Changes of testicular ultrastructure of rat after clenbuterol exposure

Jing LI1, Wei-jie ZHU2
1. Department of Pathophysiology, Medical College, Jinan University, Guangzhou 510632, China
2. Department of Developmental and Regenerative Biology, College of Life Science and Technology, Jinan University, Guangzhou 510632, China

Objective To investigate effects of clenbuterol (CLB) on testicular ultrastructure of rat.
Methods Twenty adult male Sprague-Dawley rats were randomly divided into four groups (5 rats per group). CLB solved in normal saline solution was given at the dose of 0 mg/kg body weight (bw) (group A, as control), 0.4 mg/kg bw (group B), 2.0 mg/kg bw (group C), and 18.5 mg/kg bw (group D) for 14 d by gavage consecutively, respectively. Transmission electron microscopy was used to observe changes on testicular ultrastructure.
Results In group B, some small vacuoles were found in Sertoli cells. In groups C and D, vacuoles were common in Sertoli cells and spermatogonia. The phenomenon of vacuolation in group D was more severe than that in group C. In group D, basal membrane showed some irregular and wrinkled changes, Leydig cells had more vacuoles and increased lipid droplets.
Conclusion Testicular ultrastructure of rat had pathological changes after CLB exposure, and the alterations became more severe with the increasing doses.

Key words: clenbuterol (CLB); ultrastructure; Sertoli cell; testis; rat

Over the past decade, there has been a shift in infertility population in most countries, and male infertility is reportedly up to 50% of infertile couples[1-3]. Decreased fertility in men has a variety of etiologies such as testicular or epididymal disorders, immunologic factor, varicocele, genital tract infection, endocrine disturbance and genetic defects. At present, it is

This study was supported by Science and Technology Planning Project of Guangzhou City, China (No. 2013 00000114)
Corresponding author: Wei-jie ZHU; Tel: +86-20-85225718; E-mail: tzhuwj@jnu.edu.cn

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well recognized that the consequence of exposure to some chemicals can result in disruption of the programming of metabolic and reproductive processes, which contributes to male infertility[4-6].

Clenbuterol (CLB) is a member of the class of drugs called β-adrenergic agonists (βAR), which has the ability to promote an increase in lean muscle mass in meat-producing animals[7,8]. Because CLB can residue in the tissues of treated animals, which leads to adverse health effects in humans[9-12], it has been banned to use as a feed additive for food-producing animals in Europe, China and other countries. However, illegal use of CLB has existed in some places for economic purposes. Several reports have demonstrated that CLB has deleterious effects on reproductive organs or reproductive events[13-18]. Our previous investigations have showed that CLB inhibited the development of mouse embryo in vitro, and caused abnormal expression on testicular steroidogenic acute regulatory (StAR) protein and mRNA in rats[19,20]. In the present study, we observed effects of CLB on testicular ultrastructure of rats with transmission electron microscopy (TEM) in order to increase our understanding on testicular damage after CLB exposure.

Materials & Methods

Animals and grouping

Twenty adult male Sprague-Dawley rats (9–10 weeks old, weighing 200–220 g) were purchased from the Guangdong Medical Laboratory Animal Centre (Guangzhou, China). Rats were maintained under controlled temperature (23 °C–25 °C) and a 12 h light/dark cycle with free access to water and food throughout the experiment. The experiment was approved by the local ethics commission for the use of animals.

Rats were randomly divided into four groups (5 rats per group): a control group (group A), and three experimental groups (groups B, C and D; low, mid, and high doses, respectively). Each CLB (Bioo Scientific Co. Austin, TX, USA) dosage was dissolved 0.9% NaCl solution up to 1 ml. Based on our previous study and LD50 values [147–175 mg/kg body weight (bw)] [19], rats in groups B, C and D were treated daily by gavage with CBL dosages of 0.4 mg/kg bw (1/500 of LD50), 2.0 mg/kg bw (1/100 of LD50), and 18.5 mg/kg bw (1/10 of LD50), respectively. The animals in the control received an equivalent volume of 0.9% NaCl solution by gavage. The experimental period lasted 14 d.

Tissue samples and TEM

The rats were sacrificed by decapitation 24 h following the experimental period. Testicular tissues from the four groups were prepared for TEM analysis using a routine method as previously described [21]. Briefly, the samples were fixed in 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate/HCl buffer (pH 7.2–7.4), then postfixed in 1% osmium tetroxide, and dehydrated in graded alcohol and embedded in Emix resin.
Ultra-thin sections were cut, and double-stained with uranyl acetate and lead citrate. Ultra-structural changes of testis were observed with TEM (TELNAI-10, Philips, The Netherlands).

**Results**

Under TEM, the testicular ultrastructure in group A showed normal images. In group B, some small vacuoles were found in Sertoli cells. In groups C and D, vacuoles were common in Sertoli cells and spermatogonia. The phenomenon of vacuolation in group D was more severe than that in group C (Figure 1). In group D, basal membrane showed some irregular and wrinkled changes, Leydig cells had more vacuoles and increased lipid droplets. Some sperm heads were seen in the lumen, and did not show degenerative changes.

**Discussion**

Nowadays, food safety is becoming a public health concern in most countries, which not only greatly influences human health, but also closely associates with human reproductive health. Human infertility incidences are currently very high, which affects approximately 10% to 15% of married couples. A number of chemicals in food such as hormonally active drugs have been widely considered to be as etiological causes for decreased male fertility potential or an abnormal reproductive outcome[3-6].

Because the cycle of normal spermatogenesis in rat is about 12–14 d[22,23], the treating duration was set for 14 d in this subacute experiment. After CLB exposure, we observed that germ cells and seminiferous epithelia, especially Sertoli cells, spermatogonia, and Leydig cells
showed severe vacuolation, which indicated that testicular ultrastructure had posed pathological alterations. CLB is a βAR and acts chiefly on β2-adrenergic receptors\textsuperscript{10,11}. β2-adrenergic receptor has a high expression in the testes\textsuperscript{24}. Leydig cells and Sertoli cells have β2-adrenergic binding sites\textsuperscript{25,26}. Mammalian spermatozoa have β2-adrenergic receptors\textsuperscript{27}. Thus, the phenomenon of vacuolation of Sertoli cells, Leydig cells and germ cells could be resulted from CLB-receptor interaction. Vacuoles in Sertoli cells, Leydig cells and germ cells could mediate multiple negative effects on cellular activities, which would unavoidably lead to deleterious effects on testicular functions including spermatogenesis and androgen production. Our previous experiment showed that CLB induced significant decreases in the \textit{StAR} mRNA levels of the testis in the treated animals\textsuperscript{20}. This study demonstrated that alterations of testicular ultrastructure including vacuolation of Leydig cells would be one of reasons for \textit{StAR} mRNA abnormal expression.

In conclusion, our results revealed that testicular ultrastructure of rat had pathological changes after CLB exposure, and the alterations became more severe with the increasing doses, which influence the integrity of ultrastructure of Sertoli cells, Leydig cells and germ cells. Therefore, ingesting meat contaminated with CLB can pose a potential male reproductive health hazard.

References


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