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Short communication

Effect of metronidazole on spermatogenesis, plasma gonadotrophins and testosterone in rats

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Abstract

Metronidazole and its derivatives have both antiprotozoal and anti bacterial effects. The reproductive toxicity of metronidazole has been observed in some studies. The aim of this study was to determine the detrimental effects of metronidazole on spermatogenesis and testicular androgenesis in male adult rats. Eighteen male Wistar rats (70-90 days old) were randomly divided into three groups. Animals in group I (Control group) were administered with the water only. Animals in groups II and III were administered with metronidazol at the doses of 200 or 400 mg/kg/day for 60 days. Quantitative analysis of spermatogenesis was carried out by counting the relative number of each variety of germ-cells at the stage VII of the seminiferous epithelium cycle, *i.e.* type-A spermatogonia (ASg), pre-leptotene spermatocytes (pLSc), and step 7 spermatids (7Sd). Plasma follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were measured by radioimmunoassay (RIA). In groups II and III, there was a significant decrease in the testes, accessory sex organ weights, plasma concentrations of LH, FSH and testosterone with massive degeneration of all the germ cells at stage VII. Our data concluded that metronidazole has a suppressive influence on spermatogenesis and sex hormones in rats.

Keywords: *Metronidazole, Spermatogenesis, Gonadotrophins, Testosterone.*

Introduction

Metronidazol is used clinically to treat the genital tract infections in both men and women.

The anti-spermatogenic effect of metronidazole has also been shown in some studies. Organisms, such as flagellated protozoa, are more resistant to metronidazole and chemicals that kill these organisms might be toxic to flagellated sperm cell as well (1-2). Other derivatives of metronidazole as well as ornidazole exert a rapid and reversible anti-fertility effect in male rats (3-5). In dogs, humans and rats, one of the metabolites of ornidazole is the C_3-chloro side – chain of the nitroimidazole ring (6-7), which can produce 3-chloro-lactaldehyde and α-chloro-hydrin, the known inhibitors of the glycolytic enzymes such as;glyceraldehydes-3-Phosphate dehydrogenase (GAPDH) and triose-phospahte isomerase (TPI) in the spermatozoa (8-9). This is in accordance with the results of a study, which reported a 32% inhibition of GAPDH and a 52% inhibition of TPI activities in male rat spermatozoa after the administration of 400mg/kg/day ornidazole for 10 days (10).Therefore, the infertility action of ornidazole appears to be a result of its effect on the ability of spermatozoa to obtain ATP by the glycolytic pathway (11). Spermatogenic cells could be damaged by the increased inhibition of α–glycosidase malondialdeyde (MDA), while the sperm motility could be decreased by the inhibition of energetic transferase or non-protein substance in the epididymis (12). This study was conducted to examine the effect of metronidazole on the

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spermatogenesis, plasma gonadotrophins and testosterone levels in male rats.

Materials and methods

Animals and treatment

Adult male Wister rats, weighing 200±10g (70-90 days old), were maintained in 12 h light and 12 h dark conditions at a temperature of 21 ºC ± 1 ºC in an animal house. The standard laboratory chew and tap water were available ad libitium. The relative humidity of room was 50±5%. Metronidazole was purchased from Sobhan LDT, Iran and dissolved in sterile water. Eighteen rats were divided into 3 groups of 6 animals each. Two groups of animals were treated with 200 or 400mg/kg/day metronidazole for 60 days (Group II and III, respectively). Animals of group I were administrated with the water without metronidazole for 60 days and served as the controls. On the 61st day, between 08:00 to 10:00, the blood samples were collected from the hepatic vein under light ether anesthesia and then, the rats were killed following ethical procedure. Heparinized plasma was prepared and stored at -20 ºC until hormone RIA.

Body and organ weights

The body weight (initial) was recorded on the first day before starting the treatment and on the day of sacrifice (final). The testicles and accessory sex organs (ventral prostate and seminal vesicle) were separated through dissection after trimming off the attached tissues and weighed. The relative weight of the organs was expressed per 100g body weight. The testes of each rat were used for histological study.

Morphometrical study

After the removal of testis, it was immediately fixed in Bouin's fluid and embedded in paraffin. Sections of 5 µm thickness were taken from the middle portion of each testis, stained with hematoxylin and eosine (H-E) and examined under a light microscope. Quantitative analysis of the spermatogenesis was carried out by counting the relative number of each variety of germ-cells at stage-VII of the seminiferous epithelium cycle, *i.e.* type-A spermatogonia (ASg), pre-leptotene spermatocytes (pLSc) and step 7 spermatids (7Sd), according to the method of Leblond and Clermont (13). Stage-VII spermatogenesis was analyzed because this stage is highly susceptible to testosterone deficiency (14) and also reflects the final stages of spermatid maturation and thus, provides an evidence of spermatogenesis as a whole (15).

Hormonal assay

Plasma FSH and LH were measured by RIA, as described in the instructions provided with the kits (RADIM Ltd; Zanjan, Iran).

Statistical Analyses

Data were expressed as mean ± SD and the significance of the difference was analyzed by the student's *t*-test. The values were considered significant at $p < 0.05$.

Results

Body and organ weights

Metronidazole treatment had no effect on the survival and behavior of the animals observed. In groups II and III, the body weight was not significantly different from that of the controls. The relative weights of testis, seminal vesicle and ventral prostate decreased significantly ($p < 0.001$) after the treatment of metronidazole (200 or 400 mg) (Table I).

Table I. Effect of metronidazole on body weight (g) and organ weights (mg % body weight) in rats.

Group	Body Weight	Testis(Pair)	Seminal Vesicle	Ventral Prostate
Control (I)	210.1± 1.26	1581.12±12.01	521.75 ± 3.71	286.25± 7.33
200mg/kg (II)	204.25±1.61	1452.87±9.51*	412.75±2.11*	193.01±4.84*
400mg/kg (III)	202.87±1.41	1423.25±4.52*	407.51±2.19*	176.75±1.91*

(Mean ± SD; n=6). *$p < 0.001$, compared with control, Student's *t*-test.

Plasma hormonal levels

Plasma levels of FSH and LH were significantly decreased in treatment groups compared to the controls ($p < 0.001$). The changes were more prominent in the third group, which received 400 mg/kg metronidazole. Plasma level of testosterone was also showed a significant decrease in the treatment groups compared to the control ($p < 0.001$) (Table II).

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Table II. Effect of metronidazole on the plasma level of FSH, LH and testosterone in rats.

Group	FSH (mIU/ml)	LH (mIU/ml)	Tsstosterone (ng/ml)
Control (I)	12.07 ± 1.41	9.87 ± 3.38	6.12 ± 2.52
200mg/kg (II)	7.81 ± 1.68 *	6.93 ± 1.94*	3.51 ± 1.63*
400mg/kg (III)	6.32 ± 1.81*	5.43 ± 1.71*	2.62 ± 2.41 *

(Mean ± SD; n=6).*p <0.001, compared with control.

Morphometrical findings

Metronidazole treatment significantly reduced the number of PLSc and spermatids in the treatment group compared with the control (table III).

Table III: Effect of metronidazole on the number of germ cells per tubular cross section at stage-VII of seminiferous tubules cycle in rats (mean ± SD; n = 6). ASg = spermatogonia A, PLSc=Preleptotene spermatocytes and Sd = Step 7 spermatid.

Group	ASg	PLSc	7Sd
Control (I)	1.83 ± 0.04	20.75 ± 5.48	84.62 ± 2.94
200mg/kg (II)	1.71 ± 0.05	15.75 ± 2.29 *	73.68 ± 2.79*
400mg/kg (III)	1.85 ± 0.02	11.18 ± 1.42 *	63.51 ± 3.01*

(Mean ± SD; n=6). *p < 0.001, compared with control.

Discussion

The mutagenic and toxic potentials of the drugs or environmental chemicals on the male germ cells have become an important area of environmental concern (16). Metronidazole, a 5-nitroimidazole drug has been reported to decrease testicular weight, testicular and epididymal spermatid counts and to cause abnormal sperm morphology with degeneration of seminiferous tubules within 6 weeks of administration at metronidazole 400 mg/kg (2). The use of metronidazole is increasing, however its carcinogenecity has not been discarded (17). Our results demonstrate that the daily treatment of 200 and 400 mg/kg/day metronidazole, for 60 consecutive days, significantly decreased the weight of the testes and accessory sexual organs, (prostates and seminal vesicles). Previous studies have shown that a single oral dose of metronidazole 250 mg/kg drastically reduces the testicular weight and causes infertility in rats after 2–3 weeks, lasting for 3–4 weeks (18). High doses of metronidazole produced infertility in male rats (1). In our study, the effect of metronidazole administration resulted in a persistent decrease in testes weight and testosterone level in rats killed after 2 months of administration. The decrease in weight of testes and accessory sexual organs may be attributed to the decreased testosterone levels at all periods of the experiment in this study. In addition, intra-peritoneal administration of metronidazole (400 mg/kg/day), for 30 days, reduced the hormone levels of testosterone, FSH and LH in rats (2). In the present experiment, metronidazole caused a significant decrease in the gonadotrophins and testestrone levels after 2 months of the administration. Moreover, Joshie *et al* (1977) found that a single dose of 700 mg/kg of 2 thiazolyl-5-nitroimidazole resulted infertility in mice after 3 weeks of administration, with a return of fertility by week 7 (19). The reduction in testosterone and gonadotrophins might be due to metronidazole, which reaches the blood-testis barrier and gains access to the germ cells in the seminiferous tubules. Dixon and Lee (1977) reported that the blood testis barrier was possibly an important aspect when considering reproductive and mutagenic effects of drugs and environmental chemicals (20). The permeability characteristics of the blood-testis barrier are generally similar to those, which limit the membrane penhetration in the central nervous system (21). Metronidazole is distributed to all the tissues including the blood-brain barrier and seminal fluid (22, 23). The results of these studies and our experiment might explain the direct hazardous effects of metronidazole on the germ and Leydig cells, *i.e.,* a decreased testosterone secretion after penetration of into the blood-testis barrier.

Conclusion

The results of this study indicate that 1) metronidazole administration (200 or 400 mg/kg), for 60 days, caused a harmful effect one the fertility in male rats. 2) It appears that the primary site of metronidazole action may be on the brain or pituitary gland, however; direct action of drug on the germ cells can not be ruled out and further studies are required to clarify these points.

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