Asian J. Pharm. Res. Vol 4, Issue 1, 7-11, 2014.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

CALCIUM CHANNEL BLOCKERS: EFFECT ON THE SERUM TESTOSTERONE LEVELS

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ABSTRACT

Calcium channel blockers (CCBs) are the most commonly prescribed drugs for the treatment of cardiovascular diseases; they produce their effect by blocking the transmembrane calcium influx through calcium channels. Calcium is vital in many biological processes including hormonal secretion, mitosis, reproduction, fertility, and regulation of gene expression. Thus, when CCBs are used for cardiovascular conditions they might affect calcium ion concentration at these sites including the endocrine glands and the hormones secreted by them. Therefore this study was undertaken to elucidate the effects of CCBs on serum testosterone levels in male albino rabbits. Serum Testosterone levels were taken at the commencement of the study as control values This study was performed on thirty male albino rabbits. The rabbits were divided into three groups of ten each. Each group received one of the calcium channel blocker- Verapamil, Diltiazem or Nifedipine for three months continuously. At the end of each month the serum testosterone levels were evaluated by chemiluminiscence and these levels were compared with control values. On daily administration of CCBs for three months serum testosterone levels were lowered in comparison to the control values. Calcium channel blockers are prescribed very commonly and this study proposes the importance of monitoring serum testosterone hormone levels in patients receiving these drugs for long-term since the fall in serum testosterone levels brought about by this group of drugs may have serious clinical implications.

Key words: Calcium channels, Calcium channel blockers, Testes, Testosterone.

INTRODUCTION

Calcium ions are important for many biological processes such as excitation-contraction coupling, excitation-secretion coupling, fertilization and regulation of gene expression, but so far the effect of CCBs is confined to cardiovascular conditions. Thus the effect of change in calcium ion concentration by drugs like CCBs in organs other than cardiovascular system goes unnoticed.

It is an established fact that calcium plays an important role in the synthesis, release and functioning of several hormones. So the administration of calcium channel blockers may affect these hormones at some level.

Extensive study of membrane properties of endocrine cells were performed and it is revealed that some cells are electrically coupled and that the membrane properties of several types of endocrine cells, especially those of aminergic or peptidergic cells, have features common to those of neuronal cells. Some kind of endocrine cells elicit action potentials in response to electrical and/or chemical stimuli and release of bioactive substances such as hormones or transmitters from the cells strongly depends on the intracellular concentration of calcium ions [1].

Some endocrine cells are reported to possess voltage dependent calcium channels in the membrane, activation of which causes depolarization and generation of action potentials resulting in release of transmitters and hormones. [2].

Although the process of hormone production and

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Asian J. Pharm. Res. Vol 4, Issue 1, 7-11, 2014.

the mode of secretion in endocrine cells which secrete steroid hormones may be different from those aminergic or peptidergic cells [3], the participation of calcium ions in regulation of the production of steroid hormones in the cells may also occur via voltage gated calcium channels [4].

Despite exhibiting substantial cardiovascular selectivity, reports exist that suggest that calcium-channel blockers may have anti-reproductive effects in males on long-term treatment. Moreover, there are reports of infertility in males who are using CCBs [5, 6]. The underlying mechanism of CCBs in causing infertility is not well established. Although there are evidences that CCBs possess antiproliferative activity, which may interfere with fertility [7-9].

Besides this, the Ledyig's cells are known to have at least two kinds of voltage dependent calcium channels in the membrane. These calcium channels may be activated by physiological changes in membrane potential leading to influx of calcium ions. The calcium dependent K ion channels hardly seem to be activated unless the internal calcium ion concentration increases remarkably. It is presumed that intercellular coupling may play role in synchronizing or intensifying the endocrine activities of Ledyig cells located within a cluster [10].

Recent studies reveal that the extracellular calcium affects the magnitude of stimulation of testosterone production in response to LH in mouse Ledyig cells and the Verapamil (which affects the slow calcium channels) significantly decrease testosterone production in response to LH from 45% to 73% of control [11]. Based on these findings, it was logical to investigate whether the administration of CCBs interfere with the gonadal steroid–testosterone levels. Thus this study was designed and carried out to elucidate the serum testosterone levels after administration of calcium channel blockers and to observe the resultant side effect on the testes.

MATERIAL AND METHODS

This study was conducted in the Department of Pharmacology, Dr. Sampurnanand Medical College, Jodhpur after acquiring the approval from Animal Ethic Committee (Registration number 692/02/a/CPCSEA).

Thirty adult male albino rabbits weighing about 1.5 to 2.5 kgs were used. Throughout the study the animals were maintained under standard laboratory conditions at room temperature of 25ºC to 30ºC with food and water ad libitum. They were divided into three groups with ten rabbits in each group. Group I received Verapamil orally, in a dose of 9.6 mg/kg/day, Group II received Diltiazem orally, in a dose of 4.8mg/kg/day and Group III received Nifedipine orally, in a dose of 1.2 mg/kg/day.

Blood samples for hormone assay were obtained from ear veins of the rabbits of all the three groups and the serum was then separated for the estimation of levels of testosterone prior to drug administration. These levels served as control values. Thereafter, drug administration was started and continued for three months and at the end of each month the serum levels of testosterone were again estimated and compared with the previous values. The serum hormonal levels of rabbits of all the three groups were evaluated by chemiluminisence using ADVIA Centaur system, which is an automated immunoassay analyzer. The arithmetic mean and standard deviation of all the observations were calculated. The difference in the mean among the control and the treated group was calculated by 't' test. The difference is considered significant if p-value was found less than 0.05.

Histopathological examination of the testes releasing these hormones were performed at the end of study (Figure 2, 3,4 and 5).

RESULTS

The serum levels of hormones were estimated after the end of each month and compared with control levels. It was observed that all the three CCBs i.e. Verapamil, Diltiazem and Nifedipine cause gradual fall in the serum testosterone levels (Table 1).

HISTOPATHOLOGICAL RESULTS

At the end of three months histopathological examinations of tests were done. These studies revealed that the administration of calcium channel blockers for three months continuously brought marked changes in the histology of the testes. The testes of rabbits on Verapamil showed early maturation arrest of spermatozoas, testes of rabbits on Diltiazem showed focal maturation arrest of spermatozoa while the testes of rabbits on Nifedipine exhibited maturation arrest with edema.

Table 1. Effect of different CCB_S on serum testosterone level. (Mean+SEM) in ng/dl

S. No.	Drugs	Control	After one month	After two months	After three months
1	Verapamil	320.215±66.089	306.522±64.128	276.654±63.015	248.249±59.220
		P <0.01	P <0.01	P <0.01	P <0.01
2	Diltiazem	342.850±61.446	326.414±63.126	308.677±58.021	227.191±58.000
		P <0.01	P <0.01	P <0.01	P <0.01
3	Nifedipine	340.096±56.575	284.493±60.425	257.731±50.724	224.290±51.651
		P <0.01	P <0.01	P <0.01	P <0.01

p= Level of significance *p< 0.01 statistically significant as compared to control group

Asian J. Pharm. Res. Vol 4, Issue 1, 7-11, 2014.

Fig 1. Effect of different CCB_S on serum testosterone level

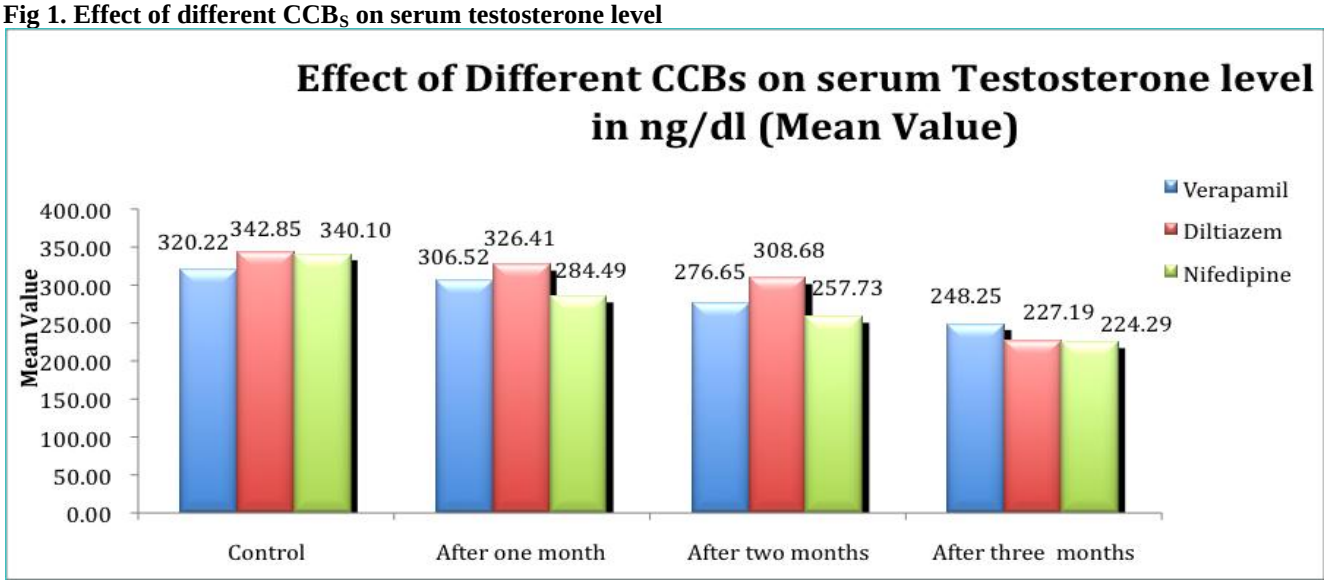


Fig 2. Normal Testes of Rabbit: Composed of seminiferous tubules showing various stages of speramatogenesis

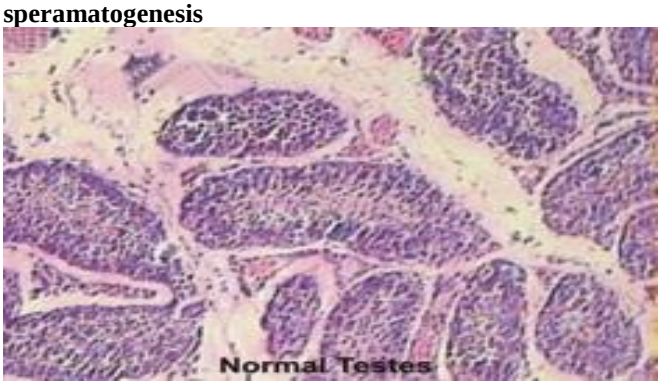


Fig 3. Effect of three months of continuous therapy with Verapamil: Early maturation arrest of spermatozoas

Fig 4. Effect of Three Months of Countinuous Therapy With Diltiazem: Focal maturation arrest of spermatozoas

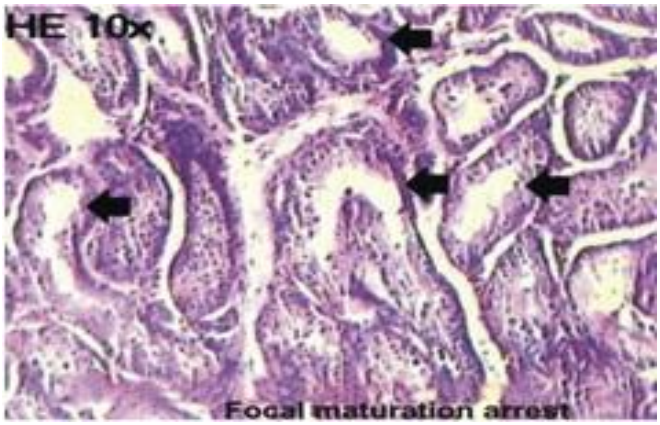
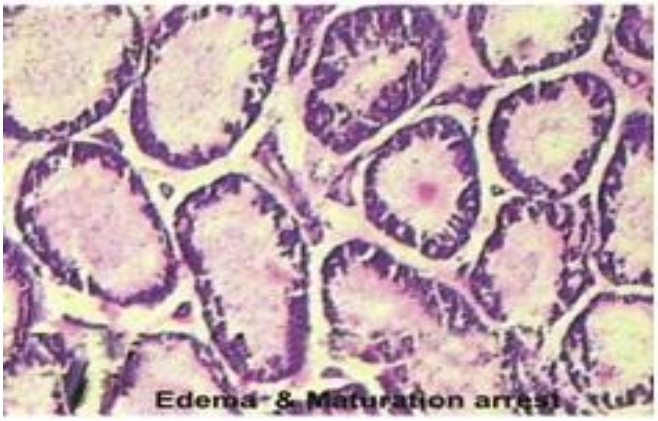


Fig 5. Effect of three Months of Continuous Therapy with Nifedipine: Early maturation arrest of spermatozoas and edema



Asian J. Pharm. Res. Vol 4, Issue 1, 7-11, 2014.

DISCUSSION

As the name itself suggests, that the calcium channel blockers produce their effects mainly by blocking the calcium channels, thus altering the calcium concentrations across the cells. Therefore to understand the cause of change in the hormonal levels after administration of calcium channel blockers, it is important to establish the presence and role of calcium channels in the synthesis, release, regulation and action of hormones.

It is well known now that calcium channels are present in the secretory tissues and the calcium plays an important role in the endocrine glands [12]. It is estabished that the Ca^{2+} influx and outflux should be tightly regulated to maintain the intracellular Ca^{2+} homeostasis, and any alteration in the Ca^{2+} transport across the cell membrane could result in a drastic impact on spermatogenesis and steroidogenesis [13]. Thus on administration of calcium channel blockers this activity of calcium in promoting steroidogenesis and/or spermatogenesis is hampered and as a result the formation of testosterone and/or spermatazoa is reduced.

Present study also favors the perception that calcium ion and calcium channels play important role in the process of steroidogenesis, that is why with the administration of calcium channel blockers the process of steroidogenesis is slowed resulting in lowering of serum steroid levels.

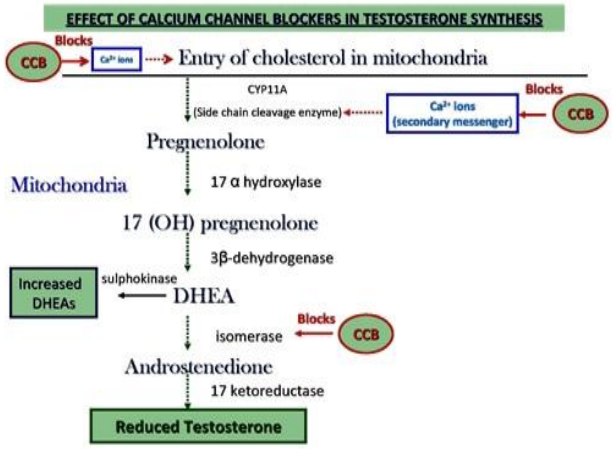
The present study shows that on continuous administration of CCBs like Verapamil, Diltiazem and Nifedipine, the serum testosterone levels exhibit a gradual fall. Although to delineate the exact mechanism behind this is a matter of further elucidation but in light of the available literature and the findings of the present study, the following mechanisms are proposed to justify the fall in serum testosterone levels with CCB administration.

1. CCBs affect the synthesis of testosterone i.e. the process of steroidogenesis by altering availability of calcium ions in the concerned tissues [14].
2. CCBs alter the histopathology of testes thus hampering spermatogenesis and steriodogenesis.

The precursor for the steroid synthesis is cholesterol and the rate-limiting step in the process of steroid synthesis is the entry of cholesterol in mitochondria and its conversion into pregnenolone by the enzyme cholesterol side chain cleavage enzyme (CYP11A). Here calcium acts as a second messenger and stimulates steroidogenesis by:

1. Affecting cholesterol transport to mitochondria [15].
2. Affecting the activity of cholesterol side chain cleavage enzyme (CYP11A) [16]

There are reports that when calcium channel blockers are administered, the serum DHEA and DHEA-s levels are increased. DHEA is an intermediate in the synthesis of testosterone. It is either converted to DHEA-s by sulphokinase or to androstenedione in the presence of enzyme 3 β –DH Δ [4,5] isomerase and this androstenedione is then converted to testosterone by the enzyme 17 ketoreductase [14].



On administration of calcium channel blockers, the conversion of DHEA to DHEA-s is increased and that to testosterone is reduced. This indicates that the calcium channel blockers reduce serum testosterone levels probably by inhibiting enzyme isomerase.

The changes in serum testosterone levels on administration of CCBs can also be attributed to the change in histology brought about by these drugs. These changes at cellular levels may lead to altered hormonal production by these glands. Whether these histopathological changes seen are due to direct chemical assault of calcium channel blockers on gland tissues or it is due to blockade of calcium channels in glands, remains a subject of further elucidation.

CONCLUSION

It is an established fact that calcium channels are widely distributed in our body system including the endocrine glands. Thus when CCBs are used for long term therapy for some other clinical conditions, there is a possibility that they affect the functioning of endocrine glands in synthesis, secretion, release or regulation of hormones. In the present study the administration of CCBs- Verapamil, Diltiazem and Nifedipine for three months continuously in male albino rabbits exhibited a gradual fall in serum testosterone levels. There are several clinical reports of occurrence of reversible infertility with CCBs. Thus, based on the findings of the present study along with the clinical observations with use of CCBs, the importance of serum testosterone level follow up in patients on long term CCB therapy is proposed.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the guidance and support provided throughout this entire research work by Dr. Adesh K Mathur, former Professor and Head, Department of Pharmacology, Dr. S.N. Medical College, Jodhpur India.

Asian J. Pharm. Res. Vol 4, Issue 1, 7-11, 2014.

REFERENCES

1. Ozawa S and Sand O. Electrophysiology of excitable endrocrine cells. *Physiological reviews*, 66, 1986 887-952.
2. Rorsman P and Traube G.Calcium and delayed potassium currents in mouse pancreatic β- cells under voltage clamp conditions. *J of Physiology*, 374, 1986, 531-550.
3. Christensen AK. Leydig cells: In handbook of physiology, section 7. *Endocrinology*, 5, 1975, 57-94.
4. Aguilera G and Catt KJ .Participation of voltage-dependent calcium channels in the regulation of adrenal glomerulosa function by angiotension II and potassium. *Endocrinology*, 118, 1986,112-118.
5. Benoff S. Voltage-dependent calcium channels in mammalian spermatozoa. Fro*nt Biosci*, 3, 1998,1220–40.
6. Nowak R. Antihypertension drug may double as male contraceptive. *J NIH Res*, 6, 1994, 27–8.
7. Stepien O, Iouzalen L, Herembert T, Zhu DL, Marche P. Amlodipine and vascular hypertrophy. *Int J Cardiol*, 62, 1997, S79–84.
8. Tang J, Liu Z, Ren D, Wu C. Effect of amlodipine on the growth of vascular smooth muscle cells of spontaneously hypertensive rats. *J Tongji Med Univ*, 17(3), 1997, 165–7.
9. Latif R, Lodhi GM, Aslam M. Effects of amlodipine on serum testosterone, testicular weight and gonado-somatic index in adult rats. *J Ayub Med Coll Abbottabad*, 20(4), 2008.
10. Kawa K. Existance of calcium channels and intracellular couplings in the testosterone –secreting cells if the mouse. *J.Physiol*, 393, 1987, 647-666.
11. Wayne AM ,Xan-Hua, Liu and John DS. *J of andro*, 12(2), 1991,148-158.
12. Ueshiba H, Miyachi Y. Effects of the long-acting calcium channel blockers, amlodipine, manidipine and cilnidipine on steroid hormones and insulin resistance in hypertensive obese patients. *Intern Med*, 43(7), 2004, 561-5.
13. Yamaguchi M. Role of regucalcin in maintaining cell homeostasis and function. *Int J Mol Med*, 15, 2005, 371–89.
14. Keiichi I, Tsuyoshi I, Kouki F, Yoshinobu M, and Katsuyoshi T. Suppression of Aldosterone Synthesis and Secretion by Ca2+ Channel Antagonists. *International Journal of Endocrinology*, 2012.
15. Hall PF, Osawa S, Mrotek J. The influence of calmodulin on steroid synthesis in Leydig cells from rat testes. *Endocrinology*, 109, 1981, 1677-1682.
16. Suulvian MHJ, Cooke BA. The role of calcium ions in steroidogenesis in Leydig cells: stimulation of intracellular calcium ions by lutropin (LH), lubiberin (LHRH) agonist and cyclic AMP. *Biochem J*, 236, 1986, 45-51.