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Original Article

Effect of Metronidazole on Spermatogenesis, Plasma Gonadotrophins and Testosterone in Male Rats

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Abstract

Metronidazole and it's derivatives are drugs that have both antiprotozoal and anti bacterial effect.The reproductive toxicity of metronidazole has been shown in some studies. To investigate the effect of metronidazole on spermatogenesis in adult male rats, this study was designed.

Eighteen wistar male rats (70-90days old) were randomly divided into three groups. Animals in group I (Control group) were administered the water. Animals in groups II, III were administered metronidazole at doses of 200 and 400 mg/kg/day for 60 days. Different varieties of germ cells at stage VII seminiferous epithelium cycle, namely, type A spermatogonia (ASg), preleptotene spermatocytes (pLSc) and step 7 spermatids (7Sd) were quantitatively evaluated, along with radioimmunoassay of plasma follicle-stimulating hormone (FSH), luteiniging hormone (LH), testosterone assessment.

In the 200 and 400 mg/kg groups, there were significant decreases in the testes and accessory sex organ weights, plasma concentrations of LH, FSH and testosterone with massive degeneration of all the germ cells at stage VII.

It is concluded that metronidazole has a suppressive influence on spermatogenesis and sex hormones in rats.

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

Introduction

Metronidazole is used clinically to treat genital tract infection in both men and women. The antispermatogenic effect of metronidazole has also been shown in some studies (1-2). Other derivatives of metronidazole as well as ornidazole exert a rapid and reversible antifertility 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 may produce 3-chlorolactaldehyde and α-chlorohydrin known inhibitors of the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and triosephospahte isomerase (TSI) in spermatozoa (8-9). This is in accordance with the results of a study that reported a 32% inhibition of GAPDH and a 52% inhibition of TPI activities in male rat spermatozoa after administration of 400 mg/kg/day ornidazole for 10 days (10). Therefore, the infertility action of ornidazole appears to be result of its effect on the ability of spermatozoa to ultimately obtain ATP by the glycolytic pathway (11).

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Spermatogenesis cells could be damaged by the increase of inhibiting alpha–glycolsidase malondialdeyde (MDA), while sperm motility could be decreased by inhibiting energetic transferase or non protein substance in the epididymis (12). This study was conducted to examine the effect of metronidazole on spermatogenesis, plasma gonadotrophins and testosterone in male rats.

Experimental

Animals and treatment

Adult male wistar rats weighing 180±10g (70-90 days of age) were maintained in 12 h light and 12 h dark animal house at a Temperator of 21 C° ± 1 °C and standard laboratory chew and tap water were available a*d libitum*. The relative huminity of room was 50 ± 5%. Metronidazole was purchased from Sobhan LDT and dissolved in sterile water. Eighteen rats were divided into 3 groups of 6 animals each. Two groups of animals groupsII and III were treated with either 200 or 400mg/kg/day metronidazole for 60 days. Animals of groupI were administered water without metronidazole for 60 days and served as the controls. On the 61st day between 08:00 to 10:00, blood samples were collected from the hepatic vein under light ether anesthesia and after that the rats were killed following ethical procedure. Heparinized plasma was prepared and stored at -20 °C until hormone radioimmuno-assay.

Body and organ weights

The body weight was recorded on the first day before treatment (initial) and the day of sacrifice (final). The testicles and accessory sex organs (venteral prostate and seminal vesicle) were dissected out, trimmed off the attached tissues and weighed. The relative weight of organs was expressed per 100g body weight. The testis of each rat was used for histological study.

Histological study

After their removal, the testes were immediately fixed in Bouin's fluid and embedded in paraffin. Sections of 5 µm thickness were taken from the mid portion of each testis and stained with hematoxylin and eosine (H-E) and then examined under a light microscope. Quantitative analysis of 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), preleptotene 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 evidence of spermatogenesis as a whole (15).

Hormone assay

Plasma follicle-stimulating hormone (FSH) and luteiniging hormone (LH) were measured by radioimmunoassay (RIA) as described in the instructions provided with the kits (RADIM LTD) from the Ahmadi Atomic Research Centre, Zajan, Iran.

Statistical Analysis

Data were expressed as mean±SD and the significance of difference was analyzed by the student T- test. Values were considered significant at $P<0.05$

Results and Discussion

Body and organ weights

Treatment with metronidazole has no effect on the survival and behaviour 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 were significantlly decreased ($P<0.001$) after administration of 200mg or 400mg doses (Table 1).

Plasma hormonal levels

Plasma levels of FSH and LH were significantly decreased in treatment groups compared to controls ($P<0.001$). The changes were more prominent in the third group which received 400 mg/kg metronidazole. Also plasma level of testosterone was significantly decreased in the treatment groups compared to the control group ($P<0.001$) (Table 2).

Table 1. 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 T-test.

3.3 Histological findings

Metronidazole treatment significantly reduced the number of PLSc and spermatids in the treatment group compared with the control group (Table3).

The mutagenic and toxic potentials of drugs or environmental chemicals on 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 of metronidazole at 400 mg/kg dose (2). The use of metronidazole is increasing. However, its carcinogencity has not been discarded (17).Our results demonstrate that daily treatment with metronidazole at 200 and 400 mg/kg/day doses for 60 consecutive days significantly decreases the weight of the testes and accessory sexual organs, (prostates and seminal vesicles) . Previous studies have shown that a single 250 mg/kg oral dose of metronidazole drastically reduces testicular weight and results in infertility in rats after 2-3 weeks, lasting for 3-4 weeks (18). High doses of metronidazole produce infertility in male rats (1). In our study the effect of metronidazole administration resulted in persistent decrease in testes weight and testosterone level in rats killed after 2 months. The decrease in weight of testes, accessory sexual organs in this study may be attributed to the decrease in testosterone levels at all stages of the experiment. In addition, intraperitoneal administration of metronidazole at 400 mg/kg/day dose 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 from the start of administration. Moreover, Joshie (19) found that a single dose of 700 mg/kg b.wt. of 2 thiazolyl-5-nitroimidazole resulted in infertility in mice after 3 weeks, with a return of fertility by week 7. 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 (20) reported that the blood testis barrier was possibly an important aspect when considering reproductive and mutagenic effects of drugs and environmental chemicals. The permeability characteristics of the blood testis barrier, are generally similar to those that limit penetration of membranes of the central nervous system (21). Metronidazole is distributed to all tissues including the blood brain barrier and seminal fluid (22, 23). The results of these studies and and does of our experiment might explain the direct hazard effects of metronidazole on germ cells and Leydig cells, i.e. decreased

Table 2. Effect of metronidazole on plasma level of FSH,LH and testosterone in rats.

Group	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (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, Student T-test.

Table 3. Effect of metronidazole on 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: &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, Student T-test.

testosterone secretion after penetration of the blood testis barrier by metronidazole.

Conclusion

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

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