Effects of Tributyltin Chloride(TBTCl) on Seroid Hrmone and Seroidogenic Ezymes in Sprague-Dawley Male Rat.

Tae Seok KANG[1], Su Jung Lee[1], Jae-Ho Shin[1], Tae Sung Kim[1], Hyun Ju Moon[1], Ho Yun Ki[1], Hoon Bae[1], Mi Sook Dong[2], Yong-Dal Yoon[3], Soon Young Han[1]

[1]Endocrine Toxicology Division, National Institute of Toxicological Research, Korea FDA, Seoul
[2]Korea University, Seoul
[3]Hanyang University, Seoul

Introduction

Tributyltin (TBT), organotin compound is used a wood preservative, a stabilizer of poly-vinyl chloride (PVC), a bactericide, a vermicide, and antifouling agent in maritime paint. TBTCl has been known to bioaccumulate through the food chain and induce imposex in female gastropods.[1]

Testosterone was synthesized from cholesterol in Leydig cell. In male rat testes, cholesterol in in the inner membrane of mitochondria is converted to prognenolone by cytochrome P450 side-chain cleavage (P450scc), and prognenolone is consequently converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD). Progesterone is converted to testosterone by cytochrome P450 17α-hydroxylase/C17-20 lyase (P450c17), and 17β-hydroxysteroid dehydrogenase (17β-HSD). Testosterone is converted to estradiol by P450 aromatase.

Some studies have been reported that steroidogenic enzyme and steroid hormone were affected by TBT. In a two-generation study of tributyltin chloride, serum estradiol was decreased in F1 and F2 male rats. But serum concentration of testosterone and luteinizing hormone (LH) were not changed in F1 and F2 male rats.[2] When pregnant rats were orally administrated by TBTCl, serum progesterone was decreased and serum estradiol was increased in female litters. Also litters were affected on development of reproductive organs and sexual of differentiation by TBTCl.[3] Tributyltin increased serum progesterone in granulosa cells, but serum testosterone and estradiol were diminished.[4] TBT has been known that it repressed P450 aromatase activity.[4]

In this study, we investigated effect of tributyltin chloride (TBTCl) on the mRNA expression of steroidogenic enzymes and steroid hormone in male rat.

Material And Methods

Animals and Treatment: Male Sprague-Dawley rats (3weeks old) were purchased from Samtako bio KOREA, Inc. (O Sna-Shi, Korea), were housed 3 individuals per in clear plastic cages on wood chips and given a pellet rodent diet (Shinchon Co., Seoul, Korea) and tap *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 acclimated for 7 days. All animals in the experiment were in an 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). Immature male rats (29 dayof age, 10 rats/dose) were treated by oral gavage with TBTCl (0, 1, 5, 10, and 20 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 at -20□ until serum hormone concentration were analyzed. Testes were excised and stored at -80□ use RNA isolation.

Hormonal measurements: To determine the progesterone, testosterone, and estradiol-17b levels in serum, collected serum were 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 progesterone, testosterone(1, 2, 6, 7-3H-testosterone; 96 Ci/mole), and estradiol-17β(1,2,6,7-3H-estradiol; 110Ci/mole) was used. The testosterone antiserum was developed in rabbit using testosterone-17-β-hemisuccinate;BSA as an immunogen. The estradiol-17β antiserum was developed in rabbit using estradiol 6-(O-carboxymethyl)oxime-BSA as an immunogen. Radioactivity of each sample was quantified by using 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.

Analysis of steroidogenic enzyme expression by RT-PCR : Total RNA of the testis was isolated by TRIzol reagent (Invitrogen, Carlsbad, UK) according to the manufacturer's protocol, and quantified spectrophotometrically by absobance at 260/280nm (BIO-RAD SmartSpec 3000). Isolated total RNA were confirmed with resolution in 2% agarose gel (BIO-RAD, Hercules, U.S.A) containing Orange G (Sigma & Aldrich CO. St. Louis, MO, U.S.A).

RT-PCR was used to assess mRNA levels by first reverse transcribing total RNA using SuperScript First –Strand Synthesis System for RT-PCR Kit and SuperScript ^{TM}II (Invitrogen, Carlsbad, U.K.). To amplify cDNA for P450scc, 3B-HSD, aromatase and b-actin, different primer and RT-PCR protocol was used (Invitrogen, Carsbad. U.K.). Amplified cDNA fragments generated were resolved in 2% agarose gels containing ethidium bromide, and visualized using a digital imaging system.

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 the effect of TBTCl administration on steroidogenesis by steroidogenic enzyme mRNA expression in testes and stroid hormone level in serum. TBTCl significantly affected the serum progesterone and serum estradiol levels (Figure 1). Serum progesterone level was significantly decreased at 20 mg/kg/day TBTCl-treased group, and serum estradiol level was significantly decreased at 10 and 20 mg/kg/day TBTCl-treased groups . Harazono et al. (2000) have shown that TBTCl 16.3mg/kg and above decreased in serum progesterone level and estradiol level.[5] In two-generation study, the serum estradiol level was decreased by TBTCl(125 ppm) in the F1 and F2 generation male.[2]

TBTCl significantly affected the expression levels in P450scc, 3β-HSD and aromatase mRNA (Figure 2). P450scc mRNA level was significantly decreased at 20 mg/kg/day TBTCl-treated group. The expression levels of 3β-HSD mRNA were decreased at at all treated animals, but it was not dose-dependent. The 3β-HSD mRNA level was significantly decreased at 20 mg/kg/day TBTCl-treated group.

P450 aromatase mRNA level were decreased at 5, 10 and 20 mg/kg/day TBTCl-treated group at dose-dependent. Significant decreases in P450 aromatase mRNA level were observed at 10 and 20 mg/kg/day TBTCl-treated group. The expression levels of both P450scc and 3β-HSD mRNA were significantly decreased at 20 mg/kg/day TBTCl-treated group. It has been not reported of effects of TBTCl on expression levels of P450scc and 3β-HSD mRNA, but study of 3β-HSD activity was reported. Mark et al. (2003) have shown that TBTCl (12 and 59uM) inhibited 3β-HSD activity. [6]

From these results, we found that TBTCl altered mRNA expression of enzymes related steroidogenesis, P450scc, 3β-HSD and aromatase and serum progesterone and estradiol level. So, we suggest that decrease in the serum progesterone concentration may be related to decrease in expression level of P450scc and 3β-HSD mRNA and decrease in the serum estradiol concentration may be related to decrease in expression level of aromatase mRNA. In addition, these suggest that the change of hormone levels may be due to the alteration of mRNA levels of steroidogenic enzyme in testes by TBTCl.

Acknowledgments

This work was supported by NITR/Korea FDA Grant for Endocrine Disruptors Research.

References

1. Bryan GW, Gibbs PE, Burt GR 1986, A comparison of the effectiveness of tri-n-butyltin chloride and five other organitin compounds in promoting the development of imposex in the dog-welk, Nucella lapilus. *J. Mar. Biol Ass UK.* 68: 733-744.
2. Omura M, Ogata R, Kubo K, Shimasaki Y, Aou S, Oshima Y, Tanaka A, Hirata M, Makita Y, Inoue N. 2001. Two-generation reproductive toxicity study of tributyltin chloride in male rats. *Toxicol. Sci.* 64(2):224-32.
3. Ogata R, Omura M, Shimasaki Y, Kubo K, Oshima Y, Aou S, Inoue N. 2001, Two-generation reproductive toxicity study of tributyltin chloride in female rats. *J. Toxicol. Environ. Health. A.* 25;63(2):127-44.
4. Schoenfelder M, Schams D, Einspanier R. (2003) Steroidogenesis during in vitro maturation of bovine cumulus oocyte complexes and possible effects of tri-butyltin on granulosa cells. *J Steroid Biochem Mol Biol.* 84(2-3):291-300.
5. Harazono A. Ema M. 2000, Suppression of decidual cell response induced by tributyltin chloride in pseudopregnant rsts : a cause of early embryonic loss. *Arch. Toxicol.* 74(10):632-7.
6. Mark J. McVey, Gerard M. Cook (2003) Inhibition of rat testis microsomal 3B-HSD activity by tributyltin. *J Steroid Biochem Mol Biol.* 86: 99-105.

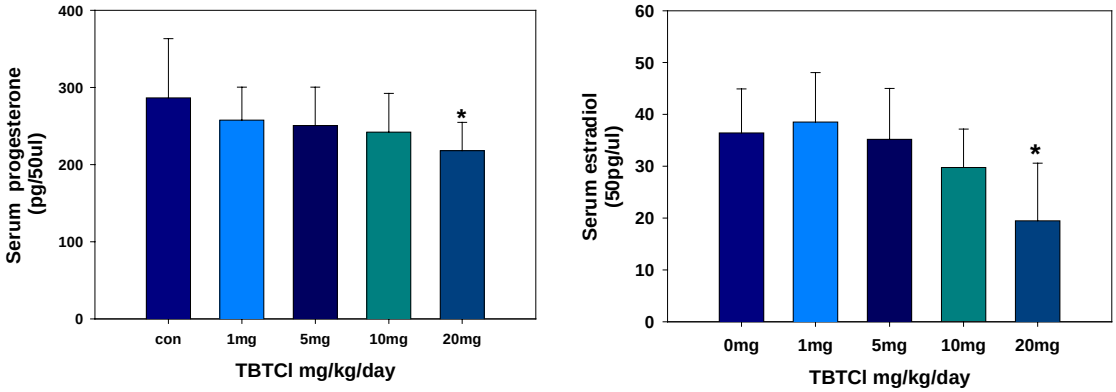

Figure 1. Effect of TBTCl on serum level of progesterone and estradiol. *: Significantly different from the control (P<0.05).

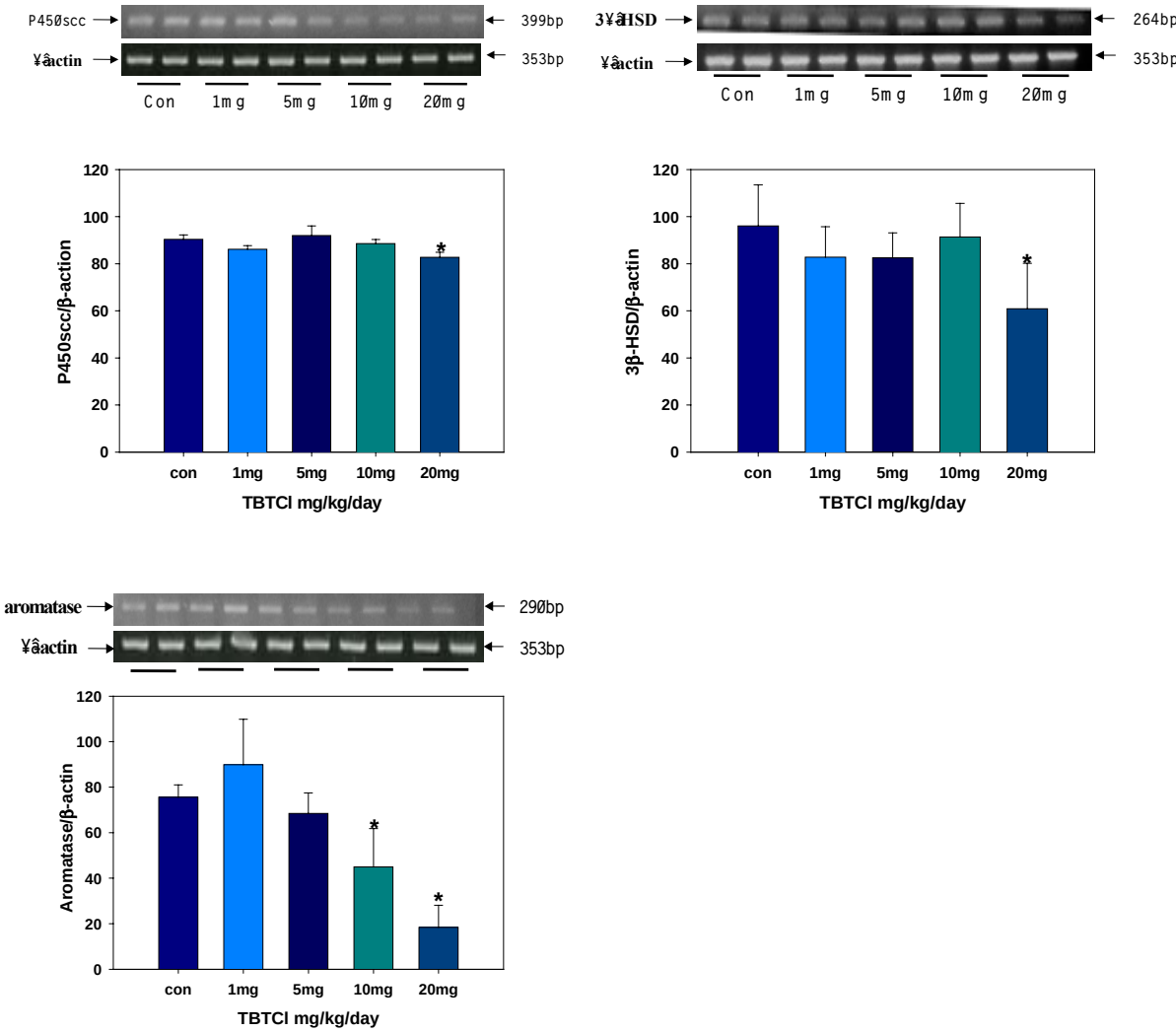

Figure 2. RT-PCR analysis for the effect of TBTCl on expression of cytochrome P450 scc, 3β-HSD and aromatase mRNA in rat testis. *: Significantly different from the control (P<0.05).