Toxicity and Mechanism Analysis of Bisphenol a on Male Reproductive Development of Rats Based on Information Technology Statistics

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Abstract. In this study, BPA was selected as the research object to explore the effect of BPA on the development of reproductive system and its damage mechanism in the weaning F1 male offspring SD rats. The development of sexual organs and the change of sex hormone level in F1 male offspring were observed from the day of pregnancy when BPA of 50mg / kg, 100mg / kg and 200mg / kg was administered to the mother rats to the weaning period. The results showed that the serum PRL level of F0 parent rats in BPA group increased significantly at the end of weaning period, and the serum E2 level of 100 mg / kg and 200 mg / kg groups increased significantly. In F1 offspring, the dose of BPA was positively correlated with the level of serum E2, negatively correlated with the level of serum T, FSH decreased in 200mg / kg BPA group, and the organ coefficient of testis decreased significantly in 100mg / kg and 200mg / kg groups compared with the control group.

Keywords: Bisphenol A, Reproductive development, Male rats

1. Introduction
The lactation of offspring is a sensitive period of the development of reproductive system. In this period, the Sertoli cells of testis proliferate rapidly and reach a certain number in a short period of time[1]. Because the Sertoli cells mainly provide nutrients necessary for the development and maturation of spermatogenic cells, and act as the attachment scaffolds of spermatogenic cells at all levels, the number of Sertoli cells is directly related to the quantity and quality of spermatogenic cells[2]. In addition, the spermatogenic epithelium in the seminiferous tubules also develops from monolayer primordial spermatogenic cells at birth It is a multi-level spermatogenic cell. Exposure to environmental endocrine disruptors during lactation will damage the metabolism balance of sex hormones in the body, affect the content of serum testosterone, interfere with the rapid proliferation of
supporting cells in the early stage, affect the development and maturity of seminiferous tubules, and seriously lead to permanent and irreversible abnormal reproductive system development. In this study, the effects of BPA on the gonad development of male offspring were observed by histopathological analysis.

2. Materials and methods

(1) Material

Experimental animal:
10 male SD rats, weight 300-350g, 20 female SD rats, weight 230-260g.

(2) Experimental method

Preparation of bisphenol a solution:
BPA was dissolved in edible corn oil to prepare 20mg / ml, 40mg / ml, 80mg / ml, and the volume of gavage was 0.1ml/100g.bw, i.e.[3-4]. the doses of the three experimental groups were 50mg / kg, 100mg / kg, 200mg / kg, respectively. The control group was gavage with edible corn oil as the reagent control group at 9am every day.

Dose grouping and exposure of experimental animals:
Male and female SD rats were raised in separate cages. After one week's observation, they were mated in cages at 8:00 p.m. every day according to the female / male 2:1. Sperm was examined on the vaginal smear of female rats in the morning of the next day. All the female rats found sperm and in the estrus stage were determined as the day 0 of pregnancy[5-6]. They were randomly divided into 4 groups, 5 rats in each group, namely 50mg / kg, 100mg / kg, 200mg / kg BPA three dose group and a blank control group The pregnant rats and male rats were killed 20 days after birth.

Sample collection:
On the 20th day of postpartum lactation, the female rats were killed. Blood was taken from the femoral artery first, and then left in a 37 °C constant temperature water bath for 30 minutes. The upper serum was centrifuged for 10 minutes at 2500r / min for the determination of serum LH, FSH, PRL and E2[7-8]. In each dose group and the control group, two males were selected from each litter in the five litter weaning offspring, i.e. ten males in each dose group. Blood was taken from the femoral artery first, and serum was collected for the determination of LH, FSH, t and E2.

Serum sample analysis
The concentrations of LH, FSH, E2, PRL in serum of pregnant rats and LH, FSH, E2, t in serum of offspring rats were measured by radioimmunoassay.

Histopathological analysis
The testis development, seminiferous tubules, spermatogenic cells and stroma were observed under light microscope.

Image analysis and statistical analysis method
The gray value was analyzed by image analysis system[9]. The experimental data were analyzed with statistical software package sas8.02. The measurement data were analyzed by variance analysis and linear correlation analysis. χ2 test and trend test were used for counting data.

3. Result analysis
3.1. The body weight and serum hormone level of female rats on the 20th day of lactation

Table 1 shows that the body weight (before execution) of each dose group has no statistical significance compared with the control group. The PRL of BPA group was significantly higher than that of the control group (P < 0.05). The E2 of BPA group was significantly higher than that of the control group (P < 0.05).

Table1. Body weight and concentration of serum hormone in F0 female rats

| Groups (mg/kgBFA) | n | Body weight(g) | LH mlu/ml | FSH mlu/ml | E2 pg/ml | PRL mlu/ml |
|------------------|---|----------------|----------|------------|----------|------------|
| control          | 5 | 281±18.79      | 0.643±0.194| 1.516±0.204| 2.726±0.247|
| 50               | 5 | 274±14.27      | 1.060±0.380| 1.516±0.399| 3.070±0.701|
| 100              | 5 | 268±12.94      | 0.996±0.220| 1.392±0.225| 3.590±0.547|
| 200              | 5 | 273±13.82      | 0.906±0.252| 1.328±0.214| 3.861±0.763*|

*Compared with the control, P<0.05

3.2. Serum hormone level of male offspring of 20 day old F1

As shown in Table 2, the serum FSH concentration of 200mg/kg BPA group was significantly lower than that of the control group (P < 0.05). The concentration of E2 in BPA group was significantly higher than that in the control group (P < 0.05). The serum T concentration of each BPA experimental group was significantly lower than that of the control group (P < 0.05).

Table2. Concentration of serum LH, FSH,E2 and T in 20 day-old F1 male rats

| Groups (mg/kgBFA) | n | LH mlu/ml | FSH mlu/ml | E2 pg/ml | T ng/ml |
|------------------|---|-----------|------------|----------|---------|
| control          | 10| 0.768±0.279| 1.215±0.291| 3.750±1.063| 2.055±0.243|
| 50               | 10| 0.977±0.285| 1.344±0.339| 5.841±1.492*| 1.731±0.231*|
| 100              | 10| 0.956±0.198| 1.118±0.216| 8.020±2.104*| 1.408±0.174*|
| 200              | 10| 0.627±0.191| 0.890±0.157*| 11.76±2.974*| 1.009±0.169*|

*Compared with the control, P<0.05

3.3. Body weight, organ coefficient of testis and epididymis of F1 male offspring

Table 3 shows that the body weight of 50 mg/kg, 100 mg/kg and 200 mg/kg BPA group is significantly lower than that of the control group (P < 0.05). The organ coefficient of testes in BPA group was lower than that in the control group (P < 0.05). There was no significant difference in epididymis weight between the two groups.

Table3. Organ coefficient of 20 day-old F1 male rats

| Groups (mg/kgBFA) | n | Body weight(g) | Organ coefficient (g/100g body weight) |
|------------------|---|----------------|----------------------------------------|
|                  |   |                | testis                                | epididymic     |
| control          | 10| 45.14±3.39     | 0.574±0.058                           | 0.049±0.008    |
| 50               | 10| 41.50±2.56*    | 0.542±0.048                           | 0.047±0.007    |
| 100              | 10| 37.91±3.09*    | 0.493±0.061*                          | 0.051±0.005    |
| 200              | 10| 33.61±3.63*    | 0.463±0.032*                          | 0.049±0.009    |

*Compared with the control, P<0.05

3.4. Histopathological observation
In the control group, 20 days after birth, the structure of testis was clear, and the tubule lumen was oval. From basement membrane to lumen, there were spermatogonia, primary spermatocytes, secondary spermatocytes and spermatocytes in turn. There were many supporting cells in the testis. The connective tissue of the tubules was less, and the interstitial cells were scattered or several nuclei were relatively large; However, the testicular seminiferous tubules in the 200mg / kg BPA experimental group were shrunk, and the spermatogenic cells in each stage of the lumen were densely arranged. At the same time, a small number of exfoliated spermatogenic cells could be seen, most of the lumen central space was small or no space. The testicular tissue morphology of the 50mg / kg and 100mg / kg BPA experimental groups was relatively clear.

4. Discussion

4.1. Analysis of hormone changes

The results showed that the hormone level in serum of F0 parent rats was changed after being exposed to BPA. The level of serum prolactin (PRL) in the experimental group was higher than that in the control group, and the level of serum E2 in the high dose 200mg / kg group was significantly higher than that in the control group (P < 0.05). In this experiment, BPA may block the D2 receptor, reduce the response to Da, thus increasing the serum PRL level. Meanwhile, the serum E2 level is also significantly increased, and the increase of E2 is positively related to the dose of BPA, which may be caused by the competitive binding of BPA with ER.

Compared with the control group, the level of serum hormone in the experimental group at weaning stage of F1 offspring also changed. BPA can slightly raise FSH and LH at low dose, but the high dose of BPA can slightly lower LH and FSH, and FSH in the high dose group of 200mg / kg is significantly lower than that in the control group. BPA can significantly increase the level of serum E2 and significantly reduce the level of T (P < 0.05). In this experiment, low dose BPA may slightly increase the levels of FSH and LH in F1 offspring at weaning stage, while high dose BPA may decrease both FSH and LH, suggesting that BPA may inhibit the secretion of gonadotropin releasing hormone (GnRH) in hypothalamus, decrease the response of pituitary to GnRH, and inhibit the levels of FSH, LH and T in serum, and BPA may compete with ER in hypothalamus and anterior pituitary, resulting in E2 rises.

4.2. Analysis of morphological development index results

From the point of view of the body weight, which is the overall development index of F1 offspring at weaning stage, the dosage of 50mg / kg, 100mg / kg and 200mg / kg in the experimental group were significantly lower than that in the control group (P < 0.05), suggesting that BPA may have a certain toxic effect on the growth and development of F1 offspring. From the organ coefficients of testis and epididymis on the 20th day after the birth of F1 male offspring, the organ coefficients of testes in 100mg / kg and 200mg / kg BPA experimental groups were significantly lower than those in the control group (P < 0.05). There was no statistical significance between the organ coefficients of epididymis and the control group, suggesting that BPA may have a certain impact on the growth and development of gonads in F1 offspring.
4.3. Analysis of histopathological observation results
In the control group of F1 male offspring at weaning stage, the testis tissue structure was clear and the lumen was large, while the testis seminiferous tubules in the 200mg / kg BPA experimental group were narrow and the seminiferous cells in each phase of the lumen were densely arranged. At the same time, a small number of exfoliated seminiferous cells were seen, and the tissue structure was not clear. It has been reported [15] that estradiol (E2) can cause severe development disorder of testicular seminiferous tubules and has the effect of inhibiting testicular development in male rats. The main mechanism is that the high concentration of estrogen in serum can reduce the level of testosterone in vivo by antagonizing the effect of testosterone. Because testosterone is an essential hormone for testicular development, the lack of testosterone directly leads to the development of testicular seminiferous tubules and spermatogonic cells at all levels. The results showed that BPA has estrogen like effect on testicular dysplasia, and its mechanism may be the same as E2.

5. Conclusion
After BPA exposure, the serum E2 and PRL of the parent rats were significantly increased in 100mg / kg and 200mg / kg groups. There was a positive correlation between the dose of BPA and the level of serum E2, a negative correlation with the level of serum T, a decrease of FSH in 200mg / kg BPA group, a decrease of body weight and a negative correlation with the dose of BPA, and a decrease of testicular organ coefficient in 100mg / kg and 200mg / kg BPA groups.

Acknowledgement
Study on the relationship between the methylation of jam3-m4 gene locus, p16 protein and the incidence of female cervical cancer in Hunan Province, general topic of Hunan Provincial Department of education, 18c1182;Effects of bisphenol A on reproductive function of adult male rats, national innovation and entrepreneurship training program for college students, 20191082301

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