Effects of Triphenyltin Chloride (TPTCl) on Development of Reproductive Organs and Leydig Cell T-Production in Male Rats

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Introduction

Organotin Compounds have become important commercial organometals. They have been used widely as agricultural pesticides, as antifouling paints for ship hulls and fishery firm nets and as stabilizers to lessen the effects of heat and light in polyvinyl chloride plastics.

Not only tributyltin but also triphenyltin(TPT) has been shown to have a strong effect on the development of imposex in the rock shell, *Thais clavigera*.[1] In terrestrial mammals, triorganotins have pronounced immunotoxic effects. In guinea pigs and rats, TPT reduced lymphopoiesis and caused atrophy of the white pulp of the spleen and thymus. [2] Also TPT are highly embryotoxic, peripheral myopathy and genotoxic effects.[3, 4] TPT appears to cause reproductive effects in rats and developmental toxicity in rats, rabbits, and hamsters. In a two-generation rat study of TPT, an increase in the number of dead F1 pups and a decrease in mean litter size, pup weight, and relative spleen and thymus weights in the weanlings were observed. When TPT was dosed by gavage on gestation day, TPT prevented implantation in rats.

But there are scarcely reports identifying effects of TPT on development of reproductive organs in male rats before and after puberty period. Therefore, in this studies, we compared the effects of TPTCl on development of reproductive organs, serum testosterone level, and Leydig cell testosterone production between 5-week-old and 7-week-old SD male rats and found that TPTCl affected the development of reproductive organs in male rats and was more sensitive to 5-week-old than 7-week-old and that serum testosterone level and Leydig cell testosterone production was decreased by TPTCl in only 7-week-old SD rat.

Material And Methods

Animals and Treatment: Male Sprague-Dawley rats (4 and 6-week-old) were purchased from Samtako bio KOREA, Inc. (O Sna-Shi, Korea), and were housed 3 per cage in clear plastic cages on wood chips. They were fed a pellet rodent diet (Shinchon Co., Seoul, Korea) and tap water ad libitum. Environmental conditions were controlled, i.e., 21-25℃, a relative humidity of 50-60%, a frequency of ventilation change of >15 air exchanges/h, and a 12 -h light/dark cycle (light on; 7:00-19:00). Prior to treatment, all animals were acclimated for 7 days. The study was conducted in a accredited Korea FDA aninmals facility in accordance with the guidelines for animal experiment of the Association for Assessment and Accredtation of Laboratory Animal Care (AAALC) International Animal Care Policies (Accredited Unit-Korea Food and Drug Administration: Unit Number -000996). Four and 6 -week-old male rats (10 rats/dose) were treated by oral gavage with TPTCl (0, 1, 5, 10, and 15 mg/kg/day; Sigma Co., St. Louis, MO) for 14 days. Corn oil (Sigma Co., St. Louis, MO) was used as a vehicle, at a dosage of 5ml/kg body weight. One day after the last dose, all animals were euthanized under light ether anesthesia and blood was collected from the abdominal aorta. Blood was allowed to clot at room temperature after serum collection, and stored in aliquots in capped vuals a -20℃ until serum hormone concentration were analyzed. Testes were excised and used for purification of Leydig cell and testosterone production test (T -production). The epididymides, prostate, and seminal vesicle were carefully dissected and weighed.

Hormonal measurements:

To determine the testosterone levels in serum, collected serum was extracted three times with 3 volume of diethyl ether. Ether extracts were dried in speed vacuum evaporator and reconstituted with gelatin containing phosphate

buffered saline (GPBS, pH=7.2). Recovery rate of these steroids in the plasma was relatively constant (95.2%, n=10). Labeled testosterone (1, 2, 6, 7 -3H-testosterone; 96 Ci/mole was used. The testosterone antiserum was developed in rabbit using testosterone -17-β-hemisuccinate;BSA as an immunogen. Radioactivity of each sample was quantified by using a liquid scintillation analyzer (Packard, TR -2900). Routinely, two sets of standard (5 - 2000 pg) were included in each assay. Steroid concentration calculated with a Riasmart program (Packard) by personal computer.

Purification of Leydig cell and T-production test:

Leydig cells were isolated from testes by following a modified procedure.[5] Briefly, after removing the testicular capsule, the testes were placed in a dissociation buffer. Tissues were mechanically dispersed. Seminiferous tubules from dispersed testes were allowed to settle for 5 min, and the supernatants were collected by aspiration with a pipette. The same procedure was repeated on the settled seminiferous tubules. The combined supernatant were centrifugated at 80xg for 10 min to collect the Leydig cells. After washing, the crude Leydig cells were purified by continuous Percoll density gradient centrifugation. The purity of Leydig cells as assessed by 3β-hydroxysteroid dehydrogenase staining was shown to be greater than 80%. To measure the rates of T production, aliquots of 0,1-0,2 x 10^6 Leydig cells were incubated in microcentrifuge tubes in 1ml culture medium. Incubations were conducted at 34℃ for 3h using the maximally stimulating dose of 100ng/ml LH. Leydig cell T production values were normalized to nanograms per 10^6 cells.

Statistical analysis:

All values are expressed as mean and standard deviation (SD). The means were compared using Dunnett's test after one-way analysis of variance using a computer program (SigmaStat V 2.03, SPSS, Inc., Chicago). Significant differences between values were set at $p<0.05$.

Results and Discussion

In this study, we investigated effects of TPTCl on development of reproductive organs, serum testosterone level and Leydig cell testosterone biosynthesis in SD male rats before and after puberty period.

In 5-week-old rats, TPTCl(10 and 15mg/kg/day) significantly decreased the weight of testes, epididymis, and prostate. The weight of seminal vesicle was significantly decreased in a dose-dependent manner at the dose of 1mg/kg/day and above (Figure 1). In 7-week-old rats, TPTCl(15mg/kg/day) significantly decreased the weight of epididymis and seminal vesicle. The weight of prostate was significantly decreased at the dose of 5mg/kg/day and above (Figure 2). Seminal vesicle was the most sensitive organ in 5-week-old rats, wheras the prostate was the most responsive in 7-week-old rats. TPTCl significantly affected reproductive organs at the dose of 1mg/kg/day in 5-week-old rats, but not in 7-week-old rats. These results were similar to other reports demonstrating that TPT (6, 12mg/kg/day) decreased the weight of epididymides, prostate, and seminal vesicle (K. Grote et al., 2004).[6]

K. Grote et al. (2004) observed that TPT (6mg/kg/day) decreased serum testosterone levels and increased serum LH levels.[6] In the present study, TPTCl significantly decreased serum testosterone levels at 15mg/kg/ only in 7-week-old SD rat (Figure 3). TPTCl also inhibited testosterone production at the dose of 10 and 15mg/kg/day in Leydig cell from 7-week-old SD rats, but not in 5-week-old rats (Figure 4).

From the above results, we found that TPTCl affected the development of reproductive organs in male rats and was more toxic to 5-week-old than 7-week-old rats. Serum testosterone level and Leydig cell testosterone production was decreased by TPTCl in only 7-week-old SD rat.

Acknowledgments

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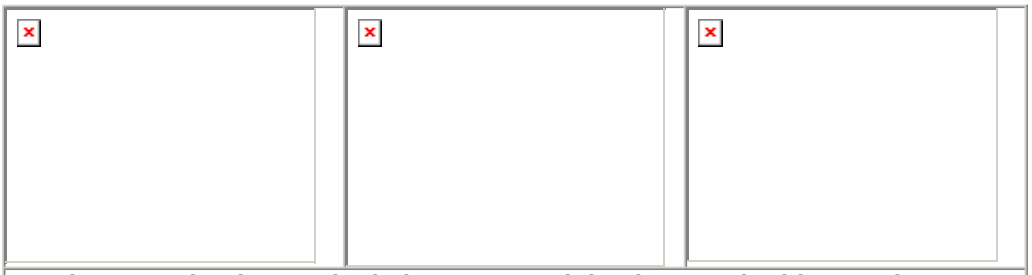

Figure 1. Absolute and relative organ weights in 5-week-old SD male rats treated with TPTCl for 14 days. *: Significantly different from control by Dunnett's Test (P 0.05)

Figure 2. Absolute and relative organ weights in 7-week-old SD male rats treated with TPTCl for 14 days. *: Significantly different from control by Dunnett's Test (P 0.05)

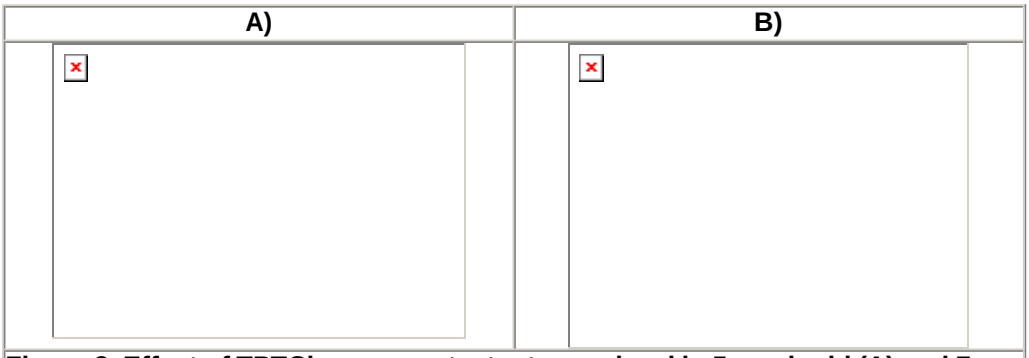

Figure 3. Effect of TPTCl on serum testosterone level in 5-week-old (A) and 7-week-old (B) male rats treated with TPTCl for 14 days. *: Significantly different from control by Dunnett's Test(P 0.05)

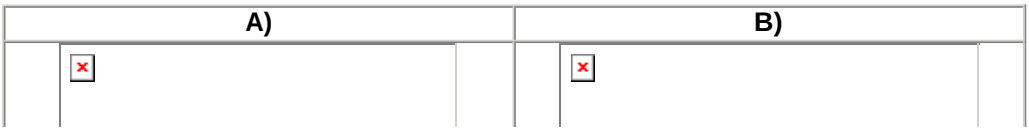

Figure 4. Effect of TPTCl on testosterone production in Leydig cells in 5-week-old (A) and 7-week-old (B) male rats treated with TPTCl for 14 days. Each column represents the mean±SD. *: Significantly different from control ($P<0.05$)