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Effect of vitamin E on reproductive function in the mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin*

JP Xu, YP Yin and XQ Zhou

Department of Laboratory Animal Science, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China

The study was conducted to determine the effects of vitamin E on reproductive performance in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The 75 female and 40 male mice were randomly assigned into five groups. The levels of both TCDD and vitamin E given by gavage were 0 and 0 (Control group), 100 and 0 (experimental group I), 100 and 20 (experimental group II), 100 and 100 (experimental group III), and 100 ng/kg/day and 500 mg/kg/day (experimental group IV), respectively. Males and females were mixed to mate at the ratio of 1:2 after 4-week experiment. The gavage treatments were continued until the end of gestation in female mice after mating. The results showed that the litter number, survival rate, and body weight at birth of offspring in experimental group I had significantly decreased, and the females’ pregnancy rate and pup sex ratio in experimental group I had the decreasing tendencies when compared with the control group. The litter number in experimental group III, survival rate in experimental group II and III, body weight at birth in experimental group III and IV exhibited significant increase compared with experimental group I. The female pregnancy rate in both experimental group III and IV recovered to 100%, but there were no significant differences when compared with experimental group I. The pups’ sex ratio had a gradually increasing tendency with increase of vitamin E level, but there was no significant difference among experimental group I–IV. The results suggest that TCDD could induce reproductive toxicity in mice, whereas vitamin E alleviated adverse effects on reproductive performance in mice caused by TCDD. Toxicology and Industrial Health 2008; 24: 595–601.

Key words: 2,3,7,8-tetrachlorodibenzo-p-dioxin; mice; reproduction; vitamin E

Introduction

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which exists widely in nature and is difficult to decompose, is one of the most potent environmental contaminants. TCDD is lipophilic, possesses a low rate of metabolism and excretion, prone to bioenrichment and has potentially adverse effects on human and animals (Salisbury and Marcinkiewicz, 2002). Also, TCDD is a known environmental female hormone which has an adverse effect on sexual maturation and reproductive ability in female rats. It could lower the response to gonadotrophic hormone in ovary and estrogen level and decrease the number of ovulation as well as disturb the regular cycle of the sex drive. More and more research showed that reproductive organs and endocrine system of rats are very sensitive to the toxicity of TCDD during the developmental period, specially during the time of sexual maturation. TCDD has effect on development of reproductive function of immature female rats through disturbing their reproductive endocrine system (Tanaka, 2002).
et al., 2006). If the rats were exposed to TCDD during the periods of embryonic stage or/and nursery time, their ability to reproduce upon reaching adulthood will be significantly compromised (Salisbury and Marcinkiewicz, 2002).

Vitamin E is an essential lipidsoluble vitamin in the human body and plays multiple important physiological roles. Vitamin E is not only closely related to the function of reproduction but also is an important antioxidant and immunomodulator in the human body (Moriguchi and Muraga, 2000). Vitamin E deficiency in male rats induces testicular atrophy, degeneration of epithelium tissue in the seminiferous tubules, sperm catagenesis, and even prevents spermatogenesis. In female rats, Vitamin E deficiency causes embryo and placenta atrophy, hypofunction of ovaries, abnormal sexual cycle, and habitual abortion (Patnode and Curtis, 1994). Vitamin E succinate alleviates the retardation of fetal growth and inhibits the increases of lipid peroxidation in embryonic and placental tissues in mice induced by TCDD (Hassoun, et al., 1997). The combination of both vitamin E and selenium relieves the decreases of both dams body weight and progeny survival in rats caused by Methylmercury (Beyrouty and Chan, 2006). Evidence has been proved both in-vitro and in-vivo experiment that vitamin E plays an important role in the function of reproduction. In addition, vitamin E level has close relation with gene expression, mitochondrial metabolism, cell differentiation, and immune regulation (Marquez, et al., 2002). However, little information is known about the effects of vitamin E on reproductive performance in TCDD-treated mice and its ability to ameliorate the reproductive toxicity of TCDD. The present study was designed to evaluate the effects of different levels vitamin E on reproductive performance in TCDD-treated mice.

Methods

Animals and treatment

In all, 28-day-old clean mice of ICR were used in the present study and were obtained from Beijing Vital River Laboratory Animal Technology Limited Corporation, China. Each mouse was raised in a mouse box of 26 cm × 15 cm × 15 cm. They were maintained at a constant temperature of 22 ± 2 °C and a humidity of 50 + 5% with a 12:12 light/dark cycle and given a standard commercial pelleted diet except vitamin E and tap water ad libitum. The nutritional ingredients in the pelleted diet are protein 20.6%, fat 4.16%, carbohydrate 53.17%, crude fiber 4.92%, ash content 7.5%, and moisture content 9.65%. The experiment was initiated after 1 week acclimatizing to laboratory conditions. TCDD (97% purity) was provided by Research Center for Eco-Environmental Sciences, the Chinese Academy Sciences. Vitamin E (95% purity) was obtained from Sigma Company.

Experimental design and methods

Healthy 75 female and 40 male with an initial body weight of 16.4–24 g and 19–33.3 g were selected, respectively. They were randomly assigned to five groups, and each group contained 15 females and 10 males. The levels of both TCDD and vitamin E were 0 and 0 (Control group), 100 and 0 (experimental group I), 100 and 20 (experimental group II), 100 and 100 (experimental group III), and 100 ng/kg/day and 500 mg/kg/day (experimental group IV), respectively. Vitamin E and TCDD were given by gavage. TCDD was dissolved in corn oil and given 2 h before vitamin E was administrated. The mice in the control group received an equal volume of corn oil. After 4-week experiment, females and males were mated at the ratio of 1:2. Once vaginal plug was found, the females were selected out and housed individually and were consecutively given TCDD and vitamin E to the end of gestation. Pups were weighed at birth. The pregnancy rate in female mice, litter number, survival rate, and sex ratio of offspring were observed and recorded. All mice could take their food and drinking water freely during acclimation and experiments.

Statistical analysis

All data were analyzed by SPSS 11.5, a statistical software. The pregnancy rate in female mice, the survival rate, and sex ratio in the offspring were determined by chi-square test. The litter number and body weight in newborn pup between control group and experimental group I were determined by t-test, and those among experimental group I–IV were analyzed by one-way analysis of variance (ANOVA) followed by LSD’s multiple range test to detect significant differences between groups. All
values were mean ± SE. The results were considered significant at $P < 0.05$.

Results

The change of pregnancy rate in female mice

TCDD had no significant effects on the pregnancy rate of female mice ($\chi^2 = 2.154, \ P = 0.142$). Although the pregnancy rate in experimental group I had a decreasing tendency compared with control group, there was no significant difference between control group and experimental group I (Table 1). The pregnancy rate among experimental group I–IV had no significant difference ($\chi^2 = 3.734, \ P = 0.292$). Although the pregnancy rate in both experimental group III and IV recovered to 100%, there were no significant difference compared with experimental group I (Table 1).

The change of the litter number in offspring from the mice

TCDD showed a significant influence on the litter number ($t = 2.175, \ P = 0.040$). The litter number in experimental group I was less than that of control group ($P < 0.05$) (Figure 1). Vitamin E had a significant effect on the litter number in offspring from TCDD-treated mice ($F = 2.829, \ P = 0.049$). The litter number in both experimental group II and IV had no significant difference compared with experimental group I ($P > 0.05$) but one in experimental group III was more than those in experimental group I and IV ($P < 0.05$) (Figure 1).

The change of the survival rate in offspring from the mice

The survival rate of pups in experimental group I showed a significant decrease compared with the control group ($\chi^2 = 11.23, \ P = 0.001$) (Table 2).

Vitamin E had significant effects on the survival rate in pups from TCDD-treated female mice ($\chi^2 = 15.141, \ P = 0.002$). The survival rate in experimental group II and III significantly increased compared with experimental group I ($P < 0.05$), but there was no significant difference between experimental group I and IV ($P > 0.05$) (Table 2).

The change of the body weight in newborn pup from the mice

TCDD showed a significant effect on the body weight in newborn pups ($t = 2.428, \ P = 0.023$). The pup’s body weight at birth in experimental group I was significantly lower than that of control group (Figure 2). Vitamin E had significant effect on the pup body weight at birth from TCDD-treated mice ($F = 4.047, \ P = 0.012$). The pup body weight at birth in experimental group II had no significant difference compared with experimental group I ($P > 0.05$), but the ones in experimental group III and IV were higher than those in experimental group I and II ($P < 0.05$) (Figure 2).

The change of the sex ratio in pups from the mice

TCDD had no significant effect on the sex ratio in offspring ($\chi^2 = 0.483, \ P = 0.487$). Although the sex

Table 1. Effects of vitamin E on the pregnancy rate in TCDD-treated female mice (mean ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pregnant females</th>
<th>Nonpregnant females</th>
<th>Death females</th>
<th>Total females</th>
<th>Pregnant rate(%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>100</td>
<td>0.142</td>
</tr>
<tr>
<td>Experimental group I</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td>85.7</td>
<td>0.292</td>
</tr>
<tr>
<td>Experimental group II</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td>86.7</td>
<td></td>
</tr>
<tr>
<td>Experimental group III</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Experimental group IV</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>15</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
ratio in experimental group I had a decrease tendency compared with control group, there was no significant difference between them ($P > 0.05$) (Table 3). Vitamin E had no significant effect on the sex ratio in offspring from TCDD-treated mice ($\chi^2 = 3.524$, $P = 0.318$). The sex ratio in pups had a gradually increasing tendency with increase of vitamin E level, but there was no significant difference among experimental group I–IV ($P > 0.05$) (Table 3).

**Discussion**

**Effect of vitamin E on pregnancy rate in TCDD-treated female mice**

The present results have showed that the female mice in TCDD-treated group had a decrease tendency of pregnancy rate compared with control group, which recovered to 100% in the two groups supplemented with vitamin E (100 and 500 mg/kg) plus TCDD. TCDD is an estrogen-like environmental toxin and has severe toxicity of reproduction and development. Guo, *et al.*, (2000) reported that 10 of 12 macaques miscarried from day 22 to 32 of gestation after acute exposure to TCDD. The evidence in vitro indicates that TCDD causes the loss of reproductive cells and decreases the number of primordial follicle of ovaries during the embryonic period by activating pathway of cell apoptosis (Matikainen, *et al.*, 2002). TCDD inhibits the ovary response to gonad stimulating hormone, lower estrogen level, and disorders sexual cycle, which significantly decreases the number of ovulating and pregnancy rate (Patnode and Curtis, 1994; Roby, *et al.*, 2001). The toxic effects of TCDD vary and are dependent upon the species, sex, age of the animal studied. The mechanism of TCDD toxicity may be associated with inducing the activation of enzymes promoting the hydroxylation and metabolism of estrogen and thus decreasing the level of estrogen (Patnode and Curtis, 1994; Roby, *et al.*, 2001).

Vitamin E is a potent antioxidative agent against toxic chemistry both in vitro (Farris, 1990; Hassoun, *et al.*, 1995b) and in vivo (Bagchi, *et al.*, 1993).

**Table 2. Effects of vitamin E on the survival rate of offspring from TCDD-treated mice (mean ± SE)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival pups</th>
<th>Death pups</th>
<th>Total pups</th>
<th>Survival rate (%)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>144</td>
<td>1</td>
<td>145</td>
<td>99.31</td>
<td>0.001</td>
</tr>
<tr>
<td>Experimental group I</td>
<td>85</td>
<td>9</td>
<td>94</td>
<td>90.43*</td>
<td>0.002</td>
</tr>
<tr>
<td>Experimental group II</td>
<td>122</td>
<td>2</td>
<td>124</td>
<td>96.30a</td>
<td></td>
</tr>
<tr>
<td>Experimental group III</td>
<td>139</td>
<td>1</td>
<td>140</td>
<td>99.29b</td>
<td></td>
</tr>
<tr>
<td>Experimental group IV</td>
<td>78</td>
<td>3</td>
<td>81</td>
<td>99.29b</td>
<td></td>
</tr>
</tbody>
</table>

*Represents significant difference between control group and experimental group I. Completely different superscript letters represent significant difference among experimental group I–IV.

**Table 3. Effects of vitamin E on the sex ratio of offspring from TCDD-treated mice (mean ± SE)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Male pups</th>
<th>Female pups</th>
<th>Total pups</th>
<th>Sex ratio</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>73</td>
<td>72</td>
<td>145</td>
<td>1.01</td>
<td>0.487</td>
</tr>
<tr>
<td>Experimental group I</td>
<td>43</td>
<td>51</td>
<td>94</td>
<td>0.84</td>
<td>0.318</td>
</tr>
<tr>
<td>Experimental group II</td>
<td>55</td>
<td>69</td>
<td>124</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Experimental group III</td>
<td>75</td>
<td>65</td>
<td>140</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Experimental group IV</td>
<td>44</td>
<td>37</td>
<td>81</td>
<td>1.19</td>
<td></td>
</tr>
</tbody>
</table>
1993; Hassoun, et al., 1995a). Vitamin E reacts to hydroxy radical in the membrane phospholipids and effectively attenuates testicle DNA damage and the production of lipid peroxidation induced by Aspergillus flavus (Verma and Nair, 2002). Vitamin E provides a protection against TCDD through enhancing activities of superoxide dismutase (SOD) and catalase (CAT), inhibiting DNA damage and depressing the productions of the lipid peroxidation, superoxide anion, and reactive oxygen in the brain after subacute exposure to TCDD in rats (Hassoun, et al., 2004; Hassoun, et al., 2006). Vitamin E significantly suppresses the p53 phosphorylation and p53-dependent oxidative stress and inhibited DNA damage in human trophoblastic cell (JEG-3) by benzopyrene-induced (Drukteinis, et al., 2005).

Effect of vitamin E on the litter number in offspring from TCDD-treated mice

The litter number is correlated to the development and maturity of oocytes, ovulation as well as the number and vital status of spermatozoa. In addition, it is also closely associated with the nidation rate of oosperm, functional status of uterus, and the maintenance of progestogen during gestation. Our data have shown that 100 ng/kg TCDD significantly decreases the litter number of offspring in mice, whereas 100 mg/kg vitamin E alleviates this decrement induced by TCDD. There is no significant effect beyond the doses of vitamin E, indicating that optimal dose of vitamin E antagonizes the effect of TCDD on the litter number. TCDD disrupts the regulation of sexual hormone to endometrium, inhibits estradiol-induced endometrial hyperplasia, obstructs nidation of oosperm, and development of blastocysts as well as synchronization of endometrial (Safe, et al., 1991). The action mechanism of TCDD-induced ovarian dysfunction is related to increase in some metabolic enzyme activity to promote hydroxylation of estradiol, as well as to decrease the expression of estrogen receptor (Romkes and Safe, 1998). TCDD disturbs the development and ovulation of oocytes in ovary by directly acting on ovaries or through disrupting follicle-stimulating hormone and luteinizing hormone release from pituitary secretion (Gao, et al., 2000). Vitamin E may alleviate the reduction of litter number by regulating the progesterone secretion. Li, et al., (2006) showed that TCDD decreases the level of serum progesterone in mice, whereas Yin, et al., (2008) have showed that vitamin E alleviates the decrease of plasma progesterone in mice acutely treated with TCDD.

Effect of vitamin E on the survival rate in offspring from TCDD-treated mice

Sufficient vitamin E provides a support for normal reproductive capacity of female mice and the survival rate of pups. The present study showed that there was a significant decrease in the survival rate of pups from TCDD-treated mice, whereas vitamin E at doses both 20 and 100 mg/kg provided a protective effect on the survival rate of pups from mice exposed to TCDD. No effect was observed on survival rate of pups at 500 mg/kg vitamin E. The reason that TCDD caused the decrease of survival rate in pups might be associated with the retardation of embryonic development and fetal death during female mice exposed to TCDD (Hunt and Hunt, 1977; Johnson, et al., 1992). The effect mechanisms of TCDD on fetal development are not well understood, though several mechanisms have been proposed.

Vitamin E inhibits TCDD-mediated lipid peroxidation, prolongs life span to 25 days in rats after exposed to a lethal dose of TCDD (Stohs, et al., 1984). Hassoun, et al., (1997) showed that vitamin E succinate and ellagic acid significantly decrease the retardation of fetal growth, and lowers the increase of fetal death, and inhibits lipid peroxidation in mice induced by TCDD, but there were no significant ameliorating effects on TCDD-induced malformations including cleft palate and hydronephrosis. The combination of both vitamin E and selenium relieved the reduction of body weight, extended gestational period, and reduced death rate of pups exposing to methyl mercury (Beyrouty and Chan, 2006). Kitagawa, et al., (2004) showed that vitamin E effectively prevented the oxidative stress damage and ameliorated development in the swine embryos.

Effect of vitamin E on the body weight in offspring from TCDD-treated mice

The present study showed that the pup body weight at birth in TCDD-treated group significantly reduced by 8.88% compared with control group,
whereas notable increased in two groups supplemented vitamin E (100 and 500 mg/kg) with TCDD compared with TCDD-treated alone. These results suggested that vitamin E enhanced the normal growth and development of pups. This is similar to results from previous studies of Wen and Lin, (1996). Alsharif and Hassoun, (2004), in which they showed that vitamin E alleviated the decreases of body and thymic weight and lowered the increases of liver weight, superoxide anion, and DNA damage induced by TCDD in C57BL/6J mice. Oxidative stress may play a potent role in the TCDD-induced toxicity, because TCDD causes dose dependent the increase of reactive oxygen species, whereas vitamin E attenuates production of reactive oxygen species by suppressing the activation of extracellular signal-regulated kinase (ERK-1/2) and mitogen-activated protein kinase (MAPK) pathway or NMDA receptor induced by TCDD (Kim and Yang, 2005).

**Effect of vitamin E on the sex ratio in offspring from TCDD-treated mice**

The present studies have shown that TCDD resulted in a decreasing tendency of offspring sex ratio in mice, whereas vitamin E 100 and 500 mg/kg recovered the sex ratio to normal level. Hunt and Hunt, (1977) have showed that environmental hormones and their metabolin resulted in the changes of avian sexual ratio. In a epidemiologic survey, Moriguchi and Muraga, (2000) discovered that the number of females was more than that of males in the offspring exposed to dioxins in Sevoso, Italy in 1976, and the males only accounted for 38% of newborn babies. It is possible that TCDD disrupts physiological function and alters cellular signal transduction, which in turn causes the change of the sex ratio.

In summary, our results support that vitamin E antagonizes reproductive toxicity caused by TCDD, and vitamin E 100 mg/kg is the optimal dose in the present experiments, which provides a novel way for preventing the reproductive toxicity caused by TCDD.

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**References**


