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

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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, whereas 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[13-15]. 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...
Effects of 3, 4-DCA on Testicle Enzymes

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=testis weight, LW=liver weight, SW=spleen weight, and Their Coefficient of Rats (x±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.

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-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.
TABLE 2

<table>
<thead>
<tr>
<th>3,4-DCA Concentration (mg/kg)</th>
<th>ALP (µ/mg prot)</th>
<th>ACP (µ/mg prot)</th>
<th>LDH (µ/mg prot)</th>
<th>LDH-X (µ/mg prot)</th>
<th>SDH (µ/mg prot)</th>
<th>G6PDH (µ/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.81±5.65</td>
<td>31.64±3.58</td>
<td>218.50±45.81</td>
<td>80.25±13.81</td>
<td>19.72±1.75</td>
<td>31.06±2.01</td>
</tr>
<tr>
<td>39</td>
<td>42.11±3.20</td>
<td>44.41±7.88</td>
<td>186.34±29.01</td>
<td>34.29±16.23</td>
<td>31.61±5.90</td>
<td>32.05±6.61</td>
</tr>
<tr>
<td>81</td>
<td>64.69±11.61</td>
<td>53.77±16.82</td>
<td>123.94±24.21</td>
<td>19.38±5.82</td>
<td>10.41±2.05</td>
<td>38.29±5.24</td>
</tr>
<tr>
<td>170</td>
<td>51.80±4.79</td>
<td>30.39±4.03</td>
<td>86.18±10.61</td>
<td>12.74±4.61</td>
<td>8.17±1.44</td>
<td>18.71±6.53</td>
</tr>
<tr>
<td>357</td>
<td>35.45±3.61</td>
<td>21.17±2.48</td>
<td>55.78±8.39</td>
<td>3.39±1.25</td>
<td>7.89±5.40</td>
<td>10.56±4.41</td>
</tr>
</tbody>
</table>

Note: *P<0.05, **P<0.01, ***P<0.001.

The activity of SDH increased significantly at a lower concentration of 3,4-DCA (39 mg/kg), and decreased significantly at a higher concentration of 3,4-DCA (equal to or higher than 81 mg/kg). The activity of G6PDH remained unchanged at a lower concentration of 3,4-DCA (39 and 81 mg/kg), and decreased significantly at a higher concentration of 3,4-DCA (equal to or higher than 170 mg/kg).

DISCUSSION

The results of our study indicate that 3,4-DCA is toxic to rats and the target organs are the kidney, liver, and urinary bladder[16]. In this study, 3,4-DCA caused changes in testicular enzymes and affected the functions of the reproductive system of male rats. Testicular enzymes are sensitive biochemical endpoints and may be used as a marker reflecting 3,4-DCA toxicity to the reproductive system of male rats. The production and maturity are a complex process that is related to the activities of testicular enzymes. 3,4-DCA is a precursor for synthesis and a degradation product of some herbicides, and is commonly present in environment. It was reported that 3,4-DCA has acute effects on blood of Wister rats and also splenotoxicity and nephrotoxicity to mammals[3, 6, 8]. In this study, 3,4-DCA was found to have effects on testis enzyme activities.

The activity of ALP is related to the mitosis of spermatogenic cells and glucose transport. ACP located in lysosome of leydig cells is involved in the protein synthesis by abduction of sex hormones. Changes in the activity of ALP and ACP may be used as an indicator of spermatogenesis function. In this study, ALP and ACP activities increased significantly at a lower concentration of 3,4-DCA and decreased significantly at a higher concentration of 3,4-DCA. Changes in the activity of testicular ALP and ACP of 3,4-DCA-treated rats also reflect testicular degeneration, which may be a consequence of suppressed testosterone and indicative of lytic activity[17]. The activities of SDH and LDH in testicular tissue are associated with the maturation of the germinal epithelial layer of seminiferous tubule. SDH, mainly located in chondriosome of sertoli cells and spermatogenic cells, plays an important role in energy metabolism of sperms. SDH activity is related to fructose transferred into sorbic alcohol and glucose that provides energy to sperms. The SDH activity increases markedly throughout the maturation of germ cells and decreases during the depletion of germ cells[18-19]. LDH, widely present in sertoli and spermatogenic cells, plays an important role in testis energy production and biotransformation. LDH-X is a special enzyme produced at the phase of primary spermatogenic cells. Since inhibition of LDH and LDH-X activities may induce denaturalization of spermatogenic cells[20]. LDH and LDH-X activities can be used in evaluating the function of spermatogenic cells. In this study, the activity of LDH and LDH-X reduced significantly at a concentrations equal to or higher than 81 mg/kg of 3,4-DCA and equal to or higher than 39 mg/kg of 3,4-DCA, respectively, indicating that the function of spermatogenic cells can be affected by 3,4-DCA, the LDH and LDH-X activity is sensitive to 3,4-DCA, and LDH-X activity is the sensitive endpoint to measure the toxicity of 3,4-DCA to testis.

In this study, the SDH activity increased dramatically at a lower concentration of 3,4-DCA (39 mg/kg), and decreased markedly at a concentration equal to or higher than 81 mg/kg of 3,4-DCA, suggesting that the movement of sperms may be affected by 3,4-DCA. The decreased activity of LDH, LDH-X, and SDH in the treated rats suggested a deterioration of germinal epithelium due to chemical exposure.

G6PDH, present in leydig, sertoli, and spermatogenic cells, is more active in leydig cells. The activity of G6PDH is associated with the function of leydig cells. In this study, the G6PDH activity decreased dramatically at a concentration equal to or higher than 170 mg/kg of 3,4-DCA,
suggesting that 3,4-DCA can injure the function of leydig cells.

In summary, the activities of ALP, ACP, LDH, LDH-X, SDH, and G6PDH are changed significantly after treatment with 3,4-DCA. The function of reproductive system in male rats may be impaired by 3,4-DCA. 3,4-DCA has potential toxicity to mammal reproduction system. The activities of ACP, SDH, LDH, and LDH-X are sensitive to 3,4-DCA and can be used as biomarkers to evaluate the function of testis.

REFERENCES


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