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Systemic Effects of Orally Administered p, p′-DDE on Immature Male Wistar Rats during Pubertal Period

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Abstract: Systemic Effects of Orally Administered p, p′-DDE on Immature Male Wistar Rats during Pubertal Period: Yuji MAKITA, *et al.* Department of Hygiene, Graduate School of Medical Sciences, Kyushu University—Systemic effects of p, p′-DDE (1, 1-dichloro-2, 2 bis (p-chlorophenyl) ethylene ; DDE) on immature male rats were investigated in pubertal Wistar rats after oral administration of DDE. Special rat chow containing 125 ppm DDE (approximately 10 mg/kg DDE) had been administered daily for 42 d since 6 wk of age and its effects had been observed until 12 wk of age. The administration of DDE did not produce any overt signs of toxicity. Neither physical development nor sexual maturation was affected, and serum biochemistry was not impaired at the dose used in this experiment. Moreover, the male reproductive organs and epididymal sperm count were not affected by the administration of DDE during the pubertal period. Our results showed that even immature male rats were resistant to DDE exposure at the daily dose of ca. 10 mg/kg, but metabolic and immunological changes still remained uncertain. Further investigation should be conducted to reveal all the effects of DDE on immature male rats.
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Key words: p, p′-DDE, Immature male rat, Pubertal exposure, Endocrine disruptor, Systemic effects

Some industrial chemicals and environmental pollutants can disrupt reproductive development in wildlife and humans by mimicking the action of native hormones. These substances are called endocrine disrupting chemicals (EDCs) to date[1].

p, p′-DDE (1, 1-dichloro-2, 2 bis (p-chlorophenyl) ethylene; DDE) is a persistent metabolite of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) which was used as a pesticide for over 30 yr and banned in the 1970s in developed countries. DDE is still used in some parts of the world such as tropical countries[2, 3]. DDE is accumulated in the environment and found in food. Moreover, recent evidence about global distribution of some organochlorine pollutants has indicated migration through the atmosphere from warmer to colder countries[4]. General human exposure to DDE will mainly be from consumption of contaminated food and minor exposure may also occur through the manufacture and use of DDT as an insecticide. Recent investigations of organochlorine residues have revealed high levels of DDE accumulation in human beings and wildlife due to an elongated half-life in the body or environment[5, 6]. DDE concentrations have certainly declined from those in the mid-1960s[3, 7], but several reports suggest that human DDE levels can exceed those that inhibit human androgen receptor *in vitro* even now[8, 9]. A recent monitoring study of organochlorine residues in humans revealed that the median serum DDE level was 12.6 ppb in women in the USA[10] and the maximum level is over 600 ppb in human adipose tissues[6, 7, 10].

DDE is strongly suspected to be one of the EDCs. DDE was recently reported to be a potent antiandrogen rather than an estrogen compound[8]. Previously, an experiment was conducted in our laboratory to examine the direct effects of DDE on male reproductive organs[11]. But, in the experiment, the method of administration was a single intraperitoneal injection and the dosed concentration was quite high at 220 mg/kg at one time. Besides, only the effects of DDE on male reproductive organs were examined in this study. DDE exposure is usually continuous at low levels through food. Therefore, the present study was conducted to investigate not only male reproductive effects but also systemic effects of DDE on immature male Wistar rats caused by oral administration of DDE.

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Materials and Methods

Animals

Twelve male Wistar rats, 4 wk of age were purchased from Kyudo Breeding Co., Ltd. (Tosu, Japan). The rats were acclimatized to animal facilities for two weeks and kept at a temperature of 20–24°C and a humidity of 30–60% in a controlled room with a 12-h light cycle (12-h light/12-h dark). They had free access to laboratory rat chow, CE-2 (Clea Japan Inc., Tokyo, Japan) and tap water. Three rats were housed in a plastic cage with laboratory grade pine shavings as bedding. Because normal sexual development is completed by 8 wk of age in the Wistar rat, the pubertal period was considered to be around this time.

This experiment was approved by the Committee of Ethics on Animal Experiments in the Faculty of Medicine, Kyushu University and conducted under the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University and the Law (No.105) and Notification (No.6) of the Japanese Government.

Treatment

p, p′-DDE (1, 1-dichloro-2, 2 bis (p-chlorophenyl) ethylene) (DDE) with a purity of 99% was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Special rat chows containing DDE were prepared as follows; DDE was dissolved in a small amount of ethanol and well mixed with CE-2, forming food pellets. During this process, ethanol was deprived by evaporation. About 0.21 ppb DDE (0.21 ng DDE/g) was already contained in a normal rat chow, CE-2.

Immature male Wistar rats were randomly divided into 2 groups; namely a control group and a DDE group with 6 rats in each. The dose of DDE administered was determined as follows: the acceptable daily intake (ADI) of DDE for humans is 2 mg/d. Therefore, the daily DDE intake in the rats was adjusted approximately to the ADI for humans. The control group was fed only CE-2, and the DDE group, CE-2 containing 125 ppm DDE (125 μg DDE/g). The rats had been fed each prepared chow for 42 d from 6 wk to 12 wk of age. The body weights of rats were measured every 7 d.

The rats were sacrificed at 12 wk of age by CO_2 inhalation and autopsied. Blood samples were collected from the posterior vena cava. The serum was separated by centrifugation and stored at –80°C until measurement. Organs which were resected and weights measured at autopsy included liver, kidney, spleen, thymus, testis, epididymis, seminal vesicles and prostate. These organs were fixed in 10% neutral buffered formalin solution and then embedded in paraffin, cut into 6-micrometer sections and stained with hematoxylin and eosin for ordinary histopathological study. In the case of testis, it was fixed in Bouin's solution, embedded in paraffin, sectioned at 3 micrometers and stained with periodic acid Schiff (PAS) and hematoxylin. The left epididymis was used for the sperm count. Decapsulated epididymis was homogenized in saline containing 0.05% (v/v) Triton X-100 in a blender and homogenization-resistant sperm were counted using a hemocytometer.

Testosterone was measured with a No-Extraction Coat-a-Count RIA kit obtained from Diagnostic Products Company (Los Angeles, CA). Measurements of LH and FSH were performed with a rat lutenizing hormone (rLH) and follicle stimulating hormone (rFSH) assay kit obtained from Amersham International plc (Amersham, UK) by RIA.

Statistical significance was determined by Fisher's least significant difference procedure after a one-way analysis of variance.

Results

Daily mean consumption of rat chow (daily consumption per cage/3) was 23.8 ± 2.3 g in the control group and 23.3 ± 0.7 g in the DDE group, respectively. There were no differences in rat chow intake between the control and DDE groups. Daily intake of DDE was ca. 10 mg/kg, based on daily chow consumption. The administration of DDE did not produce any overt signs of toxicity. The body weight changes in both groups during the observation period are shown in Fig. 1. At the starting point, 6 wk of age, the body weight was 170.5 ± 12.9 g in the control group and 171.1 ± 11.9 g in the DDE group. It reached 414.9 ± 15.2 g in the control group and 443.1 ± 50.5g in the DDE group at 12 wk of age. The body weight was slightly heavier in the DDE group than that in the control group at the end of the observation period, although statistically insignificant.

The weights of organs examined at autopsy are shown

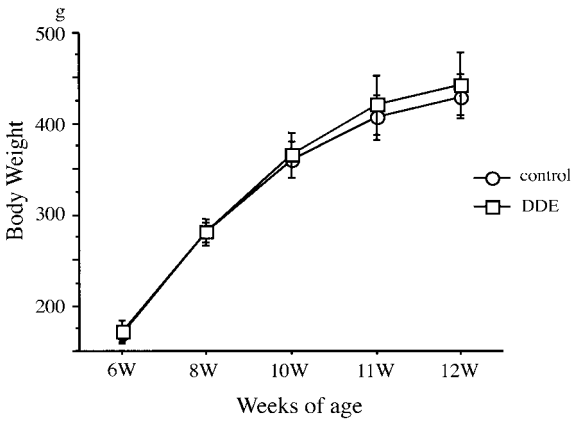

Fig. 1. Body weight changes in male Wistar rats exposed to DDE during the observation period. Vertical bars indicate standard deviations.

Table 1. Relative organ weights of male Wistar rats exposed to DDE at 12 wk of age (g/100 gBW)

	CONTROL	DDE
Liver	4.09 ± 0.41	4.40 ± 0.18
Spleen	0.21 ± 0.03	0.24 ± 0.05
Kidney	0.82 ± 0.09	0.85 ± 0.09
Thymus	0.15 ± 0.03	0.13 ± 0.03

Values (g) are means ± SD (n=6).

Table 2. Serum biochemistry of hepatic and renal function

	CONTROL	DDE
AST (IU/*l*)	79.0 ± 8.2	84.3 ± 13.1
ALT (IU/*l*)	57.8 ± 9.1	63.8 ± 8.9
Cr (mg/d*l*)	0.71 ± 0.06	0.67 ± 0.05
BUN (mg/d*l*)	26.8 ± 2.6	26.5 ± 4.3

Values are means ± SD (n=6). Abbreviations: AST, aspartate; aminotransferase; ALT: glutamate pyruvate transaminase Cr, cretinine; BUN, blood urea nitrogen

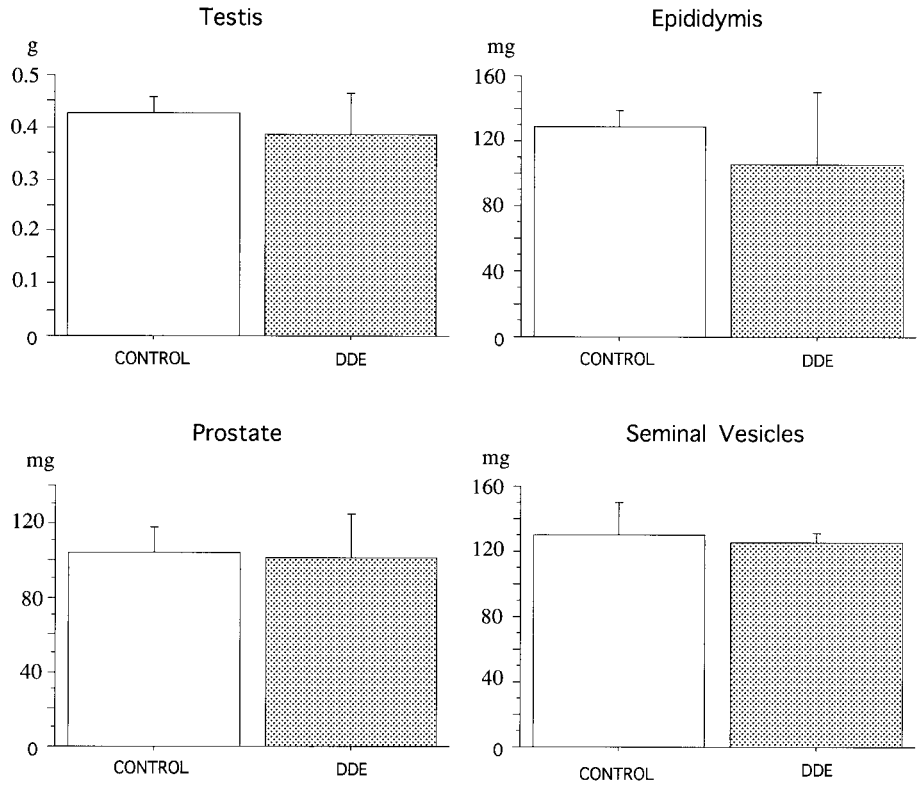

Fig. 2. Relative weights (g/100 g BW) of male reproductive organs exposed to DDE at autopsy. There were no significant weight changes in testis, epididymis, prostate or seminal vesicles in either group.

in Table 1. Relative organ weights were calculated, as the final body weight was slightly different. The liver and spleen weights slightly increased with the administration of DDE. The kidney weight also increased in the DDE group, whereas the thymus weight decreased with the administration of DDE. With regard to male reproductive organs, there were no changes in the weight of testis, epididymis, prostate or seminal vesicles, as shown in Fig. 2. In order to examine general toxicity, serum biochemical indicators of renal and hepatic functions were evaluated (Table 2). These biochemical markers were unaffected by DDE treatment, but AST and ALT slightly increased with the administration of DDE. Renal functions were not impaired by DDE treatment. The serum testosterone concentration is shown in Fig. 3. It increased slightly in the DDE group compared to the control group, but was statistically insignificant. No differences were observed between the control and DDE groups in serum LH and FSH concentrations (Table 3). No treatment-related effects were observed in prostatic acid phosphatase (PAP) concentrations (Table 3). No significant difference was observed between the control

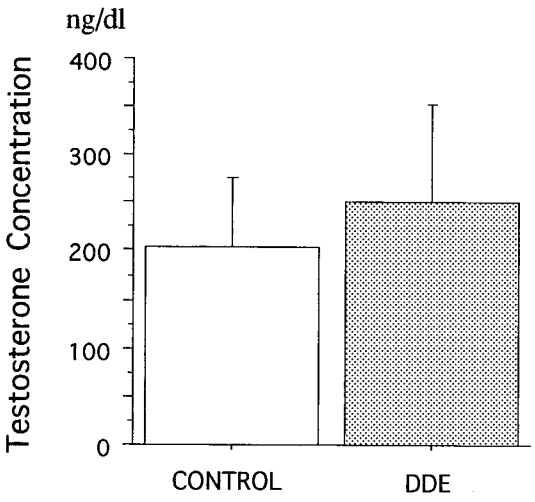

Fig. 3. Serum testosterone concentrations in male Wistar rats exposed to DDE at 12 wk of age. A relative increase in the serum testosterone concentration was observed in the DDE group.

and DDE groups, in epididymal sperm counts, as shown in Table 3. In microscopic studies, no pathological changes were recognized in the organs examined in both groups. Histologically, no necroses of the thymus were deteced by light microscopy.

Discussion

DDE is suspected to be one of the EDCs widely distributed in the environment[12–14]. If this substance were to mimic native hormone actions, its effects would be more serious in immature rats, whose sexual or reproductive organ maturation is still under development. Therefore, the systemic effects of DDE on male Wistar rats were investigated by oral administration during the pubertal period in this study. The dose of DDE administered in this study was 125 ppm, where total daily intake was estimated to be approximately 10 mg/kg DDE, based on daily rat chow consumption. The present study revealed that exposure to DDE during the pubertal period scarcely affected physical development, sexual maturation or the sperm number in male Wistar rats at the dose administered in this experiment. It is well known that DDE affects sexual development of male offspring and induces anogenital distance reduction or retention of thoracic nipples, at a dose of 100 mg/kg, when administered to pregnant rats (dams) during the gestational period[15]. Our results supported the previous report which found that the effects of DDE on male sexual maturation are minimal at a dose below 10 mg/kg/d[15]. In this experiment, DDE to which immature male rats were exposed failed to produce any obvious deterioration and therefore pubertal toxicity was not recognized in male Wistar rats.

Table 3. Serum hormone levels, PAP and epididymal sperm number

	CONTROL	DDE
LH (ng/m*l*)	4.9 ± 0.94	4.8 ± 2.33
FSH (ng/m*l*)	12.4 ± 2.20	12.9 ± 2.80
PAP (ng/m*l*)	1.20 ± 0.19	1.28 ± 0.12
Epididymal Sperm (millions)	258.4 ± 11.6	272.7 ± 25.8

Values are means ± SD (n=6). Abbreviations: LH, lutenizing hormone; FSH, follicle stimulating hormone; PAP, prostatic acid phosphatase

With regard to systemic effects, the growth of pubertal male rats was not affected at all. A slight increase in body weight was recognized. Increases in relative organ weights were observed in the liver and kidney by DDE administration. The increase in these organ weights was considered to reflect the accelerated metabolism of DDE due to overload. Serum AST and ALT levels relatively increased with DDE treatment, compared to the control. Renal functions were not impaired by DDE administration. On the other hand, thymus weight usually decreases with development. This natural thymus atrophy seemed to be accelerated by DDE administration and may be related to immunological disorders as observed in marine mammals[1, 13].

The serum testosterone concentration relatively increased with DDE administration in this study. This seemed to compensate for an antiandrogenic action of DDE as DDE was shown to inhibit the binding of androgens to the androgen receptors and to exert antiandrogenic effects in rats[8, 15].

In conclusion, a large amount of DDE comparable to the tolerable daily intake for humans was administered in this study, but immature male rats were resistant, showing no effect on sexual maturation or physical development. Immunological and metabolic changes were suspected, but these effects were not investigated in detail in our study. Further investigation should be rigorously conducted to reveal all the effects of DDE on immature male rats.

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