Comparison of the Effects of Clofibrate and Silafibrate on Sperm Parameters Quality and Sex Hormones in Male Rats

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Purpose: Fibrates are drugs widely used for the treatment of hyperlipidemic disorders. Previous studies on a novel analogue of clofibrate, called silafibrate, have shown good lipid lowering effects. This study was designed to assess the role of silafibrate as a peroxisome proliferator-activated receptors (PPARs) agonist on sperm health and spermatogenesis in adult male rats.

Material and Methods: Seventy male Wistar rats were randomly allocated into 7 groups: Cl-10, Cl-20, and Cl-40 mg/kg/day (clofibrate); Si-10, Si-20, and Si-40 mg/kg/day (silafibrate); and C, control. After a 28-day treatment, all rats were euthanized. Blood samples were taken for determination of testosterone, total antioxidant capacity, levels of malondialdehyde, and oxidized low-density lipoprotein. Reproductive organs were dissected and spermatozoa collected from the epididymis for analysis.

Result: Sperm parameters (count, motility, viability, and morphology) and total serum testosterone decreased significantly in clofibrate-treated (20 and 40 mg/kg) rats ($P < 0.05$) as compared with normal rats.

Conclusion: We conclude that PPARs agonists have significant adverse effect on sperm viability, motility, and total serum testosterone, and could be harmful for sperm parameters and male reproductive function in rats.

Keywords: clofibrate; silafibrate; spermatogensis; rats.

INTRODUCTION

Infertility is a serious life problem. Over the past decades, an increasing body of evidence has indicated a steady and dramatic increase in prevalence of infertility: 10-15% of all couples of reproductive age in our times suffer from infertility[1,2]. Approximately 50% couples' infertility is due to impaired semen parameters[3]. Multiple conditions may interfere with spermatogenesis process and cause decline in sperm quality and production[4]. Several factors—such as medicines, toxins, and diseases—lead to impaired spermatogenesis at different stages[5].

Fibrates, peroxisome proliferator-activated receptor-α (PPAR-α) agonists are effective in treatment of hypertriglyceridaemia and hypercholesterolaemia[6]. Fibrates differ in their potency, and it seems a structure–activity relationship (SAR) exists[7]. The pharmacologic and toxicologic properties of silafibrate as a potent analogue of clofibrate have been studied in experimental animals[7] but it has not been clinically trialed in human yet. Studies have been shown that silafibrate causes significant reduction in toxicity[7]. Sperm viability and motility are important for convince and are essential factors in evaluating the fertilizing ability of sperm. Relation between the velocity of sperm motility and fertilization rates have been proved[8]. The lipid and fatty acids which constitute spermatozoa can modulate their mobility and viability[9]. Several studies have been conducted to focus on the association of fibrates with male infertility. Cook et al. showed clofibrate may cause suppression of spermatogenesis at dosages ranging from 160 to 240 mg/kg[10]. However, results also show some variation, and the exact molecular mechanism(s) of action are still poorly known. It has not been clearly understood if the suppressive effect of clofibrate is cause by PPAR-α receptors or by its chemical properties. Similarly, the functional role of peroxisomes in spermatogenesis and fertility is not known exactly[11]. The second interest in the present study is to evaluate the androgenic effects of different doses of silafibrate as a novel PPAR-α agonist on sperm parameters by using hormone measurements. We assess the effects of silafibrate on sperm parameters and modulate malondialdehyde (MDA) concentration, spermatogenesis, and oxidative stress. Hence, our hypothesis is that chronic administration of clofibrate and silafibrate might lead to changes in sperm quality and hormonal situations of rats.

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Table1. The effect of clofibrate and silafibrate in comparison to control group on testis, epididymis and seminal vesicle weight.

Control	Clofibrate 10 mg/kg	Clofibrate 20 mg/kg	Clofibrate 40 mg/kg	Silafibrate 10 mg/kg	Silafibrate 20 mg/kg	Silafibrate 40 mg/kg
Testis (g)	1.74 ± 0.8	1.75 ± 0.7	1.73 ± 0.9	1.70 ± 0.5	1.72 ± 0.6	1.67 ± 0.8
Epididymis (g)	0.08 ± 0.34	0.07 ± 0.37	0.09 ± 0.37	0.04 ± 0.39	0.06 ± 0.31	0.05 ± 0.32
Seminal vesicle (g)	0.03 ± 0.52	0.04 ± 0.53	0.06 ± 0.49	0.07 ± 0.54	0.03 ± 0.52	0.04 ± 0.50

Data are presented as mean ± SD; Continuous variables were compared by independent samples *t*-test.

MATERIALS AND METHODS

A total of 70 adult male Wistar rats weighing between 280 and 300 g were used. They were obtained from the animal facility of the Pasture Institute of Iran. The animals were housed in temperature controlled rooms (22 ºC) with constant humidity (40–70%) and 12-h light and 12-h dark conditions for one week before the commencement of the experiments. They had free access to standard laboratory chaw and tap water. Experiments on animals were performed according to animal ethics guidelines of the Tabriz University of Medical Sciences Ethics Committee.

Rats were randomly allocated into seven groups of ten animals per group. Group 1 was control and received normal saline daily for 28 days[12]; groups 2–4 gavaged silafibrate (10, 20, and 40 mg/kg/ day, oral) for 28 consecutive days; and groups 5–7 received clofibrate (10, 20, and 40 mg/kg/day, oral) for 28 consecutive days. At the end of these treatments, the animals were euthanized, blood samples were taken, and reproductive organs were dissected out for biochemical and histopathological examinations.

Surgical procedure

On the twenty-eight day (at the end of the treatment period, i.e.), the rats were euthanized with diethyl ether, blood was collected into heparinized tubes, and serum was separated by centrifugation for further analysis. Testes were dissected out and spermatozoa were collected from the epididymis[13].

Testis, epididymis, and seminal vesicle weight measurement

The reproductive organs—testes, epididymis, and seminal vesicles—were dissected out from surrounding adipose and connective tissues by an expert anatomist and were weighed using analytical balance with 0.1 mg accuracy.

Epididymal sperm motility, viability, count, and sperm abnormality

Sperm from the cauda epididymis were released by cutting into 2 mL of medium (Hams F10) containing 0.5% bovine serum albumin[14]. After 5 min incubation at 37 ºC (with 5% CO_2), cauda epididymis sperm reserves were determined using the standard haemocytometric method. Sperm motility was then analyzed by microscope at 10 field and reported as the mean of motile sperm according to WHO methods[15]. Sperm abnormality was also evaluated according to the standard method of Narayana[16]. Briefly, smears of sperm suspension were made on clean glass slides and stained with periodic acid-Schiff's reaction haematoxylin. The stained smears were observed under a light microscope with 40× magnification. The sperms were classified into normal and abnormal. The total sperm abnormality was expressed as percentage incidence.

Serum total testosterone, LH, and FSH hormone

Serum concentrations of FSH and LH were determined in duplicated samples using radioimmunoassay (RIA) (Isotope Company Ltd., Budapest, Hungary). Rat FSH/ LH kits were obtained from Isotope Company Ltd. and used according to the protocol provided with each kit. The sensitivities of hormone detected per assay tube were 0.2 ng/mL and 0.14 ng/mL for FSH and LH respectively. Serum concentration of total testosterone was measured by using a double antibody RIA kit from immunotech Beckman Coulter Company, USA. The sensitivities of hormones detected per assay tube were 0.025 ng/mL.

Total antioxidant capacity and malondialdehyde concentration measurement in serum

A total antioxidant capacity (TAC) detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute, China. According to this method, the antioxidant defense system, which consists of enzymatic and non-enzymatic antioxidants, is able to reduce Fe3+ to Fe2+[14]. TAC was measured by the reaction of phenanthroline and Fe2+ using a spectrophotometer at 520 nm. At 37 °C, a TAC unit is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 mL of serum. Radical damage was determined by specifically measuring malondialdehyde (MDA). MDA was formed as an end product of lipid peroxidation treated with thiobarbituric acid to generate a colored product. Concentration of MDA was measured in se-

Table 2. The effect of clofibrate and silafibrate in comparison to control group on motility, viability and number of sperms.

	Control	Clofibrate 10 mg/kg	Clofibrate 20 mg/kg	Clofibrate 40 mg/kg	Silafibrate 10 mg/kg	Silafibrate 20 mg/kg	Silafibrate 40 mg/kg
Sperm concentration (total count) (No. of sperm/rat × 106)	66.5 ± 3.8	61.4 ± 3.9	3.6* ± 55.7	3.2* ± 48.2	65.7 ± 3.4	63.6 ± 3.7	60.2 ± 3.3
Motility (%)	48.2 ± 1.7	45.7 ± 1.2	1.9* ± 36.3	1.5* ± 30.8	46.4 ± 1.6	43.5 ± 1.8	1.5* ± 40.9
Viable Sperm (%)	66.5 ± 3.8	64.9 ± 4.4	3.8* ± 54.7	3.9 ± 50.2	65.7 ± 3.9	62.9 ± 4.2	61.3 ± 3.9

Data are presented as mean ± SD; Continuous variables were compared by independent samples *t*-test
*Significant different at $p < 0.05$ level, (compared with the control group).

Table 3. The effect of clofibrate and silafibrate in comparison to control group on LH, FSH and Testosterone.

	Control	Clofibrate 10 mg/kg	Clofibrate 20 mg/kg	Clofibrate 40 mg/kg	Silafibrate 10 mg/kg	Silafibrate 20 mg/kg	Silafibrate 40 mg/kg
Testosterone	0.18 ± 5.07	0.13 ± 5.00	0.12* ± 4.88	4.52 ± 0.12*	0.15 ± 5.09	0.13 ± 5.01	4.95 ± 0.11
FSH	0.04 ± 0.54	0.08 ± 0.60	0.07* ± 0.64	0.67 ± 0.09*	0.08 ± 0.52	0.09 ± 0.55	0.58 ± 0.07
LH	0.76 ± 0.12	0.78 ± 0.09	0.14 ± 0.77	0.72 ± 0.16	0.74 ± 0.09	0.11 ± 0.79	0.77 ± 0.13

Data are presented as mean ± SD; Continuous variables were compared by independent samples *t*-test
*Significant different at $p < 0.05$ level, (compared with the control group).

rum and testis homogenates using a method prescribed previously[17]. Testicular tissue was removed and homogenized in a Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. The lipid peroxide was measured spectrophotometrically at 532 nm and expressed as nano mole MDA per mL of serum or gram of testis tissue.

Statistical analysis

Quantitative data is presented as Mean ± SD. Sperm counts, morphology, and motility of control and experimental groups are compared using one-way analysis of variance (ANOVA); LSD test was used to find the statistical differences among their means. A value of $P < .05$ was considered to be statistically significant.

RESULTS

Tissue weight

The results of 28-day oral administration of clofibrate (10, 20, and 40m/kg/day) and silafibrate (10, 20, and 40m/kg/day) are summarized in Table 1. No significant differences can be seen among testicle, epididymis plus vas deferens of any groups administered clofibrate or silafibrate as compared to the control group ($P > 0.05$).

Sperm count, motility and viability

Table 2 shows results of 28-day oral administration of clofibrate (10, 20, and 40m/kg/day) and silafibrate (10, 20, and 40m/kg/day) on sperm motility, viability, and count in rats. Oral administration of clofibrate 20 and 40 mg/kg/day for 28 consecutive days causes considerable decline in sperm viability and motility in both experimental groups as compared to the control group ($P < .05$). Furthermore, sperm count reduced significantly as compared to the control group ($P < .05$). Oral administration of silafibrate 10, 20, and 40 mg/kg/day result in a reduction in sperm viability and concentration parameters—but these differences are not significant ($P > .05$). There is only a statistical difference in motility of sperms in group of silafibrate 40mg/kg/day ($P < .05$).

Serum total testosterone, LH, and FSH hormone measurement

Results of this study show that serum total testosterone levels are significantly low in rats receiving clofibrate 20 and 40 mg/kg/day for 28 consecutive days ($P < .05$). As shown in Table 3, silafibrate did not considerably alter levels of serum total testosterone in any of the experimental groups ($P > .05$). Administration of clofibrate 20 and 40 mg/kg/day for 28 consecutive days elevated the FSH hormone level, while 40 mg/kg/day of silafibrate increased FSH hormone level in the experimental group as compared to the control group—but this was not statistically remarkable. There was no significant difference in the level of LH hormone between the experimental and control groups (**Table 3**).

Total antioxidant capacity and malondialdehyde concentration measurement in serum

Administration of silafibrate 20 and 40 mg/kg/day for 28 consecutive days significantly decreased the level of MDA concentration in the experimental groups as compared to the control group ($P < .05$). Administration of silafibrate 40 mg/kg/day for 28 consecutive days significantly increased the level of TAC. There was no significant difference in the level of MDA and TAC between clofibrate and control groups (**Table 4**).

DISCUSSION

Dyslipidemia is emerging as an important and rising health issues in young people in the reproductive bracket. Dyslipidemia includes raised levels of triglycerides (TGs) and low density lipoprotein cholesterol (LDL), and low levels of high density lipoprotein cholesterol (HDL-C)[18]. Cholesterol is one of the most important bio-molecules: it plays crucial functions in the area of male and female reproductive physiology. According to current guidelines[19], in patients with very high hypertriglyceridemia, lowering of TGs is necessary due to the increased risk of acute pancreatitis and cardiovascular diseases [20]. Fibrates such as PPARs agonists have been prescribed to treat elevated serum triglycerides and cholesterol dyslipidemia for decades[21]. Silafibrate as a novel analogue of clofibrate has shown good lipid lowering effects in animal studies[7] but it has not been used in human yet. We estimated the safe and effective applicable dose in human (70 kg) based on Naire et al[22] formula as follow:

HED (mg / kg = Animal NOAEL mg/kg) × (Weightanimal [kg]/Weighthuman [kg])(1–0.67)

Table 4. Effects of clofibrate and silafibrate t on serum's Malondialdehyde (MDA) and Total anti oxidation concentration (TAC).

	Control	Clofibrate 10 mg/kg	Clofibrate 20 mg/kg	Clofibrate 40 mg/kg	Silafibrate 10 mg/kg	Silafibrate 20 mg/kg	Silafibrate 40 mg/kg
MDA	1.62 ± 0.09	1.72 ± 0.11	1.68 ± 0.17	1.75 ± 0.15	1.68 ± 0.08	0.11* ± 1.57	1.42 ± 0.07*
TAC	0.04 ± 0.27	0.03 ± 0.24	0.23 ± 0.05	0.24 ± 0.03	0.04 ± 0.26	0.05 ± 0.29	0.31 ± 0.03*

Data are presented as mean ± SD
*Significant different at $P < 0.05$ level, (compared with the control group).

(20 mg/kg)x (0.3[kg]/70[kg])0.33 =3.30 mg/kg x75 [kg](Patient's weight)=248 mg

Studies have also shown that PPARs agonists such as clofibrate and fenofibrate may have miscellaneous side effects, such as suppression of spermatogenesis and erectile dysfunction[10,23]. Chronic use of these drugs may result in decrease in sperm quality and in infertility.

Our study on organ weights showed that no significant changes can be discerned in the morphology and weight of testis, epididymis, and seminal vesicle between treatment and control groups over the course of 28 consecutive days. While oral administration of clofibrate 20 mg/kg/day for 28 days leads to 16.2% reduction in sperm count, clofibrate 40 mg/kg/day for the same time period decreases sperm count by 27.5%: statistically, all of these results are considerable in comparison to normal rats ($P < .05$). Additionally, along with sperm count, sperm viability and motility also decreased significantly by clofibrate as compared to the control group ($P < .05$). The decrease in sperm count, viability, and motility were not statistically significant in all doses of silafibrate in comparison to the normal control group ($P > .05$).

Testosterone is pivotal factor for spermatogenesis: it requires normal stimulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These are produced in the hypothalamus, where their secretion is controlled by the release of gonadotropin releasing hormone (GnRH)[13]. Results of this study reveal that levels of testosterone, LH, and FSH in groups treated with various doses of silafibrate do not change considerably ($P > .05$). Conversely, in clofibrate 20 and 40 mg/kg groups, testosterone hormone was increased significantly and FSH was elevated remarkably ($P < .05$). Meeker and colleagues demonstrated that FSH, LH, and testosterone are associated with human semen quality[24]. They announced that FSH and LH are inversely associated with sperm quality, motility, and morphology. FSH is produced and secreted by the anterior pituitary and acts on sertoli cells in the seminiferous tubules to initiate spermatogenesis. Spermatogenesis is regulated by testosterone and its deficiency leads to increased germ cell apoptosis. However, the mechanism by which testosterone controls spermatogenesis remains unclear[25]. Studies conducted on rats with a xenobiotic such as ethane 1,2-dimethanesulfonate (EDS) —a leydig cell toxicant— have demonstrated a rise in germ cell apoptosis[26]. Clofibrate may inhibit testosterone secretion by dispersing rodent leydig cells. It may be caused by the blood testis barrier, which inhibits entry of clofibrate to the testis across the testicular barrier; however, clofibate can enter the hypothalamus more easily. We supposed that pituitary FSH and LH secreting cells, as well as sertoli and leydig cells, may show progressive deterioration due to the high dose of clofibrate in serum. FSH rise and testosterone fall in rats can cause the depletion of mature spermatids through phagocytosis by sertoli cells[27]. Generally, these findings explain the inhibitory effect of clofibrate on serum testosterone and FSH levels. Our data shows that serum total testosterone decreases significantly in test groups ($P < .05$), and levels of FSH increase significantly only in the group that receives high doses of clofibrate ($P < .05$): nonetheless, there was no difference in the level of LH between the experimental and control groups. Our results in this study confirm the subtractive effect of clofibrate, and that silafibrate as a novel siliconized analogue has no considerable side effects on sperm quality. Sperm motility is essential for normal fertilization and one of the most important parameters in evaluating the fertilizing ability of ejaculated sperm. In this regard, correlations between the velocity of sperm movement or sperm motility and fertilization rates as proved by present results clearly indicate that clofibrate as a PPARs agonist has a subtractive effect on spermatogenesis in rats. Our results show that administration of clofibrate (20 and 40 mg/rat/day) for 28 consecutive days causes a significant decrease in sperm count, viability, and motility as compared to normal rats.

Oxidative stress was measured by malondialdehyde levels, reactive oxygen species (ROS) generation, and alterations in antioxidant defenses[13]. Sperm concentration, motility, and function are susceptible to oxidative stress: these contribute to decreased fertility[28,29]. Results of this study reveal that MDA decreases with silafibrate 20 and 40 mg/kg/day and TAC increases with the 40 mg/kg/day of silafibrate. The exact mechanism of antioxidant action of fibrates is still to be examined: the following may be possible routes for further research. First, several metabolites (not fibrates themselves) have direct radical scavenging properties[30]. Second, some experiments have shown that treatment with fibrates decreases the susceptibility of plasma proteins, especially LDLs, to oxidation[30,31]. Third, our previous studies [6,7,32,33] and several other studies have demonstrated that fibrates possess potent anti-inflammatory effects and antioxidant effects[34]. It seems that the remarkable antioxidant and anti-inflammatory effects of silafibrate may prevent adverse effects on sperm parameters in comparison to the parent analogoue. These are in accordance with other investigations, which was reported a negative correlation with MDA level and testosterone, FSH and LH[13,35]. The limitations of this study was lack of histological analysis due to research budget restrictions.

Our work demonstrates that clofibrate therapy, at 10 mg per day, has no effect on total testosterone and gonadotropin serum level. However, this significantly affects sperm parameters in rats: silafibrate as a novel analogue may be considered as a safe lipid lowering drug on sperm quality. Future research should aim towards a randomized placebo-controlled assay with a larger cohort and a longer study period to confirm silafibrate effects on human fertility.

CONCLUSIONS

In conclusion, our study shows for the first time that the administration of silafibrate to healthy and normocholesterolaemic subjects does not affect their sperm parameters (vitality, number, and motility). While considering the long duration of clofibrate treatment with 20 and 40 mg/kg—whose clinical benefit with respect to cardiovascular diseases is beyond question—potential negative consequences on reproductive functions should be taken into account when deciding to initiate such a treatment, especially for human.

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