EFFECTS OF AMLODIPINE BESYLATE ON SPERMATOGENESIS IN SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To determine the effects of chronic treatment with amlodipine besylate on spermatogenesis in Sprague Dawley rats.

Study Design: Quasi experimental study.

Place and Duration of Study: The study carried out in the department of Physiology, Army Medical College Rawalpindi in collaboration with the department of Pathology, Army Medical College and National Institute of Health, Islamabad during April 2007 and March 2008

Material and Methods: Thirty male Sprague Dawley rats were divided into two groups; each comprising of 15 rats. Group A was given vehicle whereas group B was given amlodipine besylate (norvasc) by oral and gavage for 50 days along with standard rat diet. Testes were removed 24 h after the last experimental day and sectioned. The sections were stained by hematoxylin and eosin and examined microscopically. Results were analyzed on SPSS version 13.

Results: There were significant differences in mean tubular diameter and height of germinal epithelium of testes between amlodipine and vehicle treated rats.

Conclusion: Long term treatment with amlodipine besylate (norvasc) results in suppression of spermatogenesis in male rats.

Keywords: calcium channel blocker, amlodipine besylate, testicular histology, spermatogenesis

INTRODUCTION

Spermatogenesis is an exocrine function of the mammalian testes in which male germ cells proliferate and differentiate from undifferentiated spermatogonia [1]. This process begins at puberty and involves mitosis, meiosis and spermiation. It is tightly regulated via intricate autocrine, paracrine and endocrine mechanisms in which calcium ions play crucial role. As far as hormonal control of spermatogenesis is concerned, spermatogenesis depends on hypothalamic Gonadotrophin releasing hormone (GnRH), pituitary gonadotropins, Luteinizing hormone (LH) and Follicle- stimulating hormone (FSH) [2]. LH mediates its effects via testosterone and LH induced testosterone synthesis in the Leydig cells is dependent on calcium [3,4]. Sertoli cells synthesize, secrete and proficiently distribute products that are crucial for the growth and differentiation of developing germ cells [5,6]. The secretion of sixty percent of secretory products of Sertoli cells is calcium dependent as manifested by the increase in secretion of proteins from cultured rat sertoli cells in response to agents like potassium chloride (KCl, voltage-gated calcium channel opener) and ionomycin (calcium mobilizing agent) [7]. The blood testicular barrier creates a meticulous milieu which is indispensable for germ cell survival and is regulated by an array of molecules, which include intracellular calcium [8, 9].

In view of the diverse role of calcium in regulation of spermatogenesis, it appears that calcium channel blockers can adversely affect spermatogenesis. So far, the available literature has not documented any conclusive work on the morphological changes in testes that occur during long term use of calcium channel blockers. Although few studies [10,11] are available in this respect but these have been conducted on the testes of prepubertal animals, instead of the adult age group. Since hypertension is a common problem of adults commonly treated by the calcium channel blockers, therefore present study was designed and carried out to observe the effects of long term treatment with amlodipine besylate on the morphology of testes in adult Sprague Dawley rats.

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Received: 28 Aug 2007; Accepted: 26 May 2007

MATERIAL AND METHODS

The study was conducted in the department of Physiology, Army Medical College Rawalpindi in collaboration with the department of Pathology, Army Medical College and National Institute of Health, Islamabad, from April 2007 to March 2008. Thirty adult male Sprague Dawley rats (60 to 120 days old) were used which were divided into two equal groups A and B. Group A was administered vehicle (0.5 ml distilled water/ rat/ day) orally and group B was given amlodipine (0.14 mg/ kg/ 0.5 ml/ day) orally with the help of a gavage tube for 50 days i.e one complete spermatogenic cycle. Rats were supplied with diet pellets and water ad libitum throughout the experiment. In each group, rats were sacrificed 24 h after the end of the treatment. The testes were removed and fixed in 10 % formalin. The tissues were processed routinely and sectioned at 5 micron thickness by rotary microtome. Lastly, hematoxylin and eosin staining was done. Stained sections of the testes were examined microscopically for:

a) Diameter of seminiferous tubules under low power field

b) Height of germinal epithelium under high power field

c) Germ cells study (Spermatogonia, spermatocytes and spermatids) under oil immersion lens.

d) Morphological changes in cells such as necrosis, degeneration etc.

For the calculation of tubular diameter and height of germinal epithelium, an occular micrometer was used after calibration with a standard stage micrometer. The seminiferous tubules cut at right angle to the axis of the tubule were measured. Seven observations were made in each section of an animal. In this way 105 observations were made in each group. The data regarding tubular diameter and height of germinal epithelium were analyzed statistically by using SPSS version 13. The arithmetic mean and standard deviation of all observations were calculated. Difference in mean among control and treated groups was calculated by 'independent t test'. The difference was considered significant if p value was found less than 0.05.

RESULTS

Findings of group A (control)

On gross examination, the testes appeared pink, firm, almost uniform in size and did not reveal anything abnormal. Microscopic examination of testes revealed normal morphology. Seminiferous tubules were lying back to back with little stroma in between (Figure 1). They didn't show any disruption of the basement membrane and germinal epithelium or both. Germinal epithelium did not show any degenerative change or any marker of cellular degeneration (e.g. cytoplasmic vacuoles or pyknotic nuclei), when seen under oil immersion lens. Spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids were easily visible (Figure 2). The process of spermiogenesis was within normal limits and indicated by the tails of sperms. Abnormal or multinucleated giant cells were not observed. The surrounding area of tubules showed interstitial cells of Leydig in groups and blood vessels. The mean tubular diameter was 321.07 ± 36.44 µm and the mean height of germinal epithelium was 88.41 ± 6.71 µm (Table).

Findings of group B (experimental)

Gross examination of the testes was normal looking. On microscopic examination, seminiferous tubules were arranged loosely (Figure 1). Spermiogenesis was within normal limits in all the tubules as indicated by the presence of tails of the sperms. In most of the tubules, tubular diameter and height of the germinal epithelium were reduced. Number of immature spermatogonia as well as maturing spermatids appeared reduced manifesting the suppression of spermatogenesis (figure 3). The mean tubular diameter was 268.13 ± 20.19 µm and the mean height of the germinal epithelium was 78.68 ± 8.45 µm (Table). These features were suggestive of suppressed spermatogenesis.

Statistical Analysis

In the control group, mean tubular diameter and mean height of the germinal epithelium was 321.07 ± 36.44 µm and 88.41 ± 6.71 µm respectively. Histological examination of the group B rats exposed to amlodipine besylate revealed the mean tubular diameter of 268.13 ± 20.19 µm and height of germinal epithelium as 78.68 ± 8.45 µm. The comparison of mean tubular diameter and germinal epithelium between experimental and control groups revealed a significant statistical difference (P = 0.000 and 0.002 respectively).

Table: Comparison of mean tubular diameter and height of germinal epithelium in control and experimental groups (n=15)

Parameters	Group A (Control) mean+ SD	Group B B (Experimental)	P value**
Tubular diameter (µm)	321.07 ± 36.44	268-13 ± 20.19	0.000
Thickness of germinal epithelium(µm)	88.41 ± 6.71	± 8.45 78.6	0.002

P–value is significant.

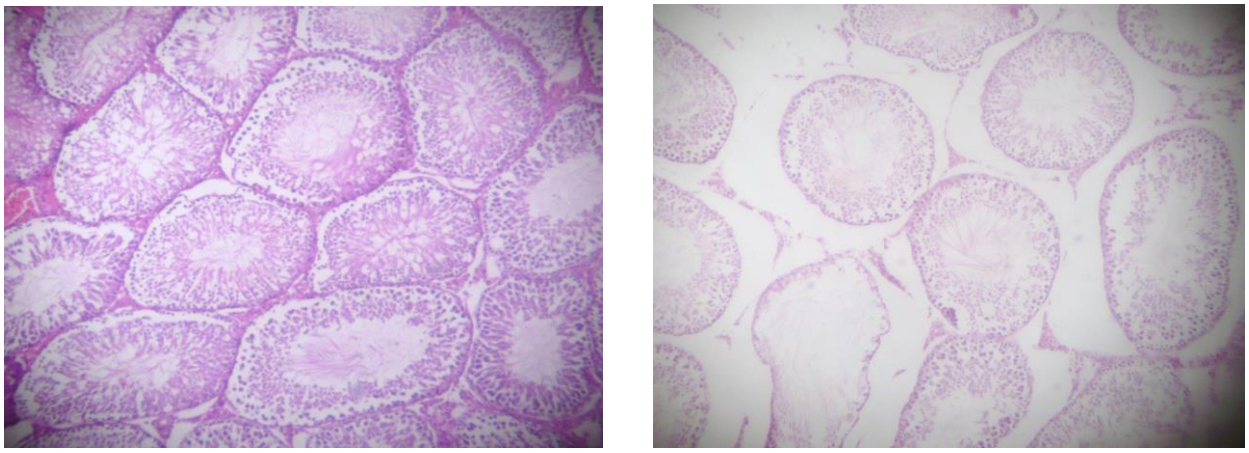

Fig. 1: Photomicrograph of testicular tissue showing normal compactly arranged seminiferous tubules in control group (H & E stain x 320 approx.) a) Seminiferous tubules b) Lumen containing tails of spermatozoa c) Interstitium containing Leydig cells and blood vessels

DISCUSSION

Living beings are defined by their ability to reproduce and spermatogenesis is the sine qua non for reproduction and continuation of life. However, this process might be insulted by various exogenous and endogenous agents e.g. radiations, chemicals and drugs etc. This study observed the effects of amlodipine besylate, a dihydropyridine calcium channel blocker on rat spermatogenesis. In control group, mean tubular diameter closely resembled the tubular diameter reported in a study conducted at Jordan [12]. Height of the germinal epithelium was similar to the data presented in Chile [13]. Histological examination of the group B rats exposed to amlodipine besylate for 50 days revealed a reduction in the mean tubular diameter and height of germinal epithelium. The comparison of mean tubular diameter and germinal epithelium between experimental and control groups revealed a significant statistical difference. These atrophic changes in the epithelium of seminipherous tubules of rats indicate either a direct action of amlodipine besylate on sertoli cell-germ cell interaction, or an indirect action affecting the hormonal milieu at the level of the pituitary-testicular axis.

Almaida et al. [11] has reported the suppression of spermatogenesis in response to amlodipine besylate administration because of the decreased FSH level which caused reduction in sertoli cell count in prepubertal rats. However it may not be the reason in present study because our experimental animals were adults. Once puberty is achieved, sertoli cell count stays

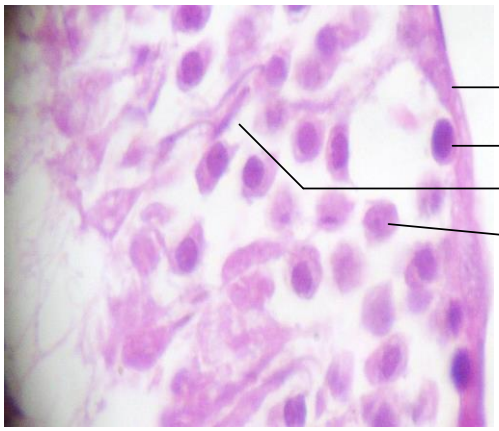

Figure 2: Photomicrograph of testicular tissue showing increased thickness of germinal epithelium in control group (H & E stain x 1280 approx.) a) Basement membrane b) Spermatogonia c) Primary spermatocytes d) Elongated spermatids

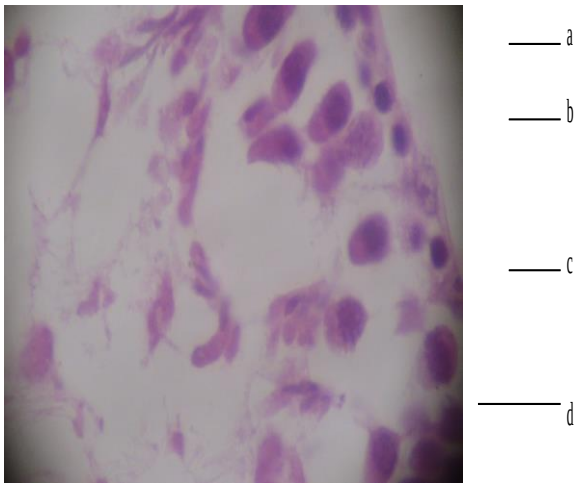

Figure 2-B: Photomicrograph of testicular tissue showing decreased thickness of germinal epithelium in experimental group (H & E stain x 1280 approx.) a) Basement membrane b) Spermatogonia c) Primary spermatocytes d) Elongated spermatids

constant [14, 15] and variations in FSH levels can no longer affect sertoli cell count [16]. Lee et al. [10] has also recently demonstrated the arrest in spermatogenesis in response to calcium channel blockers other than amlodipine such as; nifedipine in mouse but the age of the experimental animals was peripubertal.

Considering the age of animals in our study, the role of calcium in spermatogenesis, and the presence of voltage operated calcium channels (VOCCs) in germ cells, sertoli cells and Leydig cells, suppressed spermatogenesis could be either because of impaired gene transcription in germ cells as calcium ions have been shown to affect transcription process [17, 18, 19], or due to reduction in Sertoli cell protein secretion as at least 50–60% of protein secretion from Sertoli cells is calcium dependent [7].

Calcium also regulates blood testicular barrier [20]. There is a possibility that amlodipine causes detachment of spermatids by disturbing the blood testis barrier and prevents them from further maturation. Lastly, decreased serum testosterone levels have been reported with amlodipine treatment [11]. The testosterone deficiency could be the cause of germ cells depletion.

CONCLUSIONS

Based on the histological findings of experimental and control groups, it is concluded that chronic treatment of male rats with amlodipine besylate (narvase) results in suppression of spermatogenesis.

Acknowledgements: The authors gratefully acknowledge the financial support of the Higher Education Commission, Pakistan for conducting this research project.

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