

Effects of Tributyltin Chloride on the Reproductive System in Pubertal Male Rats

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Abstract

Detrimental effects of tributyltin (TBT) chloride on the reproductive system were investigated in pubertal male rats. Sixty Sprague-Dawley rats aged with 35 days were assigned to six different groups; negative control receiving vehicle, positive control receiving methyltestosterone (10 mg/kg B.W.), TBT chloride (5 mg/kg B.W., 10 mg/kg B.W., and 20 mg/kg B.W.), and a combination of TBT chloride (10 mg/kg B.W.) and flutamide (10 mg/kg B.W.). The animals were treated with test compounds by oral gavage daily for 10 days and sacrificed on the next day of the final treatment. The treatment with TBT chloride at the doses of 10 and 20 mg/kg B.W. significantly decreased seminal vesicle weights, compared to the negative control. The combined treatment of TBT chloride and flutamide caused a significant decrease in accessory sex organ weights, compared to the control and TBT chloride treatments. The treatment with TBT chloride or in the combination with flutamide increased detached debris and sloughed cells in the tubules of epididymis and narrowed seminal vesicles. In addition, the combined treatment with TBT chloride and flutamide caused a noticeable increase in serum androgen level, compared to the negative control.

These results suggest that TBT chloride exposed during pubertal period cause partial reproductive disorders in male rats.

Key words: accessory sex organ, flutamide, methyltestosterone, TBT chloride

Introduction

Organotin compounds are a broad group of chemicals widely used in agriculture and industry [14, 20]. Tributyltin (TBT) compounds have been used as antifouling agents, plastic stabilizers, wood preservative agents and in a variety of applications [20]. TBT compounds have lately attracted considerable attention, because they are directly introduced into aquatic organisms by their use as an antifouling agent in paints and they are bioaccumulated in food chain [9, 14, 23]. Though the levels of TBT compounds were not sufficiently high to have adverse effects on human health, possible exposure of humans to TBT compounds aroused a great concern about their toxic potential [28].

A variety of reproductive toxicities of TBT compounds have been reported in some laboratory and wild animals. The exposure of TBT chloride during preimplantation period produced early embryo loss and implantation failure in rats [11, 12]. In addition, TBT chloride exposure during pregnancy has been associated with increased incidence of fetuses with cleft palate and induced fetal reabsorption in rats [7, 8]. In the study of two-generation reproductive toxicity in the male rat, decreases in body weight and sex organ weights were pronounced, and sperm counts of testis and cauda epididymis were also decreased in F1 and F2 neonates [19]. Some researches on the various aqueous organisms which live in the immediate vicinity of the coast-line have clearly shown that TBT caused imposex showing male sexual characteristics in females [17, 26]. Laboratory experiments with dog-whelk gastropods proven that TBT promotes imposex at very low concentrations [10, 25]. Recently, it has been reported that TBT compounds are culprit of decline in populations of common whelks in some area of the world [2, 26, 27]. Putative mechanism of endocrine disrupting action of TBT was ascribed to the secretion of Penis Morphogenic Factor (PMF) inducing male differentiation and/or hormonal disruption by inhibition of aromatase [16].

There are scarcely reports identifying short-term effects of TBT compounds on reproductive system in male rats. Therefore, the present study was to investigate effects of

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TBT chloride after treatment during ten consecutive days on the male reproductive system by determining testicular and epididymal weights and serum testosterone levels and by observing histopathology of reproductive organs.

Materials and Methods

Animals: Twenty eight old male Sprague-Dawley (CrI:CD IGS BR) rats were purchased from Biogenomics company (Gapyeong, Korea) and allowed to be adapted for 7 days prior to beginning of treatments. Animal facilities were maintained under controlled conditions with temperature of 21 ± 2 , relative humidity of $50 \pm 10\%$, and artificially illuminated (fluorescent light) on a 12-hr light/dark cycle. They were fed with Samyang chow (Cheonan, Korea) and filtered tap water *ad libitum*. After quarantine period, sixty rats with adequate weight gain and without clinical signs were divided by computerized and stratified randomization into six experimental groups so that there were no differences of statistical significance and standard deviation among groups in body weights.

Study design: There were six experimental groups: Corn oil for negative control, methyltestosterone (MET, 10 mg/kg B.W./day) for positive control, TBT chloride (5, 10, and 20 mg/kg B.W./day), and combined treatment of TBT chloride (Aldrich Chemical Co. Inc., WI, USA., 96% pure) and flutamide (10mg/kg B.W./day, respectively). The combined treatment of TBT chloride and flutamide (Sigma Chemical Co., St. Louis, MO, USA) was set to identify that flutamide, a anti-androgen, recovers possible androgenic effects of TBT chloride. TBT chloride and methyltestosterone (Sigma Chemical Co., St. Louis, MO, USA) were prepared in corn oil (Sigma Chemical Co., St. Louis, MO, USA) and administered daily by oral gavages at around 10:00 AM during 35 to 44 days of age. The dose volume was 1.5 ml/kg B.W. The animals were sacrificed on the next day of final treatment.

Pathological evaluations: On the sacrifice day, rats were anesthetized using ethyl ether and euthanized by exsanguinations. Blood was collected from the descending vena cava and serum was prepared for hormonal analysis. For all groups, sex organs were weighed. Accessory sex organs were placed in formalin fixative and testes were placed in Bouins fixative. After normal processing for hematoxylin and eosin staining, all sex organs were examined microscopically.

Hormonal measurements: Prepared serum were stored between -65 and -85 until analysis for serum hormone concentrations. Hormone level was measured with a commercial RIA kit (Orion Co., Espoo, Finland). In this kit cross-reactivity of testosterone antiserum at 50% binding level was follows. Testosterone was 100%, 5 α -dihydrotestosterone was 4.5%, methyltestosterone was 0.45%, and other steroid hormones was less than 0.03%. Because cross-reactivity for androgens except testosterone are negligible, the steroids measured using this antiserum referred to testosterone.

Statistics: Statistical analyses of the data were performed using the SPSS 9.0 program. The data were analyzed by one-way ANOVA followed by least significant difference test when the ANOVA test yielded statistical differences ($p < 0.05$) among the groups.

Results

Clinical signs and final body weights: There were no abnormalities in clinical signs or gross findings for all animals. Mean final body weights were not affected by the administrations of TBT chloride, methyltestosterone, or combination of TBT chloride and flutamide at the dosages tested (Fig. 1).

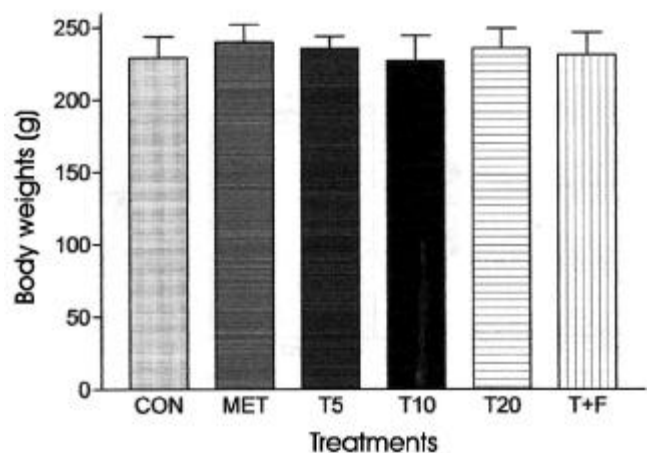


Fig. 1. Comparison of body weights in the male rat following daily oral treatments with tributyltin chloride (T5: 5 mg/kg B.W., T10: 10 mg/kg B.W., T20: 20 mg/kg B.W.), methyltestosterone (MET), a combination of tributyltin chloride and flutamide (T+F) during 35 to 44 days of age. The weight values are expressed as the mean \pm S.D. ($n=10$).

Sex organ weights: Changes in sex organ weights of rats were presented in Fig. 2. Paired testicular weights were not affected by the treatment of TBT chloride, methyltestosterone, or combination of TBT chloride and flutamide at the dosages tested. The combined treatment of TBT chloride and flutamide (0.24 ± 0.02 g) caused a significant ($p < 0.01$) decrease in paired epididymal weights, compared to the control (0.31 ± 0.02 g). The treatment of methyltestosterone and TBT chloride did not show any significant difference in prostate weights of rats from the control. However, the combined treatment of TBT chloride and flutamide (0.12 ± 0.02 g) significantly ($p < 0.01$) decreased the prostate weight of rats, compared to the control (0.17 ± 0.02 g). TBT chloride treatments caused a dose-dependant decrease in seminal vesicle weights, and there were significances at the doses of 10 and 20 mg TBT chloride/kg B.W., compared to the control. Combined treatment of TBT chloride and flutamide also caused a severe decrease in seminal vesicle weight, compared to the control ($p < 0.01$).

Histopathological findings: No TBT compound-related gross or histological changes in the testes and prostate of rats were observed in all experimental groups. In the epididymis and seminal vesicle, however, microscopic changes were induced by treatments of TBT chloride, or combination of TBT chloride and flutamide (Fig. 3 and 4). Increments of detached debris and some sloughed cells in the tubules of epididymis were observed in rats treated with TBT chloride only and combination of TBT chloride and flutamide, compared to the control rats (Fig. 3). The treatment of TBT chloride or combination of TBT chloride and flutamide also produced histological changes in the seminal vesicle of rats, that is, the vesicles were narrowed and occupied with epithelial cells (Fig. 4). The treatment of methyltestosterone did not show any histopathological changes in the epididymis and seminal vesicle of rats.

Serum testosterone levels: Treatments with methyltestosterone and TBT chloride during pubertal period did not change serum androgen levels (Fig. 5). However, the combined treatment of TBT chloride and flutamide (1.45 ± 0.67 ng/ml) significantly ($p < 0.01$) increased serum testosterone level in rats, compared to the control (0.28 ± 0.14 ng/ml).

Discussion

This study was designed to identify the androgenic actions of TBT chloride and reproductive toxicity. Male pubertal rats aged with thirty-five days was selected for treatments for 10 consecutive days. It is well known that this period is very sensitive to exposures of various pharmaceutical and environmental compounds, since rapidly interactive endocrine and morphological changes occur in this period. Major pubertal events are the changes in reproductive system. Sex organ weights and serum testosterone levels in male rats increases rapidly, and microscopic testicular changes are characterized by formation and progressive expansion of the seminiferous tubule lumen, a progressive increase in germ cell volume and numbers, and a progressive decrease in the number of degenerating germ cells [21, 24]

In this study, we investigated the effects of treatment chemicals on reproductive organs and serum testosterone changes after treatment during pubertal period. TBT chloride treatment caused various reproductive disorders in pubertal male rats. Although body weight, paired testicular

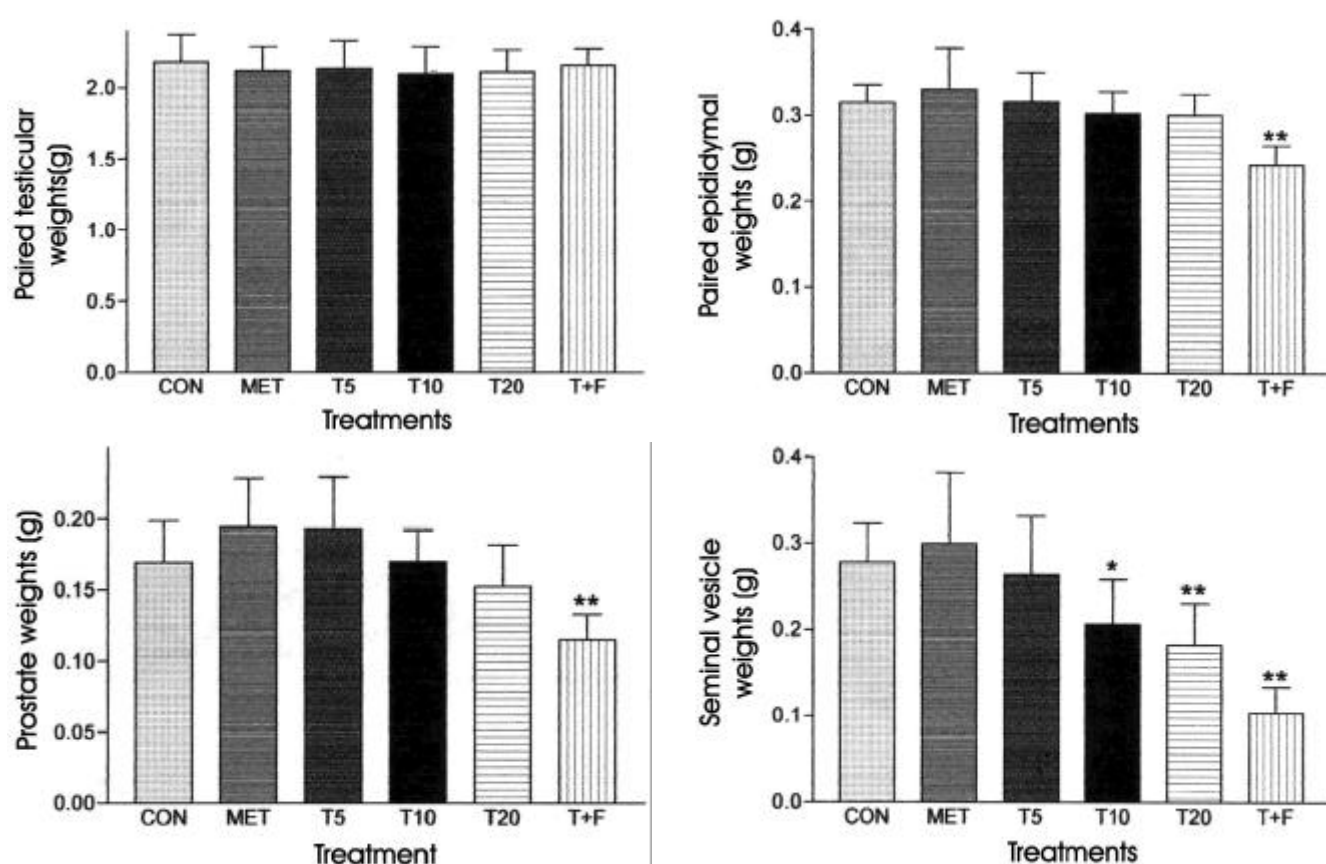


Fig. 2. Weight comparison of testis and accessory sex organs in the male rat following daily oral treatments with tributyltin chloride (T5: 5 mg/kg B.W., T10: 10 mg/kg B.W., T20: 20 mg/kg B.W.), methyltestosterone (MET), and a combination of tributyltin chloride and flutamide (T+F) during 35 to 44 days of age. The weight values are expressed as the mean \pm S.D (n=10). Asterisks on the bars mean significant difference compared to the control (* $p < 0.05$, ** $p < 0.01$).

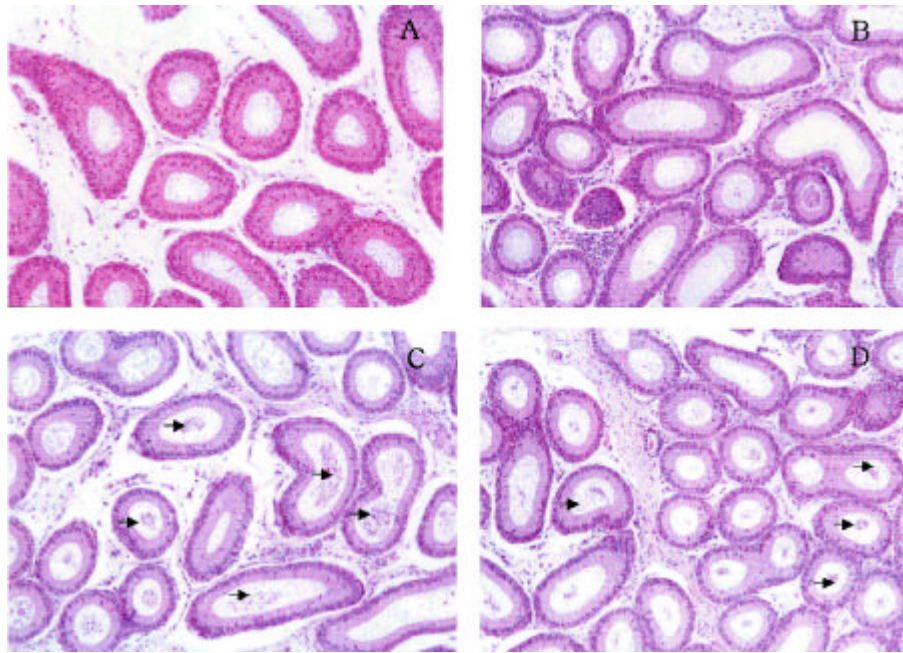


Fig. 3. Histopathology of caput epididymis in the male rat following daily oral treatments with tributyltin chloride, methyltestosterone, and a combination of tributyltin chloride and flutamide during 35 to 44 days of age. A, vehicle control. B, methyltestosterone. C, tributyltin chloride (20 mg/kg B.W.). D, tributyltin chloride (10 mg/kg B.W.) + flutamide (10 mg/kg B.W.). Arrows indicate increments of detached debris and some sloughed cells in the tubule lumens of caput epididymis in rats treated with tributyltin chloride and tributyltin chloride + flutamide, compared to controls. All magnification, $\times 100$.

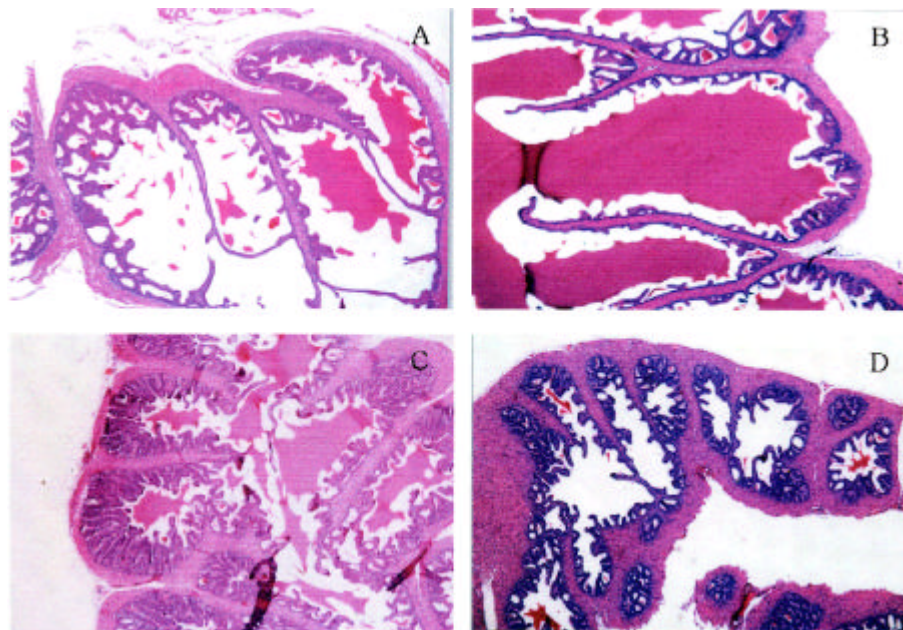


Fig. 4. Histopathology of seminal vesicle in the male rat following daily oral treatments with tributyltin chloride, methyltestosterone, and combination of tributyltin chloride and flutamide during 35 to 44 days of age. A, vehicle control. B, methyltestosterone. C, tributyltin chloride (20 mg/kg B.W.). D, tributyltin chloride (10 mg/kg B.W.) + flutamide (10 mg/kg B.W.). Significant histological changes in the seminal vesicle are showed in rats treated with tributyltin chloride and tributyltin chloride + flutamide, which are characterized by narrowed vesicles and occupied epithelial cells. All magnification, $\times 40$.

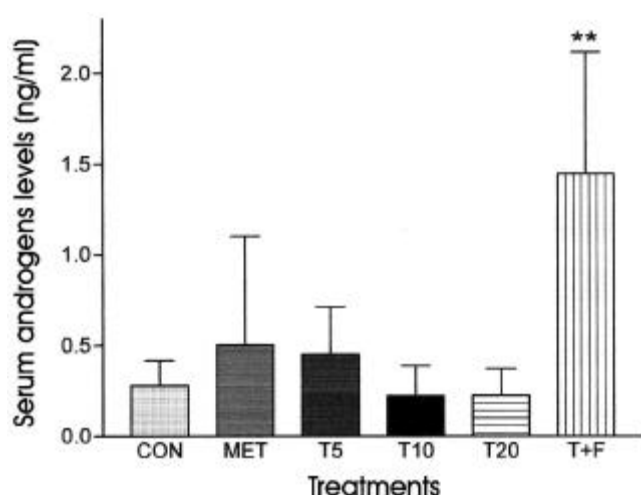


Fig. 5. The changes in serum testosterone levels in the male rat following daily oral treatments with tributyltin chloride (T5: 5 mg/kg B.W., T10: 10 mg/kg B.W., T20: 20 mg/kg B.W.), methyltestosterone (MET), and a combination of tributyltin chloride and flutamide (T+F) during 35 to 44 days of age. The values are expressed as the mean \pm S.D (n=10). Asterisks on the bars mean significant difference compared to the control (**p<0.01).

and epididymal weights were not significantly altered by the treatments of TBT chloride, these treatments decreased the weights of prostate gland and specially seminal vesicle weight in a dose-dependent manner. Recently, it was elucidated that TBT inhibits human 5 α -reductase [5] and aromatase activities [4]. In adult rats weights of seminal vesicle and prostate weight were decreased by treatments of finasteride, an inhibitor for 5 α -reductase which converts testosterone into dihydrotestosterone [18], and anastrozole, an inhibitor for aromatase which converts testosterone and androstenedione into 17 β -estradiol and estrone, respectively [18]. Major preferred hormone related to growth of these organs is not testosterone but dihydrotestosterone [22]. Lower intracellular dihydrotestosterone levels are related to decrease of these organ weights [22]. And also, it was reported that anastrozole treatment decreases prostate and seminal vesicle weights [6]. The androgen and estrogen receptors exist in these organs. It is regarded that estrogen stimulates androgen receptor expression, and maintains normal function of sex organs. Anastrozole treatment decreased the production of 17 β -estradiol, and then disturbed normal organ functions [1, 3]. Thus, decreases of prostate and seminal vesicle weights in this study were likely to be induced by inhibition activity of TBT chloride for 5 α -reductase and aromatase.

Histopathological findings in the testis showed normal in TBT treatment groups. TBT chloride intake of male adult rats in two-generation toxicity study induced mild testicular histological changes which were vacuolization of seminiferous epithelium, spermatid retention in the epithelium,

delayed spermiation, and germ cell degeneration [19]. In this study, we did not find histological disorders in the testis, but observed indirect testicular dysfunction from histopathological findings of epididymis. The caput epididymal epithelium of rats treated with TBT chloride showed the normal, but disorders characterized by increments of detached debris and some sloughed cells considered to originate from seminiferous tubules of the testis.

The combined treatment of TBT chloride and flutamide was intended to identify that flutamide, a potential antiandrogen, recovers possible masculinizing effects induced by TBT chloride suggested by other experiments [17, 26]. In this study, administration of TBT chloride and flutamide in the rats amplified adverse effects of TBT chloride. The weights of accessory sex organs were more decreased by the combined treatment than those of TBT chloride only, implying that TBT chloride is not purely ligand of androgen receptor. On the other hand, serum testosterone level was significantly elevated in the combined treatment. Based on the current result that serum testosterone level in TBT chloride regimen was similar to that in control regimen, a marked increase in combined treatment regimen was likely to be caused by flutamide treatment exclusively. This suggestion could be comparable to the previous result reported by others [13, 15]. The changes in serum testosterone level imply that TBT chloride is not a pure androgenic compound.

In conclusion, the oral application of TBT chloride to pubertal male rats during 35 to 44 days of age produces various reproductive disorders. These adverse effects of TBT chloride on reproductive system in pubertal rats are most likely to be due to its disturbing activities of 5 α -reductase and aromatase during pubertal period.

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