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Responses in reproductive organs, steroid hormones and CYP450 enzymes in female Mongolian gerbil (*Meriones unguiculatus*) over time after quinestrol treatment

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ABSTRACT

The aim of this study was to assess the effects and reversibility of the synthetic estrogen compound, quinestrol, on the reproductive organs, steroid hormones, and drug-metabolizing enzymes CYP3A4 and CYP1A2 in liver and kidney over time after two quinestrol treatments in female Mongolian gerbils (*Meriones unguiculatus*). Female gerbils were treated with 4 mg/kg quinestrol (9 gerbils/group, 3 treated group) (1 control group, 0 mg/kg) for 3 days and treated again after 25 days. Animals were killed for collection of samples at 5, 10 and 15 days after the second treatment ending. Two interval quinestrol treatments significantly increased uterine weight, with trend of increase over time, but no change could be detected in ovarian weights. Quinestrol treatment increased progesterone and estradiol levels, both with trend of decline over time. Quinestrol increased liver and kidney weights and total enzyme content of CYP3A4 and CYP1A2, with trend of decline over time. On the basis of reversible changes of detoxification enzymes or organs, interval quinestrol treatment effectively and reversibly influenced the reproductive hormone and organ to some extent.

1. Introduction

Fertility control, a second-generation pest management strategy [1], aims at reducing birth rates and overcoming the problems associated with traditional culling, such as environmental contamination, and risks to non-target species [2]. There are three main strategies of fertility control: surgical/chemical sterilization, endocrine perturbation, and immune-contraception [2,3]. Endocrine disrupting drugs have been successfully tested in both sexes of several pest rodent species in China [4]. As we all know, the inhibitory effects and reversibility of drugs are related to metabolic enzymes and detoxicating organs. However, the inhibitory effect and reversibility of two interval quinestrol treatment on rodent fertility control is still inadequate.

Quinestrol (17-alpha ethinylestradiol-3-cyclopentyl ether) is a synthetic estrogen. The cyclopentyl ethers of quinestrol has prolonged the activity, thus, exerts strong anti-fertility effects [5–7]. To date, quinestrol has been assessed as a contraceptive in rodent control in China [8–12]. For example, quinestrol increased estradiol and progesterone levels in serum, and uterine weight [13], but decreased ovarian weights in female gerbils. However, we knew little about the effects and reversibility of quinestrol treated by interval time on the reproductive organs, sex hormone during different periods in female gerbils.

Most of the peripheral catabolism occurs in the liver and to some extent in the kidneys, which are the major sites of hormone inactivation and elimination, or catabolism. Catabolism of estrogens is mediated by Cytochromes P450 (CYP450) enzymes [14,15]. For instance, the principal CYP isoforms involved in estradiol 2- or 4-hydroxylation catabolism are CYP3A4 and CYP1A2 in hamster [16] and humans [17,18]. The kidney was also responsible for the catabolism, and excretion of most drugs and xenobiotics [17–19]. The catabolism of estradiol and ethinyloestradiol was predominantly carried by CYP3A4 and CYP1A2 [20]. In addition, CYP3A4 and CYP1A2 were involved in large amount of metabolism of xenobiotics [14,15]. Many studies had focused on the enzyme activity which caused by xenobiotic compounds. However, the change of enzyme content was also very important to help understanding the action mechanism caused by xenobiotic compounds, although was always ignored in most existing studies. So we choose CYP3A4 and CYP1A2 in liver and kidney, to clarify the reaction of average or total enzyme content of both after quinestrol treatment, as the effects and its reversibility of quinestrol are directly related to the

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metabolic enzymes. Furthermore, the involvement of P450 isoforms from CYP3A4 and CYP1A2 in the catabolism of quinestrol has not been established clearly.

Mongolian gerbil (*Meriones unguiculatus*) have high reproductive rate and caused considerable damages to grasslands and the livestock in northern China [21]. Traditional lethal control is used to manage the population of gerbils, but leaving adverse effects, so new control ways are eagerly needed. Fertility control, a new means as candidate, can contribute significantly. Reports had showed that EP-1 suppressed birth rates of gerbil populations, reduced their densities, and changed their age structures [22]. In addition, doses of 0.1 to 2.7 μg/g quinestrol over 6 days completely inhibited fertility of female gerbils [23]. However, the investigations of duration of effect, reversibility and the mechanism were still deficient. So we tested the effects of quinestrol on uterine and ovarian weights, progesterone and estradiol levels in serum, average and total enzyme content of CYP3A4 and CYP1A2 in liver and kidney in female gerbils at days 5, 10 and 15 after two interval quinestrol treatment.

2. Materials and methods

2.1. Animals

Female gerbils (virgins, 3–4 months old, 60.6 ± 1.0 g) were obtained from the laboratory population maintained at the Institute of Zoology, Chinese Academy of Sciences. Animals were maintained in an air conditioned room (temperature: 23 ± 1 °C, light period: 04:00 AM to 19:00 PM) individually in plastic cages (30 cm × 20 cm × 16 cm). Food (commercial rabbit pellets, Beijing HFK Bioscience Co., Ltd) and water were supplied *ad libitum*. The experiment was carried out from March to May in 2013. All animal procedures were conducted according to the Guidelines for Animal Experiments and approved by the Animal Care and Use Committee at the Institute of Zoology, Chinese Academy of Sciences.

2.2. Experiments

Quinestrol (purity 99.9%, produced by Beijing Zizhu Medicine Co., Ltd., Beijing, China) at dose of 4 mg/kg was dissolved in peanut oil. The 36 gerbils were randomly divided into four groups (9 gerbils/group). The treated females were given with quinestrol oil solution by oral gavage for 3 days. A second round of 3 days' treatment was given again after 25 days. Females in control received 0.8 ml peanut oil by the same processing procedure. To mimic interval feeding poison bait in field, the female gerbils were treated with quinestrol two times. On the basis of Zhang et al. [24], who had showed that two interval baiting of EP-1 (the compound of quinestrol and levonorgestrel) exerted better inhibitory effects than the continuous administrations in controlling fertility of female Brandt's voles (*Lasiopodomys brandtii*), gray hamsters (*Cricetulus migratorius*) and mid-day gerbils (*Meriones meridianus* Pallas). In addition, the gestation period was 24–26 days for female gerbils, so we set up 25 days as the interval time which covered one gestation period.

Gerbils were decapitated following carbon dioxide asphyxia and blood sample was collected at days 5, 10, 15 after the second quinestrol treatment. The control females were killed at days 10 after two interval 3 days' oil treatment. Serum was separated by centrifugation at 4000*g* for 15 min at 4 °C and stored at − 20 °C until assayed. Progesterone and estradiol levels in serum were determined using radioimmunoassay (Beijing Northern Biological Technology Institute). The intra- and inter-assay variability for progesterone and estradiol levels was < 7.6% and 4.3%, respectively.

Body mass (± 0.1 g), liver and kidney weights (± 0.01 g), uterine and ovarian weights (± 0.001 g) were recorded. Liver and kidney were immediately frozen in liquid nitrogen and then stored at − 80 °C for test the content of CYP3A4 and CYP1A2. The content of CYP3A4 and CYP1A2 were assayed with commercial enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Guyan Biotechnology Company). The intra- and inter-assay variability for CYP3A4 and CYP1A2 were < 7.5% and 9.6%, respectively. For the duration of the whole experiment, some of the gerbils died of natural causes. The number of animals were 9 (Control), 8 (Day 5), 9 (Day 10), 8 (Day15) in each group at last.

2.3. Statistics analysis

All data were analyzed by using SPSS 16.0. Normality and homogeneity were tested before analysis. The body mass, uterine and ovarian weights, liver and kidney weights, estradiol and progesterone levels, the average and total enzyme content of CYP3A4 and CYP1A2 were analyzed by One-way ANOVA with LSD for *post hoc* multiple comparison analysis in different groups. Results were presented as means ± SE, differences at the 5% level or lower were considered significant.

3. Results

3.1. Organ weights

The body mass of gerbils was not significantly affected by quinestrol treatment ($F_{3,30} = 0.452$, $P = 0.718$). Liver weight was significantly increased by quinestrol at day 5 compared to the control gerbils ($F_{3,30} = 4.264$, $P = 0.013$) (Fig. 1A). Quinestrol significantly increased kidney weights at days 5 and 10 in comparison with control ($F_{3,30} = 5.051$, $P = 0.006$) (Fig. 1B).

Quinestrol treatment significantly increased uterine weights by 147%, 180% and 355% at days 5, 10 and 15 compared with control ($F_{3,30} = 20.515$, $P < 0.001$) (Fig. 1C). The mass of uterus was related to edema of this organ. Quinestrol had no significant effect on the ovarian weight compared with control in female gerbils ($F_{3,30} = 0.555$, $P = 0.649$) (Fig. 1D).

3.2. Hormone concentration

Quinestrol significantly increased progesterone levels in serum at days 5, 10 and 15 compared with control by 1012%, 816%, 768% ($F_{3,29} = 4.886$, $P = 0.007$, Fig. 2A).

Quinestrol significantly increased estradiol levels in serum at days 5 and 10 only compared with control ($F_{3,30} = 7.392$, $P = 0.001$, Fig. 2B).

3.3. Enzyme levels

Quinestrol neither significantly affect the average enzyme content for CYP3A4 ($F_{3,30} = 1.159$, $P = 0.342$) and CYP1A2 ($F_{3,30} = 1.414$, $P = 0.258$) in the liver, nor the kidney for CYP3A4 ($F_{3,30} = 1.302$, $P = 0.292$) and CYP1A2 ($F_{3,30} = 2.552$, $P = 0.076$) (Table 1).

Quinestrol significantly increased the total enzyme content for CYP3A4 ($F_{3,30} = 3.426$, $P = 0.030$) and CYP1A2 ($F_{3,30} = 7.176$, $P = 0.001$) in liver compared with control at day 5, but both of them were not significant different from control at days 10 and 15 (Fig. 3A, B).

Quinestrol significantly increased the total enzyme content for CYP3A4 ($F_{3,30} = 3.563$, $P = 0.026$) and CYP1A2 ($F_{3,30} = 6.991$, $P = 0.001$) in kidney at days 5 and 10, but both of them were not significant different from control at day 15 (Fig. 3C, D).

4. Discussion

Quinestrol treatment induced an increase in the weights of the uterus, liver and kidney. Only weight of the uterus continued to increase further up to 15 days after the end of treatment. It meant that trend of increase was found in uterine weight, while trend of decline existed in liver and kidney weights during different periods. Quinestrol increased estradiol and progesterone levels, both of them exerted trend

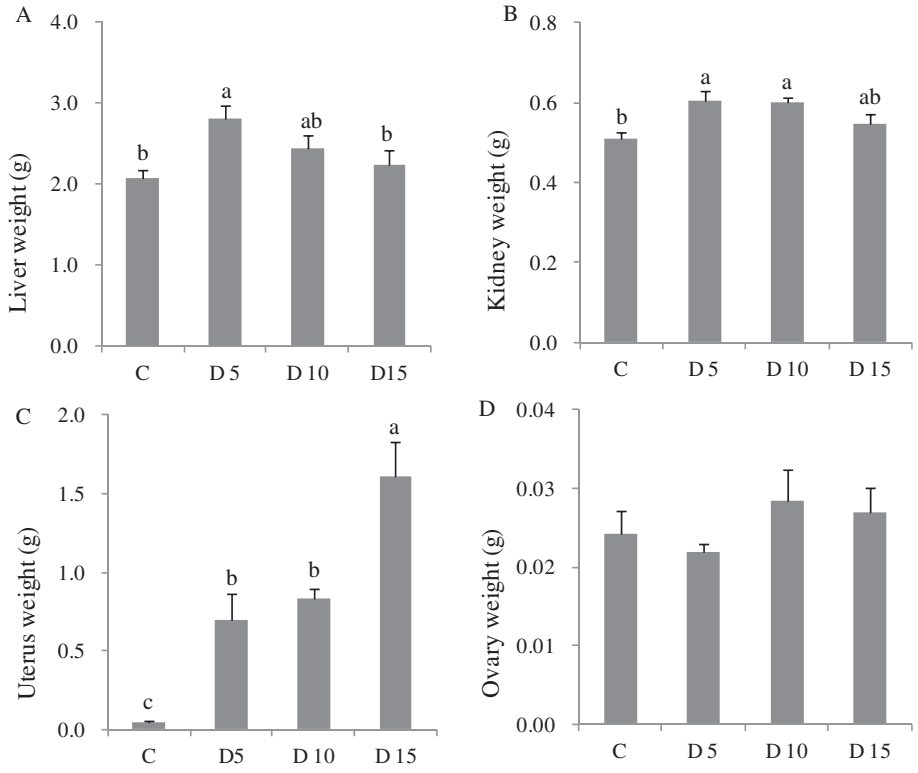

Fig. 1. Effects of quinestrol on the wet weights of liver (A), kidney (B), uterus (C) ovary (D) in female gerbils.
Note: One-way ANOVA was performed to compare the weight of liver, kidney, ovary and uterus during different periods and control. Results were presented as means ± SE. Different superscripts in each bar indicated significant differences ($P < 0.05$). Each group had 8 or 9 gerbils. D5, 10 and 15 in X-axis indicated the days when dissection was taken after the second quinestrol treatment. C was represented the Control group.

of decline during different periods, but the recovery rate was not synchronized. Quinestrol increased the total enzyme content of CYP3A4 and CYP1A2 in liver and kidney, trend of decline was found during different periods, both of them restored to control level at day 15.

Quinestrol treatment significantly increased the uterus weight at days 5, 10 and 15 in comparison with control, and it exerted continuous trend of increase, with the largest weight at day 15. Uterine edema was observed in quinestrol treated group regardless of the time. Estrogen induced a rapid increase in microvascular permeability in the rodent uterus, leading to stromal edema and a marked increase in uterine wet weight [23,25–26]. This edematous response to quinestrol has also been reported in Lv et al. [13], and most likely due to the prolonged estrogenic activity. Similarly, estradiol treatment resulted in cellular hypertrophy in uterus in female rats [26], thus, induced the dysfunctional environment of uterus. However, quinestrol had no significant effect on the ovarian weight. This was different from Lv et al. [13]. It may be related to the treatment time, and gerbils were given quinestrol for 6 days in Lv et al. [13], which indicated longer treatment period. Though ovarian weight was not markedly influenced, uterus edema was not conducive to sperm penetration, transport and implantation, and may cause breeding failure. This indicated that uterus may be sensitive to quinestrol treatment, high estradiol and progesterone levels would further contribute to the uterus edema.

Quinestrol significantly increased progesterone level at days 5, 10, 15, and estradiol level at days 5, 10 compared with control. Both of them exerted continuous trend of decline during different time. Yet, progesterone level did not return to control at day 15, estradiol was restored. High levels of active estrogen in blood would generate negative feedback on the hypothalamus-pituitary-ovary axis [27], thus, inhibited synthesis or secretion of FSH and LH. The lower levels of FSH and LH may down-regulate the expression of estrogen and progesterone receptors, furthermore, increase the concentrations of free estradiol and progesterone in blood. For example, quinestrol treatment decreased the FSH and LH levels in serum and pituitary, whereas increased the estradiol and progesterone levels in serum in female gerbils [13]. Further report showed that quinestrol up-regulated expression of estrogen and progesterone receptors in pituitary, but down-regulated expression of both in ovary in female gerbils [28]. However, the specific molecular mechanism needed further investigations. The reversibility of progesterone and estradiol was not synchronized. This asynchronized

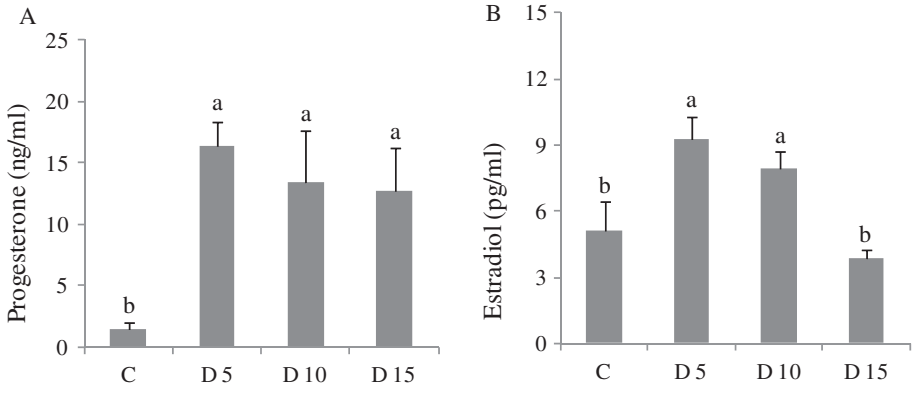

Fig. 2. Effects of quinestrol on progesterone (A) and estradiol levels (B) in serum in female gerbils.
Note: One-way ANOVA was performed to compare progesterone and estradiol levels during different periods. Results were presented as means ± SE. Different superscripts in each bar indicated significant differences ($P < 0.05$). Each group had 8 or 9 gerbils. D5, 10 and 15 in X-axis indicated the days when dissection was taken after the second quinestrol treatment. C was represented the Control group.

Table 1
Effects of quinestrol on the average enzyme content of CYP3A4 and CYP1A2 in liver and kidney.

Group	Content	Control	Day 5	Day 10	Day 15 (pmol/mg)
Liver	CYP3A4	1.873 ± 0.054	1.857 ± 0.057	1.946 ± 0.056	1.795 ± 0.058
	CYP1A2	0.350 ± 0.009	0.372 ± 0.007	0.364 ± 0.008	0.352 ± 0.008
Kidney	CYP3A4	1.238 ± 0.063	1.099 ± 0.159	1.359 ± 0.050	1.286 ± 0.073
	CYP1A2	0.226 ± 0.010	0.252 ± 0.010	0.253 ± 0.007	0.215 ± 0.010

Note: One-way ANOVA was used to analyze the average enzyme content of CYP3A4 and CYP1A2 in liver and kidney during different periods. Results were presented as means ± SE. Different superscripts in each bar indicated significant differences ($P < 0.05$). Each group had 8 or 9 gerbils. D5, 10 and 15 in X-axis indicated the days when dissection was taken after the second quinestrol treatment. C was represented the Control group.

phenomenon was worth of further research. High progesterone level regulated microvascular systolic property, thus improved endometrial blood flow and increased matrix edema and decidualization [23,25–26], which was coincident with the uterus edema.

Quinestrol increased the total enzyme content of CYP3A4 and CYP1A2 at 5 days in liver, and the total enzyme content of CYP3A4 and CYP1A2 at 5 and 10 days in kidney. The weights of liver and kidney were accordant with total enzyme content of CYP3A4 and CYP1A2. As we all know, the core structure of steroids hormone is the cyclopentanoperhydrophenanthrene, with four-ring hydrocarbon nucleus (steroid nucleus), which indicates that the catabolism of estradiol, ethinyloestradiol, estrone and quinestrol was similar to a large extent. The results were in support of reports which indicated that CYP1A2 and CYP3A4 catalyzed the main 2-hydroxylation pathway of ethinyloestradiol or estradiol at high substrate concentrations [15,16,19]. In addition, human hepatic CYP3A4 and CYP1A2 both exhibited the highest activities for the catabolism of estrone, while other isoforms showed less or no activity [29]. Western blots of renal microsomes revealed that 2-hydroxylation of estradiol catabolism were catalyzed by CYP1A2 and CYP3A families [16]. Though the structure of quinestrol was different from longbishu, they exerted similar metabolic processes. For instance, the protein expression levels of CYP3A4 and CYP1A2 were increased significantly in rat liver after intragastric administration of longbishu for two months [30]. It may indicate the plasticity of liver and kidney, by enlarging the weight of organs and furthermore increasing the total enzyme content, not by increasing the average enzyme content after quinestrol treatment, and all of these indexes showed reversible damages.

During recovery processes, weights of liver and kidney, total enzyme content of CYP3A4 and CYP1A2 all exerted trends of decline, under the catabolism and inactivation of quinestrol, all of them restored to control level at day 15. We did not test the activity of CYP3A4 and CYP1A2 in liver or kidney, so we know no changes of this after quinestrol treatment. In the future, investigations will be taken further in the activity and mRNA levels of enzymes, thus, helping us clearly understand the mechanism.

In summary, quinestrol was an effective compound for control fertility of female gerbils. The major metabolizing enzymes (CYP3A4 and CYP1A2) and metabolizing organs (liver and kidney) can be enhanced and enlarged after quinestrol treatment, and then restored to normal a few days later. However, quinestrol potently influenced the uterine weight and steroid hormone, and still did not return to control level at last. It showed that catabolism of two interval quinestrol treatment can be metabolized quickly and reversibly by metabolizing enzymes, but still inhibited the fertility of female gerbils. Low dose and toxicity of quinestrol, can be repeatedly put into practice. For the safety in the environment, the soil systems from Qinghai and Zhejiang province efficiently eliminated the estrogenic activity of quinestrol [11].

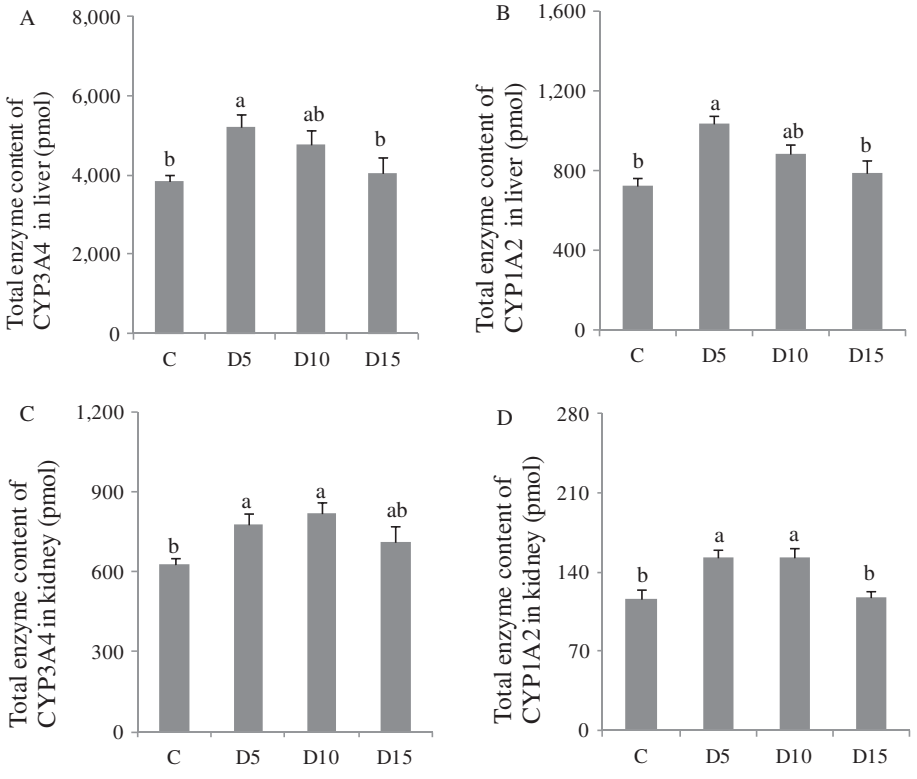

Fig. 3. Effects of quinestrol on the total enzyme content of CYP3A4 (A) and CYP1A2 (B) in liver, CYP3A4 (C) and CYP1A2 (D) in kidney in female gerbils.
Note: One-way ANOVA was performed to compare the total enzyme content of CYP3A4 and CYP1A2 in liver and kidney during different periods. Results were presented as means ± SE. Different superscripts in each bar indicated significant differences ($P < 0.05$). Each group had 8 or 9 gerbils. D5, 10 and 15 of dissection in X-axis indicated the days after the second quinestrol treatment. C was represented the Control group.

Quinestrol in aqueous solution was rapidly degraded when exposed to UV irradiation than solar irradiation, no matter for tap water in laboratory obtained in Beijing, or river water collected from Baiwang in the suburb of Beijing [31]. Other report showed that quinestrol quickly decomposed and was less of a threat to native birds in their natural environment on Tibetan Plateau [32]. In addition, many reports showed that quinestrol was also an effective agent to control male fertility [4,9–10,33–34]. That is to say, quinestrol is likely a desirable alternative to lethal management of rodent pests no matter they were males or females.

Conflict of interest

The authors declare that they have no conflicts of interest.

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