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Original Research Article

Effect of atorvastatin on spermatogenesis in rats: A stereological study

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Abstract

Purpose: *To investigate the effects of oral atorvastatin on spermatogenesis in a rat model.*
Methods: *Rats were equally assigned into control and study groups, the latter receiving atorvastatin (20 mg/kg/day). At the end of 12 weeks, spermatogenetic activity was evaluated using stereological and optical fractionator methods. Serum follicle-stimulating hormone (FSH), total testosterone (TT), and luteinizing hormone (LH) levels were measured using micro–ELISA kits. Total cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL - C), and high-density lipoprotein cholesterol levels were also measured by enzymatic colorimetric assays.*
Results: *Testicular stereological analysis revealed that atorvastatin reduced Sertoli cell numbers ($p < 0.001$), spermatogonia ($p < 0.001$), spermatocytes ($p < 0.001$), and seminiferous tubule diameters ($p < 0.001$). LDL – C ($p = 0.01$) and TG ($p = 0.01$) values were significantly lower in the study group compared with the control group. There was no significant difference in FSH ($p = 0.44$), LH ($p = 0.48$), and TT ($p = 0.06$) levels between the groups.*
Conclusion: *The findings show that atorvastatin causes deleterious effects on rat spermatogenesis. It should therefore be used with caution in clinical practice owing to its potential adverse effects, especially on male fertility.*

Keywords: *Statin, Atorvastatin, Spermatogenesis, Stereology, Testis*

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INTRODUCTION

The after-effects of drug use, varying from allergic reactions to major organ damage, have increased significantly all over the world [1]. Statins are 3-hydroxy-3-methyl-glutaryl coenzyme-A (HMG Co-A) reductase inhibitors commonly used to reduce the risk of cardiovascular disease. Statin use is growing in association with increases in cardiovascular diseases [2]. Despite being well-tolerated, statins cause side-effects such as muscular pain, hepatic enzyme elevation, and an increased risk of diabetes. Another side-effect of statins

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involves the male reproductive system, although only a few studies to date have investigated this [3].

Experimental rat studies have reported that statins increase DNA fragmentation in sperm and abnormal sperm rates, while reducing spermatozoa motility [4,5]. Similarly, studies of human males have observed that statins significantly compromise semen quality, particularly sperm motility [6,7].

Statins are thought to impair spermatogenesis through various mechanisms. Studies have reported that statins compromise spermatogenesis by causing alterations in sex hormones, particularly testosterone. They also compromise spermatogenesis by causing cell death in testis germ cells, and by damaging sperm motility and morphology through impairment of spermatozoa lipid metabolism [3]. Based on the current literature, it may be concluded that atorvastatin impacts on spermatogenesis. The aim of this study was to investigate the potential effects of atorvastatin on spermatogenesis in rats.

EXPERIMENTAL

Animals

Fourteen male Sprague-Dawley rats weighing 340.7 ± 22.6 g (range, 306 - 378 g) and aged 9 - 12 weeks were obtained from the Gaziosmanpasa Experimental Animals Laboratory (Tokat, Turkey). Following a seven-day adaptation period, all rats were assigned to control and study groups. All animals were weighed before the experiment commenced and at the end of the research. On week 12, general anesthesia was applied to fasted rats using 2.5% isoflurane in nitrous oxide, after which blood samples were collected from the inferior vena cava. All rats were sacrificed with an intraperitoneal lethal dose of ketamine after surgical removal of the testes. All animal experiments were performed in strict accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [8]. Approval for the research protocol was granted by Gaziosmanpasa University animal ethics committee (protocol ID: HADYEK - 31).

Atorvastatin dose and administration

Rats in the control group (n = 7) and the study group (n = 7) were allowed *ad libitum* access to standard chow and water for 12 weeks. However, rats in the study group also received 20 mg/kg/day atorvastatin via pipette, every day in the morning.

Serum/plasma lipid and hormone analysis

Blood samples were stored in heparin-free tubes for biochemical analysis. Serum samples were first centrifuged (2000 × g for 15 min at +4 °C) and then stored at −70 °C. The samples were subsequently used for total cholesterol (TC), low-density lipoprotein cholesterol (LDL – C), high-density lipoprotein cholesterol (HDL - C), triglyceride (TG), and glucose measurements using commercial kits in line with the manufacturers' instructions. Sex hormones were determined using rat micro - ELISA kits.

Histopathological examination

At the end of the experimental period, bilateral scrotal orchiectomy was carried out under general anesthesia. Fixation of testicular specimens was performed using Bouin's solution. The specimens were then embedded in paraffin wax. Three-millimeter sections were prepared, after which the paraffin blocks were cut to a thickness of 5 µm with the help of a rotary microtome. Hematoxylin and eosin (H&E) dye was used to study the testicular structure. The stained sections were examined under a light microscope and were also used for the stereological calculation of seminiferous tubule volumes and numbers of spermatogenic cells.

Stereological analysis of samples

Histological sections were used to assess mean testicular and tubular volumes using the Cavalieri principle as shown in Figure 1 A and B, in line with the previous descriptions [9]. The sampling strategies and counting frame size were determined on the basis of a pilot study. As a general procedure in physical dissector studies, section pairs were collected as reference and look-up sections. The study parameters were counted using an unbiased counting frame placed over these sections. The total number of cells was then calculated from the number of cells counted on these sections (Figure 1 C).

Statistical analysis

The study data were analyzed on GraphPad InStat (v3.0) (GraphPad, USA) software and were expressed as mean ± standard deviation. The Friedman and Wilcoxon tests were employed to analyze changes occurring in values. Experimental results were compared between the two groups using the Mann –

Whitney U test. Statistical significance was set at $p < 0.05$.

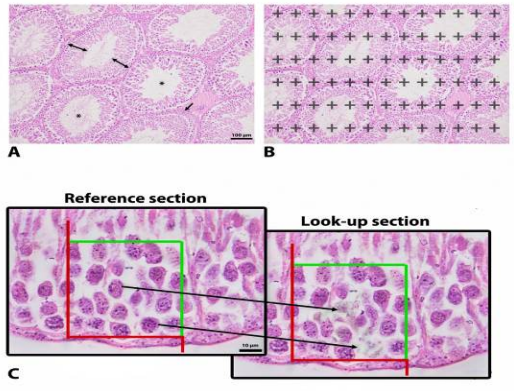

Figure 1: H&E staining of testicular specimens. (A) the general appearance of the testes, (B) the stereological Cavalieri principle applied to the sections, and (C) the application of the physical dissector cell counting method using section pairs. Double-headed arrows: seminiferous epithelium; arrow: Leydig cells; asterisks: tubular lumens

RESULTS

General

The mean pre-study weights of the rats in the study and control groups were 331 ± 25 g (range, 306 – 378 g), and 350 ± 17 g (range, 318 – 366 g), respectively (p = 0.24). Mean weights at the end of the experiment were 368 ± 22 g (range, 338 – 406 g), and 356 ± 18 g (range, 342 – 396 g), respectively (p = 0.30).

Biochemistry

The mean postoperative serum follicle stimulating hormone (FSH) levels in the study and control groups were 3.4 ± 0.3 and 3.3 ± 0.3 mIU/mL (p = 0.44), and the mean luteinizing hormone (LH) levels were 2.1 ± 0.1 and 2.0 ± 0.2 mIU/mL (p = 0.48), respectively. The mean postoperative serum total testosterone (TT) levels were 0.8 ± 0.6 and 1.2 ± 0.8 mmol/dL in the study and control groups, respectively (p = 0.06). FSH and LH levels were higher in the study group than in the control group, while TT levels were lower. However, none of these differences attained statistical significance (Table 1).

The mean postoperative serum glucose levels in the study and control groups were 168 ± 16 mg/dL and 151.1 ± 22 (p = 0.12), while TG levels were 52 ± 18.6 and 93 ± 19.8 mg/dL (p = 0.01), respectively. TC levels in the study and control groups were 47.7 ± 6.6 mg/dL and 50 ± 7.2 (p = 0.51), respectively. HDL - C levels were 41 ± 3.7 and 41.5 ± 7.8 mg/dL (p = 0.91), and LDL - C levels 3.5 ± 2.1 and 10 ± 4.4 mg/dL, respectively (p = 0.01) (Table1).

Histology

Visible cell loss and undefined cell boundaries were observed in the rat testes from the study group. However, no histological abnormalities were observed in the control group (Figure 2).

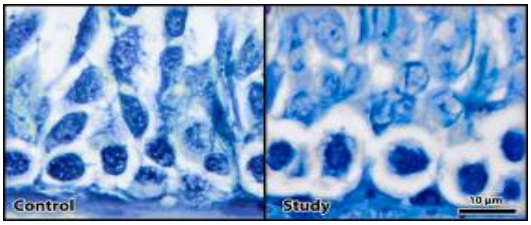

Figure 2: Light microscopic appearance of spermatogenic cells in the control and study groups

Stereology

Significant decreases occurred in the numbers of Sertoli cells, spermatogonia, and spermatocytes in the study group compared to the control group (Table 2). Mean testis tubule volumes were also significantly lower in the study group compared to the control group ($p < 0.001$) (Table 2).

Table 1: Biochemical variables of the group (mean ± SD, n = 7)

Variables	Control group	Study group	*P*-value
Blood glucose (mg/dL)	151.1 ± 22	168 ± 16	0.12
TG (mg/dL)	93 ± 19.8	52 ± 18.6	0.01
TC (mg/dL)	50 ± 7.2	47.7 ± 6.6	0.51
HDL - C (mg/dL)	41.5 ± 7.8	41 ± 3.7	0.91
LDL - C (mg/dL)	10 ± 4.4	3.5 ± 2.1	0.01
FSH (mIU/mL)	3.3 ± 0.3	3.4 ± 0.3	0.44
LH (mIU/mL)	2.0 ± 0.2	2.1 ± 0.1	0.48
TT (mmol/dL)	1.2 ± 0.8	0.8 ± 0.6	0.06

Key: FSH, Follicle-stimulating hormone; HDL - C, High-density lipoprotein cholesterol; TT, Total testosterone; LDL - C, Low-density lipoprotein cholesterol; LH, Luteinizing hormone; TC, Total cholesterol; TG, Triglyceride

Table 2: Stereological results (mean ± SD, n = 7)

Variable	Control group	Study group	*P*-value
Testicular volume (x10^2, mm^3)	291.43 ± 8.40	282.71 ± 6.28	0.18
Mean tubular volume (cm^3)	0.75 ± 0.56	0.51 ± 0.55	< 0.001
Total number of Sertoli cells (x10^6)	4.882 ± 146	3.011 ± 118	< 0.001
Total number of sermatogonia (x10^6)	1.25 ± 0.10	0.97 ± 0.08	< 0.001
Total number of spermatocytes (x10^6)	2.63 ± 0.93	1.76 ± 0.11	< 0.001

DISCUSSION

This study employed a stereological technique to investigate the effect of atorvastatin on spermatogenesis in the rat testis. Stereological methods provide essential quantitative data for the evaluation of morphological characteristics in the rat testis. The total volumes of the testes, interstitial tissues and germinal epithelium, seminiferous tubules, the diameter, length, and cross-sectional area of tubules, numbers of myoid, Leydig, and Sertoli cells, and the numbers of spermatids, spermatocytes and spermatogonia can all be calculated using this method and simulated throughout the organ [9]. Atorvastatin at 20 mg/kg per day for 12 weeks caused a significant deterioration in spermatogenesis in the present study. It also significantly reduced serum TG and LDL - C concentrations.

Infertility is associated with male-related factors, such as varicocele, undescended testis, genital infections, genetic causes, hormonal disorders, and obstructive pathologies. Other, rarer causes of infertility include radiation, surgical procedures, environmental factors, occupation, and medications [10]. The use of drugs to combat chronic diseases has increased dramatically in recent years. Numerous studies have investigated the effects of calcium channel blockers, valproate, sulfasalazine, anti-hyperlipidemics, acetylsalicylic acid, spironolactone, cimetidine, serotonin reuptake inhibitors, and antibiotics on sperm parameters [11]. Similarly to those drugs, statins are also capable of affecting semen parameters. Although the use of statins has increased recently, very few data are available concerning their effects on spermatogenesis [3].

Testosterone is the basic hormone essential for spermatogenesis. The testosterone-dependent phase of spermatogenesis, known as spermiogenesis (progression from round to elongated spermatids), constitutes the final phase of spermatogenesis. Testosterone is also essential for the transition from spermatogonia A, an early stage of spermatogenesis, to spermatogonia B. Testosterone contributes to the survival of spermatocytes and spermatids through anti-apoptotic mechanisms [12]. All steroid hormones are synthesized from cholesterol, and testosterone levels may decline under conditions of reduced cholesterol concentrations [13-16]. A decrease in testosterone levels may be associated with decreased spermatogenesis. The hypothesis in the present study was that by reducing cholesterol, atorvastatin might lower testosterone levels and subsequently impair spermatogenesis.

The study findings showed that TT levels decreased by 38.5% in the study group compared to the control group. Similarly, stereological analysis also demonstrated signify-cant decreases in numbers of spermatogonia, spermatocytes, and Sertoli cells in the study group. The decreased seminiferous tubule volume observed in the study group may probably be attributed to seminiferous tubule atrophy. Clinical studies, albeit fewer in number, have also investigated the effect of statins on testis functions. Atorvastatin also significantly affected semen parameters in another human study in which 10 mg/day of atorvastatin was administered every day for five months to 17 normospermic male patients with a mean age of 24 years. It reduced sperm vitality and total spermatozoa numbers, while increasing morphological abnormalities.

Atorvastatin also exhibited an adverse effect on the reproductive system by reducing levels of acid phosphatases, α-glucosidase and L-carnitine in seminal fluid [7]. Another study involving pre-pubertal rats examined the effect of rosuvastatin on the reproductive system. The authors reported a decrease in serum testosterone levels and delayed puberty in rats receiving rosuvastatin. A significant decrease in mature spermatids and daily sperm production numbers was also determined in rats that received rosuvastatin, indicating a significant adverse effect on sperm parameters [4]. In the present study, atorvastatin administration adversely affected spermatogenesis in the study group compared to the control group. However, the mechanism behind these effects is still unclear.

A minimal decrease in intratesticular testosterone has previously been observed, with no change in systemic hormone levels, in young patients using atorvastatin [7]. Similarly, in the present study,

TT levels decreased in rats receiving atorvastatin, although this was not statistically significant. The administration of 40 mg pravastatin and placebo has also been shown to cause no change in TT levels after 3 months [17]. In another study, even 20 mg atorvastatin administration daily for 3 months produced no change in TT levels in 16 male patients with type 2 diabetes [18]. However, numerous studies have reported statistically significant reductions in TT levels in humans following statin use [3]. Since various factors affect TT levels, different studies have elicited differing results. The decrease in TT levels noticed in experimental animal studies may also be associated with different durations of drug administration.

Stereological analysis in the present study revealed significantly impaired spermatogenesis in the testes of rats receiving atorvastatin for 12 weeks at 20 mg/kg/day. Atorvastatin has been used at between 10 and 50 mg/kg/day in previous animal studies [3]. It should be remembered that, similarly to other drugs, the effect of atorvastatin will vary depending on the dose employed.

CONCLUSION

This study shows that the administration of atorvastatin, one of the most commonly prescribed medications worldwide, particularly for the treatment of cardiovascular disease, resulted in deleterious stereological effects on spermatogenesis. Statin therapy, with its potential side-effects on spermatogenesis, should therefore be prescribed with great care, particularly in young male patients. However, further experimental research, and particularly human studies, are now needed to confirm these outcomes.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. E.A. and F.E. were responsible for the experiments and experimental design; F.F., M.B., and M.E.O. performed the experiments; E.A., M.B. and M.E.O. analyzed the data; M.E.O., A.A., O.C., M.G and M.S.B. contributed reagents/materials/ analysis tools; and E.A. and F.E. produced the manuscript. All authors read the article and approved the final version of the manuscript.

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