SPERMATOGENESIS FOLLOWING DISCONTINUATION OF CALCIUM CHANNEL BLOCKER AMLODIPINE IN RATS

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Background: The calcium channel blockers are commonly associated with male infertility. Long term administration of such drugs has been shown to suppress spermatogenesis. The present study was designed to determine the effects of cessation of calcium channel blocker (amlodipine) medication on spermatogenesis. **Methods:** The study was carried out in the Department of Physiology, in collaboration with Department of Pathology, Army Medical College, and National Institute of Health, Islamabad from April 2007 to March 2008. Thirty male rats were divided into two groups A and B each containing 15 rats. Group A rats were given distilled water (vehicle) whereas group B rats were given amlodipine by oral gavage for 50 days. After 50 days, amlodipine/distilled water was withdrawn. The rats were left for recovery to take place for another 50 days of drug withdrawal period. Testes were removed in all the rats and sectioned. The sections were stained with haematoxylin and eosin and examined microscopically. Results were analyzed using SPSS version 13. **Results:** Fifty days after drug withdrawal, there were statistically insignificant differences in mean tubular diameter and height of germinal epithelium between amlodipine treated and vehicle treated groups. **Conclusion:** Spermatogenesis in amlodipine treated rats does not differ significantly from spermatogenesis in vehicle treated rats after 50 days of drug withdrawal period.

Keywords: Calcium channel blocker, CCB, amlodipine, male contraception, testicular histology, spermatogenesis, Dihydropyridine, DHP

INTRODUCTION

Calcium signals are present not only in somatic cells but also in the germ cells.[1,2] Data accumulated over the past few years have revealed that calcium plays a pivotal role in sperm physiology. Several key functions in a spermatozoa including acrosome reaction[3], capacitation[4], motility[5,6] and hyperactivity[7,8] are regulated by cytoplasmic calcium.

Impaired calcium signalling is a common feature of male factor infertility.[9] Recently, several investigators have focused on calcium channel blockers (CCBs) as a promising target for male contraceptive development. Dihydropyridine (DHP) CCBs which are established drugs in the management of hypertension and chronic stable angina, occupy central position in such candidates because voltage operated calcium channels expressed on sperm membrane are highly sensitive to DHPs.[1,10,11]

Amlodipine, a CCB belonging to DHP group, has been shown to decrease sperm count in sperm suspensions collected from cauda epididymis of rats.[12] In humans, long term treatment with amlodipine resulted in azoospermia in semen and few non-motile sperms in testicular sperm extraction.[13] This indicates that long term use of amlodipine not only inhibits spermatogenesis but it also impairs sperm motility. Thus, amlodipine might have the potential to be developed as a safe and efficient male contraceptive. Previous experiment from our laboratory has shown that long term treatment with amlodipine resulted in decreased serum testosterone level[14] and suppressed spermatogenesis as indicated by reduction in mean seminiferous tubular diameter and height of germinal epithelium[15]. Another parameter which amlodipine must fulfil before being characterized as male contraceptive agent is reversibility, therefore present study was designed to see whether the spermatogenic suppression caused by amlodipine is reversible or not.

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. Thirty adult male Sprague Dawley rats (60 to 120 days old) were divided into two groups A and B each containing 15 rats. Groups A and B were designated as control recovery and experimental recovery respectively. 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/ rat/ 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. After 50 days, drug/vehicle was withdrawn. Animals from both groups were kept for recovery and sacrificed after another 50 days. The testes were removed and fixed in 10% formalin. The tissues were processed routinely and sectioned at 5 micron thickness by rotary microtome. Lastly, haematoxylin and eosin staining was done. Stained sections of the testes were examined microscopically for diameter of seminiferous

tubules under low power field and height of germinal epithelium under high power field

For calculation of tubular diameter and height of germinal epithelium, an ocular 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 subgroup. 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

In recovered groups, seminiferous tubular diameter was 293.60±8.32 µm in group A (control recovery) and 285.20±14.90 µm in group B (experimental recovery) (Figure-1).

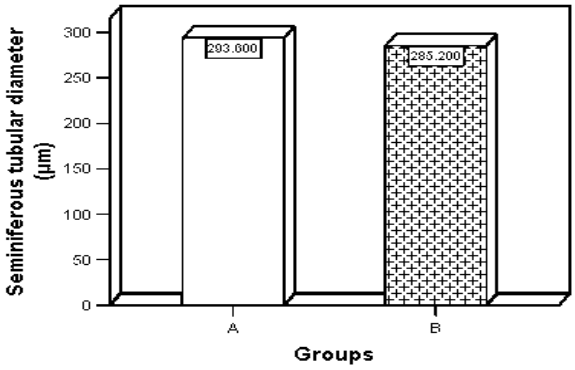

Figure-1: Seminiferous tubular diameter in groups A (Control recovery) and B (experimental recovery)

Height of germinal epithelium in group A (control recovery) was 84.30±2.87 µm and in group B (experimental recovery) was 83.61±3.02 µm (Figure-2).

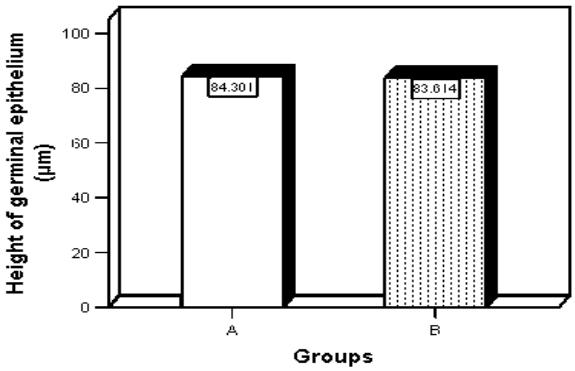

Figure-2: Height of germinal epithelium in groups A (Control recovery) and B (experimental recovery)

Comparison of mean seminiferous tubular diameter and height of germinal epithelium between group B (experimental recovery) and group A (control recovery) revealed statistically insignificant differences ($p>0.05$).

DISCUSSION

In the present study, differences between seminiferous tubular diameter and germinal epithelium height of group B (experimental recovery) and group A (control recovery) were statistically insignificant. This shows either amlodipine administration has not resulted in any change in seminiferous tubular diameter and germinal epithelium height, or if at all amlodipine caused a change, that was completely reversible on drug withdrawal. Previously published results from our laboratory have clearly shown that long term administration of amlodipine causes a significant reduction in seminiferous tubular diameter and height of germinal epithelium.[15] Therefore, the data presented herein reveals that the reduction in seminiferous tubular diameter and height of germinal epithelium caused by amlodipine administration is completely reversible on drug withdrawal.

The results presented herein compare favourably with previously published studies in which investigators have reported that anti- reproductive effects of CCBs are reversible on discontinuation of therapy. Hershlag *et al*, assessed sperm fertilizing potential in sperms from infertile male patients taking DHP CCB as antihypertensive treatment. He reported subnormal expression of sperm head-directed mannose ligand receptors and failure of spontaneous acrosomal reaction in the sperms of such patient. The effect was completely reversible following discontinuation of medication[16].

Benoff *et al*, compared the spermatozoa from known fertile men with the spermatozoa of normospermic infertile men on CCBs. They reported that sperms from patients taking CCB as antihypertensive medication comparatively had a low frequency of mannose ligand receptors and acrosomal reaction been impaired in such patients. The sperm fertilizing potential reversed and sperm characteristics returned to normal when CCB was withdrawn and the male partner was switched to another antihypertensive, angiotensin converting enzyme inhibitor.[17] Dr. Susan Benoff strengthened the concept and declared in conjoint annual meetings of the American Society for Reproductive Medicine and the Canadian Fertility and Andrology Society that Nifedipine produces reversible male infertility by changing the cholesterol content of the sperm. She also documented that CCBs can produce infertility within a month of taking the drug. However, reversal of infertility requires 3 months of discontinuation.[18]

Literature strongly supports that long term use of CCBs is linked with infertility which is reversible on drug discontinuation. The data presented herein demonstrate reversibility of effects after 50 days of drug discontinuation. Literature has mentioned reversibility of CCBs induced adverse effects after 90 days of drug withdrawal.[16–18] Variation in the time period that exists between the literature and the results presented here may simply because of difference in the duration of spermatogenesis in various species. In rats, the spermatogenic cycle is of 50 days[19] as compared to humans in which spermatogenesis is of 74 days[20].

Another reason of variability might be the fact that they did not checked reversibility of drug induced adverse effects after 50 days of drug discontinuation.

The reversibility of suppressed spermatogenesis caused by amlodipine, could be the basis for the elusive male contraceptive pill. However, in contrast to amlodipine, such a product should produce prompt recovery of fertility upon discontinuation. Keeping in view, the cost of clinical trials and the development of a new product, it could take perhaps 5–6 years, before an amlodipine-based product is marketed specifically for contraception.

CONCLUSIONS

Based on the findings of study presented herein, it is concluded that by which ever mechanism amlodipine suppressed spermatogenesis either by direct toxic effects on Sertoli/germ cells or by affecting hormonal milieu at hypothalamo-pituitary-testicular axis; that mechanism was not permanent; rather it was temporary and completely reversible on drug discontinuation. However, effects of amlodipine on sperm functions such as motility, capacitation and acrosomal reactions needs to be further explored and these will finally determine the fate of amlodipine as male contraceptive agent.

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