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Research Article

EFFECTS OF AMLODIPINE ON THE TESTICULAR PARAMETERS OF ALBINO RAT

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ABSTRACT

To determine the effect of exposure of calcium channel blocker Amlodipine for different periods on the reproductive parameters of adult male Wistar albino rats Amlodipine in a dose equivalent to the normal clinical dose was administered orally to albino rats in their reproductive age for different periods ranging from two weeks to eighteen weeks and the reproductive parameters such as sperm count, motility and gonado-somatic index were verified.

Reproductive parameters such as sperm count and sperm motility were found to be adversely affected in a significant ($p<0.05$) and duration dependent manner from 28th day onward of once daily administration of Amlodipine while gonado-somatic index was decreased significantly ($p<0.05$) from 42nd day of continuous administration Potential risk of the calcium channel blocker Amlodipine on male fertility, especially on long term use must be considered while prescribing this drug to young adults.

Keywords: Calcium channel blocker, Amlodipine, male fertility, sperm count, sperm motility, gonado somatic index.

INTRODUCTION

Compared to the past infertility among married couple is a chronic problem now-a-days. Studies show that in our state 20 % of couples suffer some type of infertility due to multiple reasons. Male factor contribute to 30-50 % of these infertilities.[1]

Similarly in olden days hypertension and cardiac problems etc were considered as the diseases of the elderly. But at present many young people have to depend on drugs for controlling their hypertension/cardiac problems.[2] Calcium channel blockers (CCB) are a widely used class of drugs for their reliable antihypertensive, anti arrhythmic or anti anginal effects. But many data accumulated over the past few years have pointed towards the potential of these agents to cause infertility in males.[3] Dihydropyridine (DHP) calcium channel blockers which are the established drugs for chronic stable angina and hypertension are commonly associated with infertility.[4,5]

Literature search has revealed some experimental evidences for the potential of Calcium channel blockers to cause adverse effects on sperm characteristics when used for a short period. But it is learnt that adverse effects of an agent on the spermatogenic process may not be observed in semen evaluations or in fertility tests for a substantial time after initiation of treatment. Damage that is limited to spermatogonial stem cells would not appear in cauda epididymal sperm for 8 to 12 weeks depending on the species examined. To allow effects on spermatogonial stem cells to be observed in all evaluations of cauda epididymal sperm in sub chronic studies, treatment of adult male should be continued for a minimum of six cycles of germinal epithelium prior to sacrifice.[6] For rats one cycle of germinal epithelium requires 12.9 days. So for completing 6 cycles, 77.4 days is needed.

Based on these, in the present study an attempt is made to determine the effect of Amlodipine on the reproductive parameters of adult male Wistar albino rats when it is used for different periods, both shorter and longer than that needed for completing six cycles of germinal epithelium.

Besides, as essential hypertension is a common problem in the male population of their reproductive age and Amlodipine is a widely used drug for hypertension and other problems like Atherosclerosis, an attempt to find the potential of this drug to adversely affect the reproductive parameters when used in the clinical dose is highly relevant.[7,8] So the rat equivalent of the clinical dose of Amlodipine (Amlodipine oral - 10 mg once daily) is selected as the dose in this study [9,10]

MATERIALS AND METHODS

Ethical Clearance

The study protocol was approved by the Institutional Animal Ethical Committee, Medical College, Thiruvananthapuram. (IAEC No 02/15/2010/MCT dated 08-06-2010)

Animal Selection, Maintenance and Care

Healthy male albino rats of 5-6 months of age[11] (Wistar strain) weighing 180-220 g were used for the study. They were obtained from animal house Medical College, Thiruvananthapuram, India. These animals were fed on standard pellet diet (manufactured by Nav Maharashtra Chakan Oil Mills Ltd; Pune, India and supplied by Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. The animals were maintained under standard conditions of relative humidity, 12 h light-dark cycle, adequate ventilation and ambient room temperature. These rats were divided into different groups such as

Group I: control-distilled water in a dose of 1 ml / 100 g body weight

Group II: Treated with Amlodipine 0.9 mg / kg once daily for 14 days

Group III: Treated with Amlodipine 0.9 mg / kg once daily for 28 days

Group IV: Treated with Amlodipine 0.9 mg / kg once daily for 42 days

Group V: Treated with Amlodipine 0.9 mg / kg once daily for 91 days

Group VI: Treated with Amlodipine 0.9 mg / kg once daily for 126 days

At the end of the specified period, animals were weighed and killed by cervical dislocation, the peritoneal cavity was opened through a lower transverse abdominal incision and the testicles were removed along with the epididymis for gonado somatic index, sperm count and motility.

Sperm Analysis

Determination of Sperm Count

For sperm count the cauda epididymal semen was drawn into 0.5 mark of WBC pipette, the semen diluting fluid (prepared by dissolving 5 g of sodium bicarbonate ($NaHCO_3$) and 1 ml of 40 % formaldehyde in 100 ml of normal saline) was drawn to 11 mark which was thoroughly mixed and one drop was added to both sides of Neubauer's haemocytometer. The spermatozoa were allowed to settle down in the haemocytometer by keeping them in a humid chamber for 5 minutes. The sperm count was done with a 40 X objective as in RBC counting. The total number of sperms were counted in the 5 major squares on each side of the hemocytometer and determined the average for calculating sperm count / ml.[12] The hemocytometer is 0.1 mm deep and the 25 large squares represent an area of 1 square mm. with a volume of 0.1 µl. When 5 squares are counted, the sperm that settled out of 0.02 µl only are counted. Therefore count in 5 squares must be multiplied by 50,000 in order to determine total sperm in 1.0 ml. To get the concentration of the original sperm sample the dilution factor should also be accounted. So Sperm count / ml = (dilution factor) (count in five squares) ($0.05x\ 10^{6}$)

Assessment of Sperm Motility

The sperm motility was assayed microscopically within 5 minutes following their isolation from epididymis. The epididymal plasma was suspended in phosphate buffer saline, cleared the tissue debris and a clear solution was used for the assessment of motility of sperms. The total number of motile sperms was counted similarly as in sperm count. Motility was expressed as percentage of motile sperms compared to total cells.[13]

Determination of Gonado-Somatic Index (GSI)

Weight of the right and left testes were noted separately and Gonado-somatic index was determined with the help of formula[14]:

Gonado-Somatic Index (GSI) = (Gonad weight/total body weight) × 100
Gonad weight = (weight of the right testis + weight of the left testis) / 2

Table 1: Effects of Amlodipine on Sperm Parameters

Treatment Group	Sperm count (10^6 / ml)	Sperm motility (%)	GSI
I-Control	55.7 ± 1.58	82.3 ± 1.89	0.612 ± 0.0343
II-Aml 14 days	54.0 ± 1.48	80.2 ± 1.47	0.595 ± 0.0260
II-Aml 28 days	51.0 ± 0.966*	69.5 ± 1.75*	0.549 ± 0.0138
II-Aml 42 days	45.5 ± 1.09*	52.3 ± 1.89*	0.416 ± 0.0201*
II-Aml 91 days	40.7 ± 1.54*	43.5 ± 1.82*	0.512 ± 0.0229*
II-Aml 126 days	30.5 ± 0..957*	28.3 ± 2.29*	0.494 ± 0.0337*
F value and P value	$F_{(5,30)}$ = 53.5 P = < 0.0001	$F_{(5,30)}$ = 133, P = < 0.0001	$F_{(5,30)}$ = 7.58, P = 0.0001

Aml = Amlodipine 0.9 mg / kg body weight orally; All values were expressed as mean ± SEM. The data obtained from various groups were statistically analyzed using one-way analysis of variance followed by post hoc Dunnet test in Graph-pad prism 6. *indicates $p < 0.05$

RESULTS

Sperm Count

Table 1 shows the sperm count of the control and Amlodipine treated rats after different days of treatment. The sperm count of the control rats and the rats treated with Amlodipine in a dose of 0.9 mg / kg for 14,28,42,91 and 126 days were 55.7 ± 1.58 x 10^6 / ml, 54.0 ± 1.48 x 10^6 / ml, 51.0 ± 0.966 x 10^6 / ml, 45.5 ± 1.09 x 10^6 / ml, 40.7 ± 1.54 x 10^6 / ml, and 30.5 ± 0.957 x 10^6 / ml respectively. From 28 days onwards continuous administration Amlodipine shows a highly significant decrease in sperm count ($p < 0.05$). The above results were found to be substantiating the earlier reports made by Almeida SA and Teofilo JM. They reported a 23 % reduction in sperm count when Amlodipine is used in a dose of 0. 4 mg / rat per day for 30 days[15].

Sperm Motility

Table 1 shows the sperm motility of the control and Amlodipine treated rats after different days of treatment. The percentage sperm motility of the control rats and the rats treated with Amlodipine in a dose of 0.9 mg / kg for 14,28,42,91 and 126 days were 82.3 ± 1.89, 80.2 ± 1.47, 69.5 ± 1.75, 52.3 ± 1.89, 43.5 ± 1.82 and 28.3 ± 2.29 respectively. From 28 days onwards continuous administration Amlodipine shows a highly significant decrease in sperm motility ($p < 0.05$). The above results were found to be substantiating the reports made by Morakinyo AO and Iranloye BO. They reported a significant reduction in sperm motility when the Calcium channel blocker is Nifedipine belonging to the same group as Amlodipine is used in a dose of 0. 571 mg / kg per day for 30 days.[16]

Gonado-somatic Index (GSI)

Table 1 shows the Gonado-Somatic Index of the control and Amlodipine treated rats after different days of treatment. The Gonado-Somatic Index of the control rats and the rats treated with Amlodipine in a dose of 0.9 mg / kg for 14,28,42,91 and 126 days were 0.612 ± 0.0343, 0.595 ± 0.0260, 0.549 ± 0.0138, 0.416 ± 0.0201, 0.512 ± 0.0229 and 0.494 ± 0.0337 respectively. Unlike the other two parameters gonado-somatic index was found to be significantly decreased from 42 days of continuous administration ($p < 0.05$) only and the decrease was not fully duration dependent.

DISCUSSION

Spermatogenesis is a complex process during which primitive multi potent stem cells divide to produce daughter cells that further divide to become spermatozoa. As these processes occur within the seminiferous tubles of testis and as more than 80 % of testis volume is made up of seminiferous tubles and germ cells of various developmental stages, it can be assumed that the testicular atrophy and hypertrophy are strongly related to semen parameters. Semen analysis is an integral part of the evaluation of infertility. In this study it

was found out that a clinically equivalent dose of Amlodipine in rats is capable of causing a significant decrease in sperm count from 28 days of continuous administration. Further lengthening of therapy is found to be worsening this defect. Similarly motility also seemed to be subnormal from 28 days of administration. Motility less than 60 % is considered subnormal and that less than 40 % suggests infertility[17]. In the case of GSI, though it was significantly decreased from 42 days, further lengthening of treatment was found to be slightly increasing its value, though much less than normal. Though testis weight is a sensitive early marker of gonadal injury, this variation may arise from several factors including reactions to injury that may mask a decrease in testicular weight like edema and inflammation, cellular infiltration, Leydig cell hyperplasia etc.

CONCLUSION

The use of Amlodipine has found to be adversely affecting the reproductive parameters of male albino rats in a duration dependent manner even when given in clinically equivalent doses. So the possibility for the same in men is to be considered while prescribing it for a long duration.

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