Effects of 3,4-Dichloroaniline on Testicle Enzymes as Biological Markers in Rats[1]

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Objective To investigate the effects of 3,4-dichloroaniline (3,4-DCA) on activities of testicle enzymes as biological markers in rats. **Methods** Fifty male rats were randomly divided into 5 groups (n=10). One group was left untreated and used as a solvent control (administered orally by corn oil), while the other 4 groups were treated with 3, 4-DCA. Corn oil was used as a solvent, and 3,4-DCA was diluted into tested concentrations (39, 81, 170, and 357 mg/kg). All the groups orally administered 3,4-DCA or corn oil, once a day for 4 weeks. The testicle tissue was homogenized in a 0.1 mol/L potassium phosphate buffer (0.1 mol/L, pH 7.2). The crude homogenate was centrifuged at 6000 rpm for 5 min at 4 ℃. The supernatant obtained was used as an enzyme extract for determination of the enzyme activities. **Results** Compared with the control, the activities of ALP, ACP, and SDH were increased significantly at a lower level of 3,4-DCA, and decreased at a higher level of 3, 4-DCA, whreas the activities of LDH, LDH-X, and G6PDH were inhibited significantly with the increased 3,4-DCA concentration. Organ coefficient "organ weight/total body weight × 100" of testis, liver, and spleen increased significantly with the increased 3,4-DCA concentration. These results suggest that 3,4-DCA toxicity to the male reproductive system was associated with the activities of testicular enzymes which are the sensitive biochemical endpoints reflecting 3,4-DCA toxicity to the male reproductive system. **Conclusion** 3,4-DCA has toxicity to the reproductive system in male rats.

Key words: 3, 4-dichloraniline; Rat; Marker testicular enzymes

INTRODUCTION

Dichloroaniline is widely used as chemical intermediate in synthesis of 3,4-dichloroaniline (3,4-DCA), a precursor for synthesis and a degradation production of some herbicides (e.g.diuron, dinuron and propanil)[1-2]. DCA is toxic to spleen, blood, and has immunotoxicity to mammals[3-5] and induces acute effects on blood, kidney, liver, and bladder of rats. Studies *in vitro* and *in vivo* have shown that DCA has nephrotoxicity and splenotoxicity to mammals[6-9].

Testicle enzymes as biochemical markers have been suggested as indicators of chemical exposure. Alterations in some testicle enzyme activities have been widely used as biomarkers to evaluate the function of organs due to their important role in energy production and biotransformation. Pandey *et al.*[10] found that changes in activity of SDH, LDH, and gamma-GT may be responsible for the toxic effects of molybdenum on fertility of male rats. Pant *et al.*[11] evaluated the effects of carbofuran on the reproductive system of male rats by measuring the activities of SDH, LDH, gamma-GT, G6PDH, and beta-glucuronidase, and found that carbofuran is toxic to the the reproductive system of male rats, suggesting that the activity of testicular-cell-specific enzymes induces damage to germ cells and Sertoli cells[11-12].

3,4-DCA and chlorobenzene affect testosterone concentration and hepatic microsome enzyme activities in crucian carp and DCA is toxic to many important organs and enzymes[13-14]. Well-balanced testis enzyme activities are important conditions for the formation sperm cells. Enzyme activity assay is one of the important methods to evaluate the toxicity of xenobiotic. At present, no report is available on the effect of DCA on testis enzyme activities. This study was to investigate the effects of 3,4-DCA on testicular enzymes ALP, ACP, LDH, LDH-X, SDH, and G6PDH in Wister rats.

MATERIALS AND METHODS

Technical grade 3,4-DCA (98% purity) was

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purchased from Sigma-Aldrich (Germany). ALP, ACP, LDH, SDH, G6PDH, and Lowry protein diagnostic reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute (China).

Adult male Wister rats weighing 180-220 g, provide by Beijing Weitong Lihua Laboratory Animal Limited Company, were housed under standard environmental conditions (20-22 ℃, 40%-55% relative humidity, lights on 8:00-20:00) for 7 days before experiment with free access to water and standard commercial diet (Beijing Weitong Lihua Laboratory Animal Limited Company).

Fifty male rats were divided into 5 groups (n=10). One group was used as solvent control, while the other 4 groups were treated with 3,4-DCA. Corn oil was used as a solvent, and 3,4-DCA was diluted into tested concentrations (39, 81, 170, and 357 mg/kg). Four treatment groups orally administered 3,4-DCA at the concentration of 39, 81, 170, and 357 mg/kg, respectively. The solvent control group administered only corn oil. Rats were fasted for 24 h with free access to water prior to experiments. The treatment groups were intraperitoneally given 3,4-DCA and the control group was intraperitoneally given corn oil at 8:00, once a day for 4 weeks. After 4 weeks, the testis was isolated from each rat, washed 3 times with potassium phosphate buffer (0.1 mol/L, pH 7.2), cut into small pieces and homogenized in potassium phosphate buffer (testis/total buffer is 1:10). The supernatant was obtained after the homogenate was centrigufed at 6 000 rpm for 5 min at 4 ℃ and used as an enzyme extract for determination of the enzyme activities .

Protein concentrations in the testis of rats were estimated using the folin-phenol reagent as previously described[15]. ALP, ACP, LDH, LDH-X, SDH, and G6PDH activities in testis were determined with the diagnostic reagent kits (Nanjing Jiancheng Bioengineering Institute, China).

Results of different treatments were compared by one-way analysis of variance (ANOVA). P<0.05 was considered statistically significant. Statistical analysis was performed using SPSS 13.0 for Windows. Data were expressed as $\overline{x} \pm s$.

RESULTS

The changes in body weight and organ coefficient of rats 4 weeks after administration of 3,4-DCA are summarized in Table 1.

TABLE 1

Effects of 3,4-DCA on Body Weight, Testis Weight, Liver Weight, Spleen Weight, and Their Coefficient of Rats ($\overline{x} \pm s$)

3,4-DCA (mg/kg)	BW (g)	TW (g)	Testis Coefficient	LW (g)	Liver Coefficient	SW (g)	Spleen Coefficient
Control	427.50±14.87	3.22±0.25	0.75±0..06	15.64±1.21	3.99±0.41	1.45±0.09	0.34±0.02
39	448.74±16.97	3.39±0.34	0.75±0.08	17.93±1.90*	3.93±0.59	1.65±0.25	0.37±0.06
81	420.06±37.71	3.18±0.19	0.76±0.07	17.38±2.55	4.89±0.71*	1.52±0.23	0.37±0.07
170	409.43±19.16**	3.43±0.25	0.84±0.05*	17.97±1.90*	5.04±0.84**	1.69±0.18*	0.41±0.05*
357	378.90±42.08**	3.43±0.25	0.87±0.13*	22.71±3.05***	5.46±0.61***	1.74±0.27*	0.77±0.06***

Note. BW=body weight, TW=testitis weight, LW=liver weight, SW=spleen weight. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

The results showed that body weight changed remarkably when the concentration of 3,4-DCA was equal or higher than 170 mg/kg. Testis weight did not change remarkably while testis coefficient changed remarkably when the concentration of 3,4-DCA4-DCA was equal to or higher than 170 mg/kg. The liver weight and coefficient as well as spleen weight and coefficient changed with increased 3,4-DCA concentration.

After Wister rats were administered orally 3,4-DCA for 4 weeks, the changes in activities of ALP, ACP, LDH, LDH-X, SDH, and G6PDH in testis of rats 4 weeks after administration of 3,4-DCA are summarized in Table 2.

ALP activity changed with the concentration of 3,4-DCA and increased significantly at a lower concentration of 3,4-DCA (81 mg/kg) and decreased evidently at a higher concentration of 3,4-DCA (375 mg/kg). The change in ACP and ALP activity was similar. ACP activity increased significantly at a lower concentrations of 3,4-DCA (39 to 81 mg/kg) and decreased significantly at a higher concentration of 3,4-DCA (375 mg/kg). The activities of ALP and ACP changed in a concentration-dependent manner.

The activity of LDH remained unchanged at a lower concentration of 3,4-DCA (39 mg/kg), and decreased significantly at a higher concentration (equal to or higher than 81 mg/kg) of 3,4DCA. The activity of LDH-X decreased dramatically when the concentration of 3,4-DCA was 39, 81, 170, and 357 mg/kg, respectively.