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Downregulated StAR gene and male reproductive dysfunction caused by nifedipine and ethosuximide

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Abstract Steroid hormones that are controlled by steroidogenic acute regulatory (StAR) gene regulate sperm production. However, calcium ion is important for male fertility in vasodilation and sperm development. Calcium also serves as a second messenger to control acrosome reaction and sperm motility. Calcium channel-blockers (CCBs) as nifedipine and ethosuximide (used in hypertension and epilepsy treatment) can affect male fertility. However, little is known about the underlying mechanism of the male reproductive dysfunction and their side effects. The present study was designed to address the involvement of CCBs in inducing male infertility. Thirty-six male mice were orally treated by therapeutic dose of nifedipine and ethosuximide for 20 days followed by 10 days without treatment for drug withdrawal. Chromosome aberrations assay, sperm analysis and testicular expression level of biomarker steroidogenic acute regulatory (StAR (mRNA were measured. In addition, histological structure of the testis was investigated to the process of spermatogenesis. Our results revealed that, CCBs significantly increased the percentage of chromosome aberration and sperm shape change. StAR-mRNA expression was significantly down regulated. Sperm count and motility were significantly decreased. However, slight improvement was observed in all tested parameters after drug withdrawal. Seminiferous tubules displayed total atrophy, disruption, severe damage and elongation of tubules with disorganization of germinal epithelium that detached from the basement membrane. The lumen of seminiferous tubules showed complete absence of sperm cells.

Conclusions: Both nifedipine and ethosuximide significantly increase chromosome abnormalities, decrease sperm function, and down regulate StAR-mRNA expression. All these side effects may lead to irreversible male sterility.

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Contents

Introduction

Steroidogenic acute regulatory (StAR) protein is the protein responsible for regulation of steroid hormone biosynthesis. Steroid hormones are synthesized in steroidogenic cells of testis. StAR protein was reported to be an important factor in testosterone biosynthesis through transporting cholesterol from outer membrane to inner side of mitochondria (Miller, 2007). StAR protein expression is regulated by LH-mediated activation of cAMP-dependent pathways leading to transcriptional activation in leydig cells (Manna et al., 2016).

Calcium ion is responsible for several cellular processes and sperm function, including motility, capacitation and acrosome reaction; according to number of Ca^{2+} permeable channels and transports (Berridge, 1993; Westenbrok and Babcock, 1999; Draszon et al., 2001). Trevino et al. (2004) suggested that, calcium ion mediates sperm function and fertilization process. Two types of Ca^{2+} channels were reported; ligand-dependent Ca^{2+} channels (LDCC) and voltage-dependent Ca^{2+} channels (VDCC). VDCCs have been identified in testicular germ, and classified as L-type Ca^{2+} channels (Goodwin et al., 1999) and T-type Ca^{2+} channel (Son et al., 2002). The presence of voltage-gated Ca^{2+} channel in mammalian sperm was documented by Wennmuth et al. (2000) and Park et al., 2003.

Calcium channel blockers (CCBs) are a Class of drugs that inhibits calcium – evoked contractions in depolarized smooth muscle and used in management of hypertension, angina, migraine headaches, arrhythmias and in treatment of neurological diseases (Wassertheil et al., 2004; Karceski et al., 2005). CCBs reduce the active tone of the vascular muscles and produce vasodilation by blocking the entry of calcium. The activities of these drugs are due to their interaction at calcium mobilization process – calcium entry through a voltage-gated calcium channel of the L-type (Godfraind, 2005). CCBs are divided into 2 categories according to their target: bipyridine derivatives, that mainly expand vessels and non-dihydropyridine derivatives that, slow down cardiac activity with reduction of its muscle contraction (Frazee et al., 2014; Jez et al., 2015).

About 40–90% of male infertility is due to deficient sperm production (Purvis and Christiansen, 1992). Abnormal sperm morphology and insufficient sperm motility were reported to induce infertility (Feng, 2003). Calcium ion homeostasis, which is implicated in diverse cellular functions in both germ cells and testicular tissues, was reported to be responsible for male infertility (Berridge et al., 2003). Many regulatory functions in spermatozoa including acrosome reaction, capacitation, motility and hyperactivity were regulated by cytoplasmic calcium (Breitbart, 2002; Carson et al., 2003; Suarez and Ho, 2003; Nikpoor et al., 2004).

Amlodipine was found to decrease sperm count in rats (Almeida et al., 2000). Chronic nifedipine and amlodipine treatment resulted in azoospermia and non-motile sperms in human semen (Meacham, 2006). Furthermore CCBs not only inhibit spermatogenesis but also impair sperm motility (Latif et al., 2008). Other studies mentioned that CCBs induce reversible male infertility (Lee et al., 2011; Morakinyo et al., 2011; Chaloob et al., 2013). Growing concern for infertility – causing effects of Ca^{2+} channel blockers, particularly in children has prompted us to evaluate their effects on male sterility and induction of genotoxicity. For this purpose, we have set up an *in vivo* experiment to assess mutagenicity and sterility- causing effects of chronic administrations of both nifedipine (L-type Ca^{2+} channel blocker) and ethosuximide (T-type Ca^{2+} channel blocker).

Materials and methods

Animal and experimental design

Thirty-six adult male Swiss albino mice 6–8 weeks old and weighing 18–20 g were purchased from the Egyptian Organization for Vaccine and Biology preparations (Helwan, Cairo, Egypt). Mice were kept at 25°°C on a 12 h light/12 h dark cycle, fed on a rodent chow and drank tap water *ad libitum*.