ANTIANDROGENIC EFFECTS OF 2,2-BIS(4-CHLOROPHENYL)-1,1-DICHLOROETHYLENE (p,p'-DDE) IN NEONATAL AND FETAL RATS.

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In recent years, there has been increasing evidence that persistent organic pollutants (POPs) can alter endocrine-regulated functions resulting in toxic effects, particularly on the developing organism. This evidence consists of numerous examples of alterations in endocrine homeostasis and developmental effects in experimental animals exposed to POPs. Some examples include decreases in thyroid hormones by dioxins, PCBs and brominated diphenyl (1) estrogenic effects of PCBs and their hydroxylated metabolites (2) and antiandrogenic effects of p,p'-DDE(3). Many of these experimental effects are also observed in wildlife populations exposed to high concentrations of POPs. For example, alterations in penis size in alligators exposed to high concentrations of p,p'-DDE(4). There are also some question on the role of endocrine disrupting chemicals in human diseases. Increases in the incidence of male reproductive tract abnormalities in humans, such as cryptorchidism and hypospadias, appears to have occurred during a period of high exposure to endocrine disrupting chemicals (5). These findings have resulted in increased research on endocrine disrupting chemicals and their potential toxicities to humans and wildlife.

Initial research efforts on endocrine disruptors focused on environmental estrogens and antiestrogens and it had been proposed that the increased incidence of hypospadia and cyrptorchidism was related to exposure to environmental estrogens (6). More recent studies have demonstrated that a number of environmental chemicals demonstrate antiandrogenic actions. One such chemical is p,p'-DDE, a metabolite of the pesticide DDT. p,p'-DDE is a persistent metabolite of DDT and often accounts for almost 85% of the DDT equivalents in humans and wildlife. While o,p-DDT has estrogenic activity in rodents, p,p'-DDE is an androgen receptor antagonist with little or no activity towards the estrogen receptor (3). Developmental exposure to DDE reduces anogenital distance at birth in male rats and these animals have retained thoracic nipples, both of these responses are indicative of antiandrogenic activity (8). Postnatal exposure to p,p'-DDE delays the onset of puberty in male rats and in adult rats, p,p'-DDE exposure can decrease accessory sex gland weight (3).

The present study examines alterations in the concentration of mRNA for androgen receptor (AR) and ornithine decarboxylase (ODC), an androgen responsive gene, in the developing kidney and testes from rats prenatally exposed to p,p'-DDE and the prototypic antiandrogen, flutamide. These studies demonstrate that while *in vitro* studies demonstrate that DDE is a potent androgen receptor antagonist, its *in vivo* efficacy is limited compared to the prototypic antiandrogens.

METHODS:

Animals and Treatment:

Time pregnant female Long Evans rats were exposed to either 0, 50, 100 or 200 mg/kg of p,p'-DDE in corn oil by oral gavage on gestation days (GD) 14-18. An additional set of dams received either 0 or 50 mg/kg of flutamide in corn oil on GD 14-18. In the prenatal studies, dams were killed on GD 19 and serum collected for determination of thyroxin (T4) and triiodothyronine (T3) using a commercially available RIA kit (Diagnostic Products Inc., Los Angeles CA). Fetal testes and kidneys were isolated from 3 individual male pups/dam and used to isolate total RNA for analysis of AR and ODC mRNA concentrations. Maternal livers were removed and microsomes were prepared (9). In a second series of studies, dams received the same dosing regimen, but were allowed to give birth and the dams and pups terminated on postnatal day 21. During this period, pup body weight and anogential distance was determined on PND 1,3,8,15,21. On PND 13 male rats were examined for retained nipples. On PND 21 liver, kidney, testis, prostate, seminal vesicle and ovary weights were determined.

In a third study, the disposition of p,p'-DDE was examined following the administration of ^{14}C-ring labeled p,p'-DDE. Time-pregnant rats were administered 100 ug/kg of p,p'-DDE on GD 14-18 and terminated on GD 19. Each rat received 2uCi/day of ^{14}C-ring labeled p,p'-DDE. Maternal blood, adipose tissue, liver, placenta and fetal liver, urogenital tract and kidney were removed and analyzed for radioactivity. Radioactivity was determined by combustion (Packard 307 Sample Oxidizer, equipped with Oximate 80 robotics, Downers Grove;IL). Fetal tissue and maternal tissue concentrations were determined as previously described.

RT-PCR analysis for Androgen receptor (AR) and ornithine decarboxylase (ODC).

Total RNA isolated from adult Long Evans rat testis was used in RT-PCR reactions to generate cDNA and amplify a 412 base-pair fragment of the AR gene and 355 base-pair fragment of the ODC gene. Primer sequences were :ANDRCP5-1 (5'-GAGAACTACTCCGGACCTTA-3'); ANDRCPR3-1(5'-CAATGTGTGATACAGTCATC-3'); ODC-1(5'-GCAGATACTACGTC-GCAT-3'); ODC-2(5'-GCAGCAGCAACAGTGTAT-3'). A 165 base-pair fragment and a 77 base-pair fragment were deleted from the AR amplicon and ODC amplicon respectively using restriction endonuclease and T4 DNA ligase, followed by cloning into a pCR-Script™ Amp SK(+) cloning vector (Stratagene, La Jolla, CA). The recombinant plasmids containing the truncated amplicons were transcribed in vitro to generate truncated AR and ODC for use as internal competitors in competitive RT-PCR reactions. 100 ng of total RNA combined with specified amounts of internal standard were subjected to RT-PCR reactions and the resultant products quantitated densitometrically using an Alpha Inotech (San Leandro, CA) CCD video camera and image analysis software to estimates molecule numbers.

RESULTS

Administration of 200 mg/kg p,p'-DDE resulted in significant weight loss in the dams and dosing was stopped after the administration of the third dose on GD 16. Administration of 100 mg/kg p,p'-DDE resulted in decreased body weight gains compared to control animals. On GD 19 maternal serum total T4 and T3 were decreased to 40% and 70% of control values respectively at the high dose only. Maternal T4-glucuronidation was increased by 50% by the high dose of p,p'-DDE but was unaffected by a dose of 50 mg/kg. Androgen receptor mRNA was

decreased to approximately 60% of control values with 100 mg/kg p,p'-DDE but was similar to controls in animals treated with 50 mg/kg p,p'-DDE. Androgen receptor mRNA was unaffected in the testis. ODC is an androgen responsive gene and was unaffected by p,p'-DDE treatment in the kidney. The concentration of DDE in fetal liver, kidney, testis and adrenal on GD 19 was 33.9, 12.5, 46.5, and 141.6 ppm on a wet weight basis, respectively. Maternal liver, fat and blood concentrations were 79, 1350 and 22.9 ppm on a wet weight basis, respectively

Litter viability and weaning indices were unaltered by exposure to p,p'-DDE. Pup weights were unaffected by p,p'-DDE administration. Anogenital distance was decreased in a dose dependent manner on PND 3 and 8 but appeared to recovery at later time points. Liver/body weight ratios were increased in pups exposed to p,p'-DDE at both doses on PND 21. There was no effect on kidney or testis body weights in pups exposed to p,p'-DDE. Prostate weight/body weight ratios were decreased by exposure to p,p'-DDE but seminal vesicle weights were unaffected. In female offspring, ovary/body weight ratios were increased by exposure to p,p'-DDE in a dose-dependent manner. In males, p,p'-DDE exposure resulted in a dose dependent increase in retention of thoracic nipples on PND 13, with an incidence (# of pups affected/litter) of 33% at the highest dose. No effected pups had more than two retained nipples. No retained nipples were observed in the control animals.

The administration of 50 mg/kg flutamide from GD 14-18 did not alter maternal body weight compared to control animals. In contrast to DDE treatment, flutamide decreased serum T4 concentrations by 25% but did not alter T3 nor induce T4-glucuronidation in the dams. Anogenital distance was decreased in the male offspring on PND 1,3,8,14 and 21 in the flutamide treated rats. On PND 13, all male pups exposed prenatally to flutamide displayed thoracic nipples and the effected pups had 6-8 nipples/rat. On PND 21, flutamide exposure decreased ventral prostate and seminal vesicle weight, but did not alter ovary weight in the females.

DISCUSSION

The administration of DDE on GD 14-18 produced antiandrogenic effects in the male offspring. These effects observed were consistent with the types of effects observed in rodents following exposure to the androgen receptor antagonist flutamide. There are several differences between the effects observed with p,p'-DDE and flutamide. First, the antiandrogenic effects are more pronounced with flutamide compared to p,p'-DDE. Attempts to increase the dose of DDE to increase the antiandrogenic effect resulted in significant maternal toxicity as demonstrated by decreased body weight in the dams receiving 200 mg/kg DDE. No maternal toxicity, as measured by body weight loss, was apparent in the dams receiving flutamide. p,p'-DDE resulted in greater decreases in serum T4 and T3 compared to the flutamide treated animals. In addition, p,p'-DDE increased T4 glucuronidation in the maternal liver at the doses that decreased thyroid hormones. Flutamide slightly decreased thyroid hormones but did not increase maternal hepatic T4-glucuronidation. These data indicate that p,p'-DDE, while having antiandrogenic actions, is not a potent or efficacious antiandrogen in rats.

The tissue concentrations of p,p'-DDE reported in this study are significantly higher than concentrations reported in human tissue samples. For example, in a study of women with no known high exposure to p,p'-DDE from New York, USA, serum and milk concentrations ranged from 0.5 to 3.6 and 1.7 to 22 ppb on a wet weight basis, respectively (10). In a highly exposed population in KwaZulu, p,p'-DDE concentrations mean serum concentrations of p,p'-DDE was 103 ppb on a wet weight basis, while control means were approximately 6 ppb (11). These data suggest that the present exposures in the rodent studies are much higher than those observed in

highly exposed human populations. The antiandrogenic effects of p,p'-DDE must be viewed cautiously at this time and future studies of this chemical at steady-state concentrations would aid in future assessments of this chemical

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