Short Communication

The Effect of Chronic Administration of Methylphenidate on Morphometric Parameters of Testes and Fertility in Male Mice

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Abstract

Background: Due to common use of methylphenidate (MPH) for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and the role of the reproductive system in the production of gametes, studying the effects of this medication on the morphometry of testes, serum testosterone concentration, leydig cells function, and fertility rate was the aim of this study.

Methods: Twenty seven male mice (Balb/C), eight weeks old, were randomly divided into one control and two treated groups. After weighing the mice, the treated groups received MPH (produced in Novartis company) at the doses of 2 *mg/kg* and 10 *mg/kg* for 40 days. The control group received only normal saline. Subsequently, after weighing the animals, the weights of testes, dimensions of the testis, and the serum testosterone concentration were measured in six mice belonging to each group. After tissue processing, the samples were stained with hematoxylin and eosin, then the leydig cells were counted. In order to assess male fertility in each group, 3 male mice were chosen and each of them was kept with three female mice in a separate cage. After 10 days, the fertility rates of the male mice were determined by counting the number of embryos in uterus and the corpora lutea in their ovaries.

Results: The results of this study revealed that prescription of different doses of MPH can cause a significant decrease of the body weight. It reduces the number of leydig cells, too ($p<0.01$). Moreover, serum testosterone concentration (67.72±8.24 *ng/ml* in control group and 0.302±0.416 *ng/ml* after treatment with 2 *mg/kg/day* MPH) and fertility rate (95.42%±4.68% in control group and 64.96%±18.51% after treatment with 2 *mg/kg/day* MPH) of the male mice declined significantly in the treated groups compared with the control group ($p<0.01$), but it did not cause any changes in the weight or morphometric parameters of testes.

Conclusion: The results of this study confirmed that MPH can negatively affect serum testosterone concentration and fertility rate of the male mice by decreasing the number of leydig cells and reducing the body weight.

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Introduction

Methylphenidate (MPH), commonly known as Ritalin, is the most prescribed medication to treat behavioral disorders in children and adults (1). MPH is one of the isomers of Amphetamine (2). It is a white, odorless, and fine crystalline powder which is soluble in water. MPH is

manufactured as oral tablets of 10 and 20 *mg* in Iran (3). Some researchers have shown that MPH could induce encephalic maintenance. So, it could be used to control unfavorable signs, such as absence of concentration, attentiondeficiency, and hyperactivity. Moreover, its using in children can be continued during adulthood, too (4). Due to the extensive use of this medication to treat Attention Deficit Hyperactivity Disorder (ADHD) symptoms, many investigations have been performed to evaluate its effects on the body organs (5).

It has been shown that chronic use of MPH in female mice could cause vaginal stenosis, changes in hormone secretion, and development of ovarian follicles (6). Another study has indicated that the body weights of the male mice were significantly decreased upon MPH use in comparison to the control group. Besides, the weight of the testes and seminal vesicles decreased significantly in these mice (7, 8). In another report, disorders in the growth of the animals have been observed following long-term use of MPH (9). Also, changes in the weight of brain, heart, ovaries, spleen and prostate indicate its effects on different body organs (2).

A chemical substance similar to Amphetamine group causes reductions in body weight and testosterone concentration in rats (10, 11). Cocaine, which is structurally similar to MPH, can cause body weight reduction and harmful effects on fertility (12).

Regarding the highly frequent prescription of MPH in children and adults, the importance of the gamete producing gonads, and insufficient awareness of chronic administration of MPH with its probable effects on fertility, this study evaluates the chronic use of this medication on fertility rate in male mice.

Methods

In this study, Twenty-seven healthy adult male Balb/C mice, each varying between 25–30 *g* (eight weeks old) were examined. The mice were kept in standard conditions, like proper temperature (21–23 ℃), humidity (45%–55%), and free access to water and food in the animal lab. The male mice were initially weighed and divided into two treated and one control groups. The animals in the two treated groups were treated with doses of 2 *mg/kg* or 10 *mg/kg* of hydrochloride-MPH (produced in Novartis company). Forty days after treatment and repeated weight measurements, six male mice from each group were anesthetized and blood samples were drawn from their hearts. Serum testosterone concentrations were determined by chemo- luminescence immunoassay method. In order to determine morphometric changes of the testes, digital calipers were used to measure the length, width, and thickness of testes after opening the abdominal cavity. The same testes were later removed, washed in physiological serum, and weighed before being fixed in 10% formalin. Followed by 5-micron sectioning of the tissue blocks, the specimens were stained using hematoxylin and eosin. For histological study of the testes, eight sections from each testis were randomly selected and photomicrographs were captured by using a calibrated photomicroscope connected to a computer equipped with Axiovision 4.8 software. In each section, the number of Leydig cells was morphologically analyzed by the Vidas Image Processing System connected to a microscope (13, 14).

In order to assess the effects of MPH on fertility, from each group, 3 male mice were chosen and each of them was kept with 3 female mice in a separate cage. After 10 days, the female mice were taken out, anesthetized and the embryos in their uterus were counted. The ovaries were then removed and washed in normal saline (sodium chloride solution 0.9%). The number of corpus luteum was counted and the fertility rate was determined by dividing the number of embryos to the corpus luteum. In the cases that no embryos were found, the above experiment was repeated for three times (15).

The data were statistically analyzed by one-way ANOVA and Tukey's Post Hoc Test, and a value of $p<0.01$ was considered significant.

Results

The results of the present study in Balb/C mice showed that chronic administration of MPH can induce histological changes in testes depicted by diminishing the number of interstitial cells, declining serum testosterone levels, reduction in body weight, and also fertility rate. The assessment of weighting of the mice, before and after treatment, showed that the treated groups had a significant diminution in weight in comparison with the control group (Table 1; $p<0.01$).

A) Morphometrical findings: Regarding the dimensional characteristics of the testes, it was observed that testicular weight; width; length; and thickness were not significantly different between the control and treatment groups (Table 1). The

Table 1. Comparing the mean (Mean±SEM) of testicular dimenstions, body weight, serum testosterone concentrations, Leydig cell number, and fertility rates in different experimental groups following chronic administration of MPH

Parameters	Groups		
	Control group	Experimental group I 2 *mg/kg/day* (MPH)	Experimental group II 10 *mg/kg/day* (MPH)
Body weight differences (*g*) (before and after treatment)	12.21±1.82[a]	0.46±0.60[b]	3.73±1.41[b]
Length of testis (*cm*)	0.66±0.04[a]	0.67±0.05[a]	0.73±0.04[a]
Width of testis (*cm*)	0.39±0.02[a]	0.43±0.07[a]	0.46±0.03[a]
Thickness of testis (*cm*)	0.45±0.06[a]	0.049±0.03[a]	0.47±0.03[a]
Testis weight (*g*)	0.1150±0.0088[a]	0.1189±0.0082[a]	0.1144±0.0085[a]
Testosterone level (*ng/dl*)	67.72±8.24[a]	0.302±0.416[b]	14.583±0.4167[b]
Leydig cell count (*n*)	34.41±3.23[a]	9.81±0.60[b]	8.44±0.48[b]
Fertility rate (*%*)	95.42±4.68[a]	64.96±18.51[b]	18.53±16.72[c]

a-c Numbers with different superscript letters in each row differ significantly ($p<0.01$)

results of total counting of leydig cells, showed a significant decrease ($p<0.01$) in number, in treated groups compared with the control group (Table 1, Figures 1 and 2).

B) Biochemical findings: In another assessment the results revealed that the serum testosterone concentration in treatment groups had a significant diminution in comparison with the control group (Table 1; $p<0.01$).

C) Fertility rate: The results indicated a significant abatement in fertility rate in comparison with the control group (Table 1; $p<0.01$).

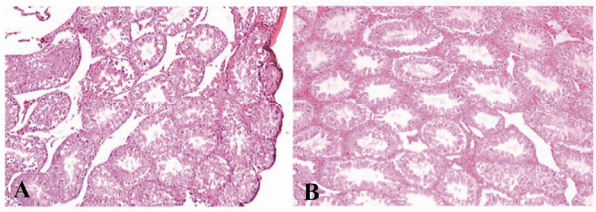

Figure 1. Photomicrographs of mice testis tissue in mice in the treated groups received methylphenidate; A: and the control group; B: (H&E×40)

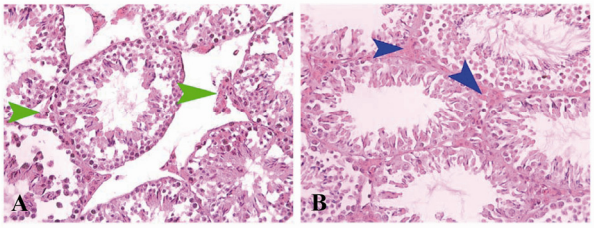

Figure 2. Photomicrographs of mice testis tissue in mice in the treated groups received methylphenidate; A: and the control group; B: Decrease in leydig cells and increase in the space between seminiferous tubules in the treated groups and comparison with the control group (H&E×40)

Discussion

MPH is a medication prescribed for treatment of ADHD in children and adults with behavioral symptoms not treated in childhood (16). Considering the possible effects of MPH on body organs, the study of its effects on body weight, testes, and fertility seems necessary. In the present study, the weights of mice in the treatment groups showed significant reduction in comparison with those in control groups. In another study, the mice receiving hydrochloride -MPH similarly showed a significant reduction in body weight (17). In fact, body weight reduction could be due to decrease of appetite. Some other chemicals, such as amphetamine could also cause body weight reduction. Weight loss after MPH treatment could be due to several factors, such as anorexia, stomach cramps, or insomnia, that reduce food intake. Weight loss caused by reduced food intake can severely affect growth and pubertal development by perturbing the maturation of not only the hypothalamo – pituitary– gonadal axis but also the axes of the hypothalamus and pituitary with the adrenals, thyroid, and growth hormone. Other nonspecific effects of weight loss that might indirectly affect growth and pubertal development include the following: gastrointestinal complications of reduced taste, delayed gastric emptying, or malabsorption (6). Therefore, the same reasons might be responsible for body weight reduction following MPH administration. It was observed that chronic oral use of MPH did not bring about any changes in weight or size of testes. However, a study on rats has shown that gavages of hydrochloride cocaine (15 *mg/kg*), which is structurally similar to MPH,

prescribed for 100 days, resulted in testicular weight reduction (12). This study showed the significant reduction of the number of leydig cells in the treatment groups compared with the control group. Some other reports, confirming this finding, have indicated that intraperitoneal injection of hydrochloride cocaine at a dose of 30 *mg/kg* could cause necrosis and also decrease in the number of interstitial cells of the testes (18).

Serum Testosterone secretion showed a significant reduction in the treatment groups of this study. A similar study on the short term effects of MPH on the production of sex-hormone in male mice showed that exposure of mice to MPH causes considerable reduction in testosterone production: Reduction in testosterone serum concentration in treatment groups compared with the control group might be due to a variety of reasons including:

1) Effect of this medication on hypothalamus, as impairment of pulsatile release of hypothalamic GnRH (6).

2) The testosterone might be naturally produced in the treated groups, but MPH through hepatic enzyme induction leads to testosterone destruction and subsequent decrease in serum testosterone concentration.

Therefore, the negative effect of MPH on the metabolism of testosterone could be concluded (19).

3) Decreasing in interstitial cell number. In this study, changes in the serum testosterone concentration showed a significant reduction in the groups receiving MPH. Reduction in the number of interstitial cells could be a reason for this significant decrease in testosterone concentration. Other studies have used similar medication to cocaine that affects brain-hypophysis-testis axis. Perhaps, chronic use of MPH could result in the reduction of the quantity of testosterone by affecting this axis. Maturation of leydig cells is induced by enzymes which any disorder in their secretion leads to disorders in testosterone synthesis (20). Moreover, studying the effects of 2,2-bis (p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) at a dose of 200 *mg/kg* in adult male rats has shown that testosterone secreted from leydig cells could be reduced (21). Fertility rates also decreased significantly in this study, which indicates that MPH could decrease testicular function. Reduction of fertility in male mice due to chronic use of MPH may have different reasons. One reason is a significant reduction in sperm motility followed by using MPH observed in treatment groups compared with the control groups (22, 23). Furthermore, decline in fertility rate because of the reduction of testosterone concentration in this and similar studies is indicative of the effects of this hormone on spermatogenesis. Similarly, a study on the effects of hydrochloride cocaine had similar negative effects on spermatogenesis and fertility. In this study after 100 days of treatment, the rats receiving daily cocaine had a pregnancy rate of only 33% versus 86% for the controls ($p<0.05$). In rats exposed to cocaine for 150 days the pregnancy rate was 50% compared with 100% for controls ($p<0.05$) (12).

Conclusion

The results of this study revealed that the long-term administration of MPH can affect fertility rates in male mice. Although this study has been performed on animals, it is not ethically possible to be done on human beings. MPH is being widely abused without prescription by the youth in recent years. As the result, restrict measures to limit its consumption needs to be taken.

Conflict of Interest

Authors declare no conflict of interest.

References

1. Solanto MV. Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. Behav Brain Res. 1998;94(1):127-52.
2. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague-Dawley rats. Toxicology. 2002;179(3):183-96.
3. Accardo P, Blondis TA. What's all the fuss about Ritalin? J Pediatr. 2001;138(1):6-9.
4. Goldman LS, Genel M, Bezman RJ, Slanetz PJ. Diagnosis and treatment of attention-deficit/ hyperactivity disorder in children and adolescents. Council on Scientific Affairs, American Medical Association. JAMA. 1998;279(14):1100-7.
5. Levin FR, Kleber HD. Attention-deficit hyperactivity disorder and substance abuse: relationships and implications for treatment. Harv Rev Psychiatry. 1995;2(5):246-58.
6. Chatterjee-Chakrabarty S, Miller BT, Collins TJ, Nagamani M. Adverse effects of methylphenidate on the reproductive axis of adolescent female rats. Fertil Steril. 2005;84(Suppl 2):1131-8.

7. Manjanatha MG, Shelton SD, Dobrovolsky VN, Shaddock JG, McGarrity LG, Doerge DR, et al. Pharmacokinetics, dose-range, and mutagenicity studies of methylphenidate hydrochloride in B6 C3F1 mice. Environ Mol Mutagen. 2008;49(8): 585-93.
8. Beckman DA, Schneider M, Youreneff M, Tse FL. Juvenile toxicity assessment of d,l-methylphenidate in rats. Birth Defects Res B Dev Reprod Toxicol. 2008;83(1):48-67.
9. Markowitz JS, DeVane CL, Pestreich LK, Patrick KS, Muniz R. A comprehensive in vitro screening of d-, l-, and dl-threo-methylphenidate: an exploratory study. J Child Adolesc Psychopharmacol. 2006;16 (6):687-98.
10. Jones JR, Caul WF, Hill JO. The effects of amphetamine on body weight and energy expenditure. Physiol Behav. 1992;51(3):607-11.
11. Tsai SC, Chiao YC, Lu CC, Doong ML, Chen YH, Shih HC, et al. Inhibition by amphetamine of testosterone secretion through a mechanism involving an increase of cyclic AMP production in rat testes. Br J Pharmacol. 1996;118(4):984-8.
12. George VK, Li H, Teloken C, Grignon DJ, Lawrence WD, Dhabuwala CB. Effects of long-term cocaine exposure on spermatogenesis and fertility in peripubertal male rats. J Urol. 1996;155(1):327-31.
13. Hess RA. Spermatogenesis, Overview. In: Knobil E, Neill JD, editors. Encyclopedia of Reproduction, Vol. 4. San Diego: Academic Press; 1998. p. 539-45.
14. Mohammad-Ghasemi F, Soleimanirad J, Ghanbari AA. A morphologic and morphometric study of adult mouse testis following different doses of busulfan administration. J Reprod Infertil. 2006; 7(1):25-36.
15. Oberländer G, Yeung CH, Cooper TG. Induction of reversible infertility in male rats by oral ornidazole and its effects on sperm motility and epididymal secretions. J Reprod Fertil. 1994;100 (2):551-9.
16. Barbaresi WJ, Katusic SK, Colligan RC, Pankratz VS, Weaver AL, Weber KJ, et al. How common is attention-deficit/hyperactivity disorder? Incidence in a population-based birth cohort in Rochester, Minn. Arch Pediatr Adolesc Med. 2002;156(3): 217-24.
17. Chapin R. Methylphenidate Hydrochloride. Environ Health Perspect. 1997;105(1):319-20.
18. Taghva M, Toutian Z, Fazelipour S. Effects of formaldehyde on morphometric structure of testis in Balb/C mice. Med Sci J Islam Azad Univ. 2007; 17(2):91-3.
19. Barroso-Moguel R, Méndez-Armenta M, Villeda-Hernández J. Testicular lesions by chronic administration of cocaine in rats. J Appl Toxicol. 1994; 14(1):37-41.
20. Adriani W, Leo D, Guarino M, Natoli A, Di Consiglio E, De Angelis G, et al. Short-term effects of adolescent methylphenidate exposure on brain striatal gene expression and sexual/endocrine parameters in male rats. Ann N Y Acad Sci. 2006; 1074:52-73.
21. Yang J, Zhang Y, Wang Y, Cui S. Toxic effects of zearalenone and alpha-zearalenol on the regulation of steroidogenesis and testosterone production in mouse Leydig cells. Toxicol In Vitro. 2007;21(4): 558-65.
22. Midzak AS, Chen H, Papadopoulos V, Zirkin BR. Leydig cell aging and the mechanisms of reduced testosterone synthesis. Mol Cell Endocrinol. 2009; 299(1):23-31.
23. Murono EP, Derk RC. The effects of the reported active metabolite of methoxychlor, 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane, on testosterone formation by cultured Leydig cells from young adult rats. Reprod Toxicol. 2004;19(1):135-46.
24. Teo SK, Stirling DI, Thomas SD, Hoberman AM, Christian MS, Khetani VD. The perinatal and postnatal toxicity of D-methylphenidate and D,L-methylphenidate in rats. Reprod Toxicol. 2002; 16(4):353-66.
25. Fazelipour S, Hadipour-Jahromy M, Tootian Z, Babaei L, Kiaei SB. Effects of nicotine on sperm motility in male mice under methylphenidate treatment. Med Sci J Islam Azad Univ. 2011;21(1):1-6.