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Abnormal secretion of reproductive hormones and antioxidant status involved in quinestrol-induced reproductive toxicity in adult male rat

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ABSTRACT

This study aimed to evaluate the effects of quinestrol, a synthetic oestrogen homologue with reproductive toxicity, on the secretion of reproductive hormones and antioxidant status in adult male rat. Our results showed that quinestrol exposure significantly decreased the weight of the testis, epididymides, seminal vesicle, and prostate, as well as the sperm counts in the cauda epididymis of rats. Quinestrol significantly reduced the size of seminiferous tubules and the total number of spermatogenic cells. Serum testosterone, follitropin, and lutropin were also significantly reduced in a dose-related manner after quinestrol exposure. Meanwhile, the activity of superoxide dismutase, glutathione peroxidase, and total antioxide capacity significantly decreased, whereas the malondialdehyde and nitric oxide concentrations significantly increased in the testes. These findings revealed that endocrine disorders of reproductive hormones and oxidative stress may be involved in reproductive toxicity induced by quinestrol in adult male rats.

1. Introduction

Numerous xenoestrogens reportedly induce male reproductive tract abnormalities, including testicular hypoplasia, tumours of the rete testis, and erectile dysfunction (McLachlan, 2001; Oliva et al., 2002). These abnormalities are induced by promoting germ cells apoptosis (Li et al., 2010), generating oxidative stress (Gong and Han, 2006), and decreasing plasma testosterone levels (Ma et al., 2008). Any factor disturbing the hypothalamo–pituitary–testicular axis may result in male gonadal dysfunction because sexual differentiation and development, as well as fertility, are under strict endocrine regulation by this axis (Dhooge et al., 2007). Many xenoestrogens such as diethylstilbestrol (Goyal et al., 2001), estradiol-17β (Jin et al., 2005), and oestrogen analogue phytoestrogens (Opalka et al., 2006) reportedly inhibit testosterone secretion in various rodents, birds, and mammals, thereby affecting their fertility. Studies have shown that xenoestrogen exposure induces oxidative stress by enhancing the generation of reactive oxygen species (ROS) (Okai et al., 2004) and depressing the activity of antioxidant enzymes (Chitra et al., 2002; Chitra and Mather, 2004) in blood and/or reproductive organs, thereby causing reproductive toxicity.

Quinestrol, a fat-soluble synthetic oestrogen homologue used as a fertility control chemical (Dipasquale et al., 1974), affects male fertility by altering the structure of reproductive organs (Zhao et al., 2007). Quinestrol also impedes follicle development and ovulation by inhibiting the release of hypothalamus GnRH and disturbing the hypothalamus–pituitary–ovary axis (Nudemberg et al., 1973; Zhao et al., 2007). Quinestrol is currently used at different doses as the major component of long-term oral contraceptives for women and sterilants in rodents (Singh et al., 1971; Dipasquale et al., 1974; Zhao et al., 2007; Lv et al., 2012). However, only a few studies have reported on quinestrol-induced changes in the reproductive system of male rat following adult exposure. The present study aimed to elucidate the influences of quinestrol on fertility by evaluating its effect on the weight of the reproductive organs, sperm characteristics, reproductive hormones, and antioxidant status of male rat. To the best of our knowledge, this study is the first to report on the toxicity of quinestrol to the reproductive hormones and antioxidant status of adult male rat.

2. Materials and methods

2.1. Animals

Eight-week-old adult male Sprague–Dawley rats (weighing 300 ± 10 g each) obtained from the Experimental Animal Centre of Henan Province (Zhengzhou, Henan, China) were used in this study. The rats were housed in polypropylene cages with cellulose

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fibre chip bedding at ambient temperature (25 °C), 30–40% humidity, and 14 h light:10 h dark cycle. All animals had ad libitum access to rodent feed and water in glass bottles with rubber stoppers.

2.2. Treatment with quinestrol

The animals were habituated by handling regularly for 1 week and then randomly parsed into five treatment groups ($n = 5$ animals per group). The rats were treated intraperitoneally (the peritoneum is highly absorptive because its area is large and has numerous blood and lymph vessels) with quinestrol in olive oil vehicle (0.2 ml) at dose levels of 0 (control group), 0.1, 1, and 10 mg/kg body weight (experimental groups) for 7 days (Ma et al., 2008). The doses used were determined based on our previous studies and those of others (Zhao et al., 2007; Ma et al., 2008; Lv et al., 2012). All procedures were conducted according to Guidelines for Animal Experiments and were approved by the Animal Care and Use Committee of China Agricultural University.

2.3. Tissue harvesting and processing

At the end of the experiment, blood samples were collected by orbital venous puncture following ether anaesthesia (20 mg/kg BW) administration before autopsy. Serum obtained from the blood samples were processed for biochemical assay of the reproductive hormones. The testes, epididymis, seminal vesicle, and prostate were then immediately removed. Their weights were recorded, and the relative weights [weight of reproductive organ (g)/body weight (g) × 1000] were calculated. The testes were processed for biochemical assay of antioxidant enzymes and histological examination. The epididymides and prostate were also processed for histological examination.

The caudal epididymides of each rat was suspended in 5 ml of 0.9% sodium chloride for 30 min to allow the sperm to immediately leave the epididymis canal after mincing. Sperm quality was then examined (Singh and Chakravarty, 2003). Spermatozoa morphology was assessed by smearing the sperm suspension and eosin staining. The number of morphologically abnormal sperm was recorded as previously described (Wyrobek and Bruce, 1975). Acrosomal integrity was evaluated by Giemsa staining as previously reported (Watson, 1975). The number of spermatozoa was counted using a hemocytometer and expressed as million per millilitre of suspension. Progressive sperm motility was evaluated under a light microscope at 400× magnification by placing an aliquot of sperm on a slide (Sönmez et al., 2005).

2.4. Assessment of antioxidant status and lipid peroxidation

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), nitric oxide (NO) content, malondialdehyde (MDA) level in the testes were assayed with commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems China Company Limited). The sensitivities by these assays were 0.1 μmol/L, 0.1 U/mL, 0.1 nmol/mL, 1.0 U/mL, and 1.0 U/L, respectively. The intra- and inter-assay coefficients of variation were <15%.

2.5. Examination of hormone concentrations

The plasma testosterone, lutropin (LH), and follitropin (FSH) concentrations in the serum were measured with commercial T, LH, and FSH ELISA kits (R&D), respectively. The sensitivities by these assays were 1.0 pg/ml, 1.0 mIU/ml, and 0.1 IU/L respectively. The intra- and inter-assay coefficients of variation were <15%.

Table 1
Body weight (BW) and paired absolute and relative weights of reproductive organs in quinestrol treated adult rats.

Quinestrol (mg/kg BW)	BW (g)	Testis weight (g)		Epididymis weight (g)		Seminal vesicle weight (g)		Prostate weight (g)	
		Absolute (g)	Relative × 10^3 (g/g b.w.)	Absolute (g)	Relative × 10^3 (g/g b.w.)	Absolute (g)	Relative × 10^3 (g/g b.w.)	Absolute (g)	Relative × 10^3 (g/g b.w.)
10	305.73 ± 16.22	2.69 ± 0.39*	8.83 ± 1.46*	0.63 ± 0.16**	2.07 ± 0.53**	0.62 ± 0.12**	2.01 ± 0.32**	0.43 ± 0.06**	1.39 ± 0.16**
1	303.51 ± 17.04	2.95 ± 0.29	9.70 ± 0.65	0.60 ± 0.12**	1.98 ± 0.31**	0.69 ± 0.07**	2.28 ± 0.24**	0.45 ± 0.07**	1.50 ± 0.23**
0.1	319.54 ± 22.04	3.19 ± 0.31	10.03 ± 1.34	1.17 ± 0.14	3.66 ± 0.29	1.16 ± 0.17	3.65 ± 0.72	0.81 ± 0.11	2.54 ± 0.21
Control	305.08 ± 12.87	3.21 ± 0.30	10.50 ± 0.64	1.24 ± 0.15	4.07 ± 0.64	1.28 ± 0.16	4.21 ± 0.70	0.86 ± 0.08	2.82 ± 0.32

Data are expressed as mean ± SD (minimum–maximum). The relative weights was calculated as weights of the reproductive organs (g)/body weight (g) × 1000.
* $P < 0.05$ as compared with control.
** $P < 0.01$ as compared with control.