Abstract

There is a close association between ischemic cardiovascular disease and hypercholesterolemia. The present study was aimed to evaluate the effect of induced hypercholesterolemia on the testis and epididymis of adult albino rats and to elucidate the possible protective role of atorvastatin and selenium.Fifty adult albino Wistar rats were divided into five groups; control group, sham control, induced hypercholesterolemia group, induced hypercholesterolemia treated with atorvastatin group and induced hypercholesterolemia treated with selenium. The effects were monitored histologicaly, ultrastructurally, Biochemically and morphometrically. Induced hypercholesterolemia produced marked histological and ultrastructural changes in the testis. Morphometrically, there was a significant decrease ($p<0.0001$) in the diameter of the seminiferous tubules, significant increase ($p<0.0001$) in the collagen fibers in the testes of rats and significant reduction ($p<0.0001$) in the polysaccharides content of induced hypercholesterolemia group rats as compared to the control groups. Atorvastatin and selenium produced marked improvement in histological and ultrastructural results as compared with group induced hypercholesterolemia. However, some ultrastructural changes were evident in the selenium treated group. Morphometrically, Atorvastatin and selenium produced significant increase of the diameter of the seminiferous tubules, significant increase in the area percent of collagen fibers, significant increase in the polysaccharides content as compared to induced hypercholesterolemia group. Hypercholesterolemia also produced many histological and ultrastructural changes epididymis. Morphometrically, the area percent of collagen fibers in induced hypercholesterolemia group was significantly increased ($p<0.0001$) as compared to control groups. Atorvastatin and selenium improved the histological and ultrastructural changes in the epididymis as compared to induced hypercholesterolemia group. Morphometrically, atorvastatin and selenium significantly decreased ($p<0.0001$) the area percent of collagen fibers as compared to group III. However, the present study didn't reveal any significant change in the optical density of PAS reaction of the epididymis among the five groups. Feeding rats with high cholesterol diet induced significant elevation ($p<0.0001$) of the serum level of cholesterol, TG and LDL as well as significant decrease in the serum level of HDL compared with control rats. Atorvastatin and selenium produced significant ($p<0.0001$) improvement in the in the serum level of LDL, HDL and triglycerides. **Conclusion**; it could be conclude that induced hypercholesterolemia produced injurious effects to both testis and epididymis and both atorvastatin and selenium could ameliorate these deleterious effects.

Keywords: Hypercholesterolemia- testis- epididymis- atorvastatin-selenium

Effect of Induced Hypercholesterolemia on the Testis and Epididymis of Adult Albino Rat and the Possible Protective Role of Atorvastatin and Selenium: Histological and Ultrastructural Study

Thesis

Submitted for Partial Fulfillment of the requirements of M.D in Anatomy and Embryology

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List of Abbreviations

ANOVA	analysis of variance
AR	Androgen receptors
CCl4	carbon tetrachloride
CREB	cAMP Response Element-Binding
Hx & E	haematoxylin and eosin
FSHR	Follicle stimulating hormones receptor
GPx	Glutathione peroxidase
HDL	High density lipoprotein
HF	Heart failure
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
LDL	Low density lipoprotein
NO	Nitric oxide
PPARγ	Peroxisome proliferator-activated receptor
SOD	Superoxide dismutase
Se	Selenium
SeP	selenoprotein P
TG	Triglycerides
TC	Total cholesterol
TR	thioredoxin reductase
VLDL	Very low density lipoprotein

Introduction and Aim of Work

Introduction

Cholesterol is a central molecule in animal physiology. It is important in the maintenance of cell structure and steroid hormone synthesis **(Saez et al., 2011).** Moreover, cholesterol is involved in the mechanism of sperm capacitation, acrosomal reaction and protection of spermatozoa against environmental affection **(Yamamoto et al., 1999; Cross, 2003).** However, when cholesterol homeostasis is deregulated, cholesterol may become a deleterious molecule associated with several pathological abnormalities (**Abou-haila & Tulsiani, 2009).**

Hypercholesterolemia is very common nowadays and it represents a high risk factor for coronary heart disease and atherosclerosis **(Shalaby et al., 2004)**. In hyperlipidimia, lipid peroxides are an important source of reactive oxygen which has been found to be extremely cytotoxic to male gonads **(Selley et al., 1991). Ramirez et al. (2000)** reported that in a group of one hundred infertile men, the incidence of hypercholesterolemia was as high as 65%.

In rats fed with high cholesterol diet, it was found that there is a marked decrease in fertility index, testicular weight, sperm cell count, and percentages of sperm motility and viability associated with a significant increase in sperm cell abnormalities **(Shalaby et al., 2004)**.

Studies on sperm maturation, epididymal histology, or epididymal tubule physiology are significant parts in reproductive researches **(Liu et al., 2009).** In spite of the valuable role of epididymis in sperm maturation, studying the effect of hypercholesteromia was concentrated mainly on Leydig and Sertoli cell functions

with no available literatures describing its effect on the epididymis **(Abdelmalik,. 2012)**.

Statins are group of hydroxy methyl glutarile coenzyme -A reductase used in the treatment of hypercholesterolemia. Statins therapy in hypercholestrolemic rats led to controversial effects on the testis and fertility measures. **Shalaby et al. (2004)** found that administration of simvastatin to hypercholesterolemic male rats improved their reproductive efficiency and produced additional protection against reduced fertility induced by hypercholesterolemia. Unfortunately, data demonstrated by **Corona et al. (2010)** showed that statin therapy might induce primary hypogonadism.

The protective role of some antioxidants against testicular injury induced by high cholesterol diet has been declared by some authors. **Ibrahim et al. (2012)** found that selenium-enriched probiotics or inorganic selenium supplementation has ameliorated at various degrees the significant adverse effects on male fertility caused by high fatty diet.

Aim of Work

The aim of the present work is to:

- Study the histological and ultrastructural changes induced by high cholesterol diet on the testis and epididymes of adult albino rat.
- Evaluate the possible protective role of atorvastatin and selenium against the testicular and epididymal changes induced by high cholesterol diet in adult albino rat.

Review of Literature

Anatomy of the testis

Gross morphology

The testis is a paired, ovoid male reproductive organ that sits in the scrotum, separated from its mate by a scrotal septum. Described by some authors as being shaped and sized like a large olive or small plum, the average volume of the adult testis is approximately 25 ml. Typically, it measures 3.5-5 cm in length by 2.5-3 cm in both width by 3cm in depth (anteroposterior diameter) **(Snell, 2000).** Smooth to palpation, the testis sits obliquely with its long axis mostly vertical with a slight anterior and lateral slant to the superior pole. Superiorly, it is suspended by the spermatic cord, with the left testis often sitting lower than the right testis. Inferiorly, the testis is anchored to the scrotum by the scrotal ligament, a remnant of the gubernaculum **(Swartz, 2006).**

The tunica vaginalis testis (a remnant of the processus vaginalis) envelopes the testis in a double layer, except at the superior and posterior borders where the spermatic cord and epididymis adhere to the testes. The visceral layer of the tunica vaginalis testis is closely applied to the testis, epididymis, and ductus deferens. On the posterolateral surface of the testis, this layer invests a slit-like recess between the body of the epididymis and the testis that is called the sinus of epididymis. The parietal layer of tunica vaginalis is adjacent to the internal spermatic fascia, is more extensive, and extends superiorly into the distal part of the spermatic cord **(Moore and Daley, 2006).**

Histology

The rat testis is composed of two compartments; seminiferous tubules compartment which is outlined by a basal membrane that separates them from the interstitial compartment. In adult, these two compartments respectively possess exocrine and the endocrine functions. The seminiferous tubules epithelium contains germ cells and Sertoli cells. It is supported by a basal lamina and a wall formed of collagen fibers, fibroblasts and myoid cells which possess a contractile activity involved in the propulsion of spermatozoa **(Maqdasy et al., 2012).**

In contact with the basal lamina, undifferentiated spermatogonia divide intermediate type and then type B spermatogonia. This early stage of differentiation corresponds to the proliferative phase of spermatogenesis **(Dadoune, 1994)**. Primary spermatocytes have a diploid chromosome number but duplicated sister chromatids (DNA content is thus 4N where N is the DNA content of haploid spermatozoa). Primary spermatocytes give rise to secondry spermatocytes with a haploid chromosome compelement (but 2N DNA content), the reduction division is designated as meiosis I. Secondry spermatocytes rapidly undergo the second meiotic division, where sister chroatids separate (DNA content is now N), to form haploid spermatids. Spermatids gradually mature into spermatozoa by a series of nuclear and cytoplasmic changes known as spermiogenesis **(Standring et al., 2005).** The entire genome is condensed due to the replacement of histones by protamines **(Meistrich et al., 2003)**, while much of the cytoplasm is eliminated after phagocytosis by the Sertoli cells **(Yefimova et al., 2008).** In addition, the early stages of acrosome formation and the establishment of the flagellum lead to spermatozoa **(Kierszenbaum et al., 2007)**.

Ultrastructure of Germ cells

The spermatogonia are large diploid cells which lie against the boundary tissue of the seminiferous tubules and divide mitotically. They are two types of spermatogonia are type A and type B. The type A spermatogonia are characterized by large pale ovoid nuclei containing finely granular nucleoplasm. The nuclei are usually lying with their long axis parallel to the boundary tissue and near the tubular limiting membrane, with homogenous chromatin. The cytoplasm is scanty, granular, with poor rough endoplasmic reticulum but abundant ribonucleoprotein particles. The mitochondria are spherical or ovoid and the Golgi apparatus is simple. The type B cells are slightly smaller which contain rounded nuclei with more electron dense nucleoplasmic matrix than type A and numerous chromatin clumps **(Khattab, 2007).**

The spermatocytes are two types, primary and secondary spermatocytes; The primary spermatocytes are characterized by the presence of intercellular bridges between these cells, spherical nuclei with finely granular nucleoplasm and chromatin accumulation. The cytoplasm is scanty with little endoplasmic reticulum but small clumps of ribosome are distributed throughout the cytoplasm. The mitochondria tend to aggregate in groups. The Golgi apparatus is found above the nucleus and is formed of fine vesicles and few cluster accumulated at one pole of the cell. The secondary spermatocytes are rarely seen among the germinal cells of rat, their life span is short and enter into the second meiotic division producing the spermatids. The secondry spermaotcytes are smaller in size than the late primary spermatocytes. Their nuclei are spherical with centrally located clumps of chromatin substance **(khattab, 2007).**

The early spermatids are rounded cells with large spherical nuclei which contain chromatin clumps in a lightly stained cytoplasm and the endoplasmic reticulum is membrane bounded canaliculi and flattened vesicles; mitochondria at this stage tend to aggregate at the periphery of the plasma membrane (**Ross and Pawlina, 2006).**

As spermatozoa are released from the wall of the seminiferous tubule into the lumen, they are non-motile but structurally mature. Their expanded heads contain little cytoplasm and are connected by a short constricted neck to the tails. The tail is a complex flagellum which exceeds the head in volume, and is divided into middle, principal and end pieces. The head contains the elongated nucleus with deeply stained chromatin and acrosomal cap anteriorly. The latter contains acid phosphates and hyaluronidase, neuroaminidase and proteases necessary for fertilization. The neck contains a well formed centriole. The middle piece consists of axial bundle of microtubules, the axoneme, outside which is a cylinder of nine dense outer fibers, surrounded by a helical mitochondrial sheath. The principal piece of the tail is the motile part and is formed of the axoneme and surrounding dense fibers **(Standring et al., 2005).**

The number of Sertoli cells in the adult testis determines both testicular size and daily sperm production **(Sharpe et al., 2003).** Sertoli cells are the only source of nutrients and growth factors for germinal cells. They are responsible for phagocytosis of apoptotic germinal cells and have a paracrine regulatory action on Leydig cells **(Johnson et al., 2008).**

Sertoli cells play a supportive role on germ cell and ensure the maintenance of spermatogenesis. They constitute the hemato-testicular barrier that isolates the germ cells from blood components, especially immune mediators **(Fijak et al.,**