in respect to control group (Table-4).

Table-1: Mean ± SEM level of Body weight and Testicular somatic index

Parameters	Control Group	Splenectomised Group
Initial Body Weight(gm)	58 ± 3.90	60 ± 3.41
Final Body weight(gm)	138.4± 8.35	136.8± 4.89
Weight Gain (%)	145.4± 26.77	126.8 ± 9.62
Testicular somatic index	1.476± 0.0453	1.439± 0.0427

Table-2: Mean ± SEM level of the activities of Streroidogenic enzymes and plasma Testosterone:

Parameters	Control Group	Splenectomised Group
Testicular Δ^5, *3β-HSD (unit/ mg of tissue/hour)*	23.924 ± 1.63	21.684 ± 0.503
Testicular *17β-HSD (unit/ mg of tissue/hour)*	22.424 ± 1.46	19.744 ± 0.7659
Plasma testosterone (ng/ml of plasma)	1.3 ± 0.2536 *	0.36 ± 0.092 *

Table-3: Assessment of Oxidative Stress

Parameters	Control Group	Splenectomised Group
Catalase in testis (mM of H_2O_2/mg of tissue/min)	0.5524 ± 0.054	0.5044 ± 0.038
Peroxidase in testis (Unit/mg of tissue)	0.7724 ± 0.056 *	0.50788 ± 0.106*
TBARS (nM/mg of tissue)	53.052 ± 10.4*	135.904 ± 69.9*
CD (nM hydroperoxide/mg of tissue)	170.66 ± 3.23 *	192.82 ± 8.008 *

Table-4: Histometric study and Sperm count in control and splenectomised group:

Parameters	Control Group	Splenectomised Group
STD X 100(µm)	238.55 ± 3.2	212.26 ± 3.6
Epididymal spermcount (millions/ml epididymal fluid)	12.32 ± 0.922 *	9.1 ± 1.305*

Each value represents Mean ± SEM (n=5).Two-tail t-test was performed. In each column mean with superscript * differ from each other significantly.($p < 0.05$).

Discussion

This present work provides a number of observations regarding the effect of splenectomy on testicular activities in immature male albino rats. It also explores possibility of oxidative stress originated out of removal of spleen.

From experimental observation it is clear that splenectomy has no major effect on body growth. There is no significant alteration in the testiculo-somatic index as well. The diminution in activities of Δ^5, 3β-HSD and 17β-HSD, the key steroidogenic enzymes for androgenesis, in splenectomised group may be due to an indirect effect of removal of spleen on hypothalamico-pituitary-gonadal axis. Therefore, splenectomy has caused retardation in steroid synthesis in male reproductive organ. The low plasma testosterone level after splenectomy has supported the concept of this inhibition on steroidogenic activities. Diminution in the seminiferous tubular diameter (STD) also supports this low steroid level in testis in splenectomised group as STD is considered to be a recognized indicator of plasma testosterone. Epididymal sperm count decreased significantly in experimental group after splenectomy. It also tells us that splenectomy not only retards steroidogenesis, but also inhibits spermatogenesis.

Another possibility for the inhibition in androgenesis is oxidative stress imposed in testicular tissue by removal of spleen, because, free radical generation during oxidative stress can interfere with the activities of steroidogenic enzymes. Activities of catalase and peroxidase, the two important anti-oxidant enzymes in testis have been performed. Results revealed that their activities have been reduced significantly. The low-antioxidant enzyme activity further facilitates the increased susceptibility to lipid peroxidation. Another possibility for the low activities of these two enzymes may be due to low level of testosterone, as testosterone promotes the synthesis of antioxidant enzymes in sex-organ. The quantity of TBARS in testis increased after splenectomy. The level of CD was found to be elevated significantly. Both these observations support the low anti-oxidant activity in testis.

From the above findings, two possible mechanism of inhibition of testicular activities in male albino rats after splenectomy may be framed. First one is the indirect effect of splenectomy on pituitary-testicular axis. Splenic factors may be vital for steroid synthesis and sperm production. The second mechanism may be the direct oxidative stress imposed in testicular tissue.

Reference

1. Ostensson. M., Husby. G. A. (1983), Prospective clinical study of the effect of pregnancy on rheumatoid arthritis & alkylosing spondylitis. Arthritis, Rheum, 26, 1155.
2. Takahashi. M., Saito. S., Matsuyama. S. (1988) Effect of spleen on luteal steroidogenesis in vitro or in vivo. Human Reproduction, Current Status/ Future Prospect, (Lizuka, R. and Semen, K. eds) Excerpta Medis, Amsterdam, pg- 179.
3. Saito. S., Matsuyama. S., Shiata. K., Takahashi. M. (1998) Involvement of splenocytes in the control of corpus luteum function in rat, Endocrinol. (Jap.), 35, pg-891.
4. Grossman. C.J. (1985) Interactions between the gonadal steroids and the immune response. Science, pg 227-257.
5. Fitzpatrick. F. T., Greenstein. B.D. (1987), Effects of various steroids on the thymus ,

spleen, ventral prostate and seminal vesicles in old orchidectomized rats. Endocrinol., 113,51.
6. Prakash. C., Lang, R.W. (1980) .Studies on immune infertility: A hypothesis on the etiology of the immune infertility based on the etiological role of seminal plasma immune response inhibition. Mount Senal J. Med, 47, 491.
7. Lewis. W. R., Whalen. J. J., Shrines. R.J. (1976) Mixed culture of sperms and leucocytes as a measure of histocompatibility in man. Science, 191,302.
8. Schopf. R.E., Schramm. P., Bene. P., Morsches. B. (1984) Seminal plasma induced immuno suppression of respiratory burst of PMNL and monocytes, Androl., 16,124.
9. Witkin. S.S., Rechards. J. M., Bogiovanni. A.M., Zelikonsky. J. (1983), An IgG Fc binding protein in seminal fluid. Am. J. Reprod. Immunol., 3,23.
10. Anderson. D. J., Tarter. T.H. (1982).Immunosuppressive effects of mouse seminal plasma components in vivo and in vitro. J. Immunol.,182,535.
11. Viselli SM, Reese KR, Fan J, Kovacs WJ, Olsen NJ.(1997) Androgens alter B cell development in normal male mice. Cell Immunol., Dec 15,182(2),99-104.
12. Olsen. N.J, Watson. M.B, Henderson. G.S, Kovacs. W.J. (1991)Androgen deprivation induces phenotypic and functional changes in the thymus of adult male mice. Endocrinology. Nov;129(5),2471-6
13. Talalay. P. Hydroxysteroid dehydrogenase. (1962) Methods in Enzymology, (Colowick, S.P., Kalpan, N. O.eds), Academic press, New York., 5,512.
14. Jarabak. J., Adams. J.A., Willmans-Ashman. H.G., Talalay. P. (1962) Purification of 17 β Hydroxy steroid dehydrogenase of human placenta and studies on its transhydrogenase function . J.Biol.Chem., 237,345.
15. Srivastava. T.G. (2001) Enzyme linked immunosorbent assay for steroid hormones. Orientation training course on Research methodology in reproductive biomedicine. Department of Reproductive biomedicine. National Institute of Health and Family Welfare, , p55.
16. Beers. R. F., Sizer. I. W. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem., 195, 133.
17. Sadasivam. S., Manickam. A. (eds) (1996).Peroxidase. In: Method in Biochemistry. New age International Publisher, New Delhi, p. 108.
18. Ohkawa. H., Ohishi. N., Yagi. K. (1979) Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal. Bio. Chem.,95,351.
19. Slater. T. I. (1984) Overview of methods used for detecting lipid peroxidation. Method in Enzymol.,105, 283.