TETRAMETHRIN SHOWS ESTROGEN-ANTAGONISTIC EFFECTS IN VITRO AND IN VIVO

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Introduction

Tetramethrin is one of the pyrethroids, synthetic derivatives of naturally occurring pyrethrins (Figure 1). These pyrethroids including tetramethrin have been developed as insecticides due to their high insecticidal potency and low mammalian toxicity. Tetramethrin is in worldwide use for pest control including Korea, providing potential for human exposure. It, however, could adversely affect the reproductive endocrine system if it has hormonal activity. Little is known about hormonal activity of tetramethrin throughout the world. The Organisation for Economic Cooperation and Development (OECD) proposed the immature rat uterotrophic assay as one of the screening test methods for investigating the estrogenic activities of endocrine disrupting chemicals in 2001[1]. Calbindin-D_{9k} (CaBP-9k) gene, one of intracellular calcium binding proteins, is estrogen- responsive in uterus [2,3,4]. This study investigated the potential estrogenic activity of tetramethrin by immature rat uterotrophic assay and CaBP-9k gene expression assay. In the uterotrophic assay using 18-day old female SD rats, subcutaneous treatment of tetramethrin (5 to 800 mg/kg/day) for 3 days led to statistically-significant decreases in absolute and relative uterine wet weights at all doses tested. In addition, tetramethrin shows inhibiting effect on E2-enhanced their weights, and statistical significance at certain doses. Tetramethrin reduced absolute and relative vaginal wet weights, and also inhibited the increases of their weights by E2. Northern blot analysis showed reduction of the CaBP-9k mRNA expression in response to tetramethrin as well as E2 treatment. In conclusion, our results indicate that tetramethrin shows estrogen-antagonistic activity, and might thus affect the reproductive system in immature female rats.

Methods and Materials

Animals

Immature (18-day old, about 61g) female SD rats were provided by the National Institute of Toxicological Research, KFDA (Seoul, Korea). Animals used in this experiment were handled in accordance with NITR guidelines for the care and use of laboratory animals.

Testing chemicals

Tetramethrin (CAS #: 7696-12-01, purity: 98.7%) was supplied by Riedel-deHaën (Wunstorferstrasse, Germany). Corn oil and 17β-estradiol (E2) were bought from Sigma (St. Louis, USA), and used as vehicle and positive controls, respectively.

Uterotrophic assay

Immature (18-day old, about 61g) female SD rats were randomly assigned to the control and treatment groups, and corn oil was used as a vehicle control and E2 (3 □/□/day) as a positive control. Either tetramethrin (5 to 800 □/□/day) or E2 (3 □/□/day) plus tetramethrin (5 to 800 □/□/day) were administered to the rats by subcutaneous injection from postnatal day (PND) 19 to 21. Animals were sacrificed by cervical dislocation 24 hours after the last treatment. The uteri and vaginae were removed, trimmed free of fat, and then weighed.

Northern blot analysis of calbindin-D9k (CaBP-9k) mRNA

For Northern blot analysis of CaBP-9k mRNA, uterine tissues obtained from uterotrophic assay were snap-frozen in liquid nitrogen. Three frozen uteri per group were homogenized individually, and total RNA was extracted using Trizol reagent (Life Technologies, Rockvill, USA). RNAs were denatured by heating at 65□ for 15 min, and 10□ of total RNA was electrophoresised on 1% agarose gel for 90 min at 110 V, and then transferred to a nylon membrane with a vacuum blotter. 18S rRNA was used as an indicator for quality of total RNA.

Western blot analysis of ERα protein

Proteins were extracted from the uteri obtained from uterotrophic assay by homogenization with Trizol. Western blot analysis for estrogen receptor- α (ERα) was carried out according to the manufacturer's protocol (Santa CruZ Biotechnology, Santa CruZ, CA, USA). Briefly, the protein lysates were subjected to SDS-PAGE, and separated in 12% acrylamide gels. Proteins were transferred to a PVDF membrane (Bio-Rad, Hercules, CA, USA), and probed with primary anti-ERα (1:800) and secondary anti-rabbit HRP-conjugated antibodies (1:1000). All antibodies used were obtained from the Santa Cruz Biotechnology (Santa Cruz, CA, USA). Detection was performed using ECL plus detection system (Amersham Pharmacia Biotech, Piscataway, NY, USA), and followed by analysis with a Stom 840 image analyzer (Molecular Dynamics, Sunnyvale, CA, USA).

Statistical analysis

Experimental results were presented as mean ± SD or mean ± SEM. The statistical analyses were performed by One Way Analysis of Variance (ANOVA), followed by Dunnett's Method. The statistically-significant differences were evaluated at levels of 0.01 and 0.05.

Results and Discussion

Uterotrophic assay

All doses of tetramethrin tested (5 to 800 □/□) led to significant decreases in absolute and relative uterine wet weights, and also inhibited the E2-promoting effects on uterine wet weights (Figures 2 & 3). In addition, tetramethrin reduced absolute and relative vaginal wet weights, and also inhibited the E2-induced weight increases (data not shown).

Calbindin D_{9k} mRNA expression

Northern blot analysis showed reduction of CaBP-9k mRNA expression in response to tetramethrin as well as E2 treatment (Figures 3 & 4).

ERα protein expression

Tetramethrin treatment caused significant reduction of ERα protein expression in the uteri of immature rats at all doses tested over the control group (Figure 6).

Acknowledgments

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References

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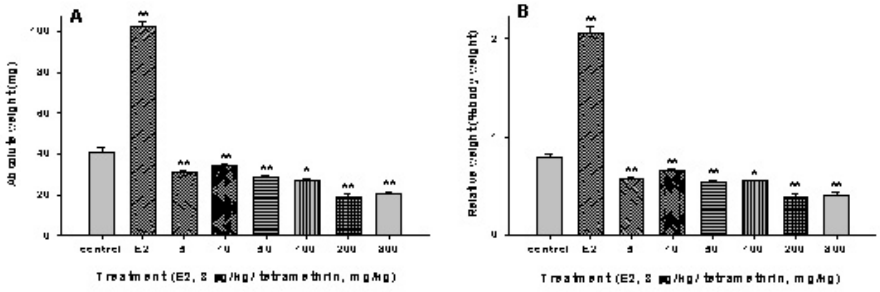
Figure 1: Structure of tetramethrin

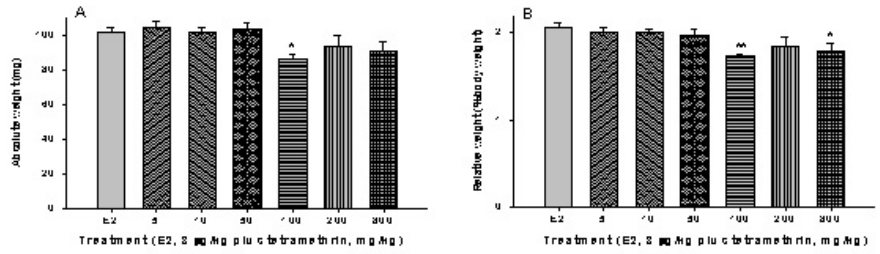
2,2-Dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylic acid (1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2-yl)methyl ester

Figure 2 : Effects of tetramethrin on absolute (A) and relative (B) uterine wet weight in immature female rats



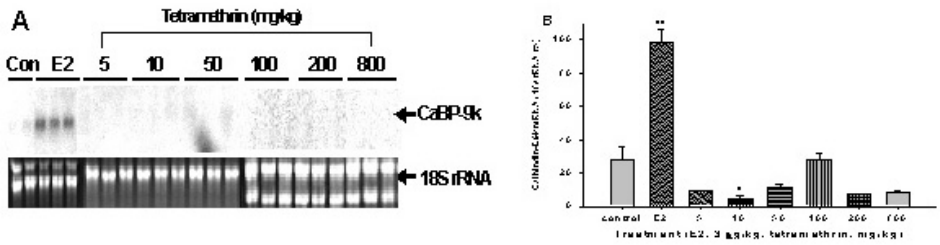
18-day old female SD rats were daily treated with tetramethrin by subcutaneous injection for 3 days. Data are presented as mean ± SEM. Significantly different from control, *P<0.05,**P<0.01.

Figure 3 : Effects of tetramethrin on E2-induced absolute (A) and relative (B) uterine wet weights in immature female rats



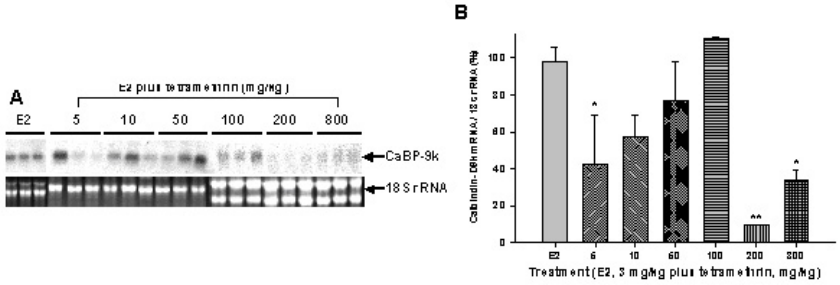
18-day old female SD rats were daily treated with E2 plus tetramethrin by subcutaneous injection for 3 days. Data are presented as mean ± SEM. Significantly different from E2, *P□0.05, **P□0.01.

Figure 4 : Expression of CaBP-9k mRNA in the uteri of immature female rats treated with tetramethrin



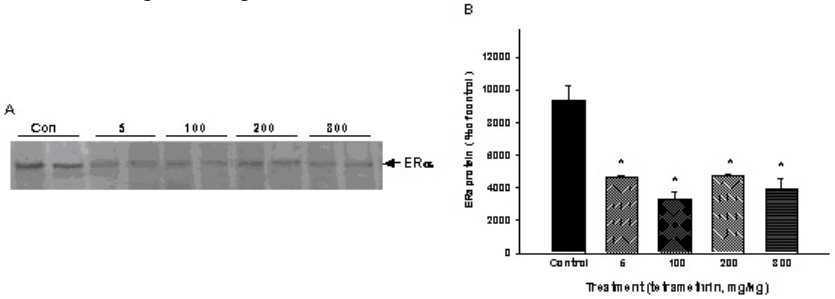
(A) Northern blot analysis of CaBP-9k mRNA in the uteri of immature female rats treated with tetramethrin. 18-day old female SD rats were daily treated with tetramethrin by subcutaneous injection for 3 days. Total RNA was individually isolated from the three uteri per group. Lower panel indicates 18S rRNA used as an indicator for quality of total RNA. (B) Schematic representation of CaBP-9k mRNA expression by tetramethrin. Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **P<0.01.

Figure 5 : Expression of CaBP-9k mRNA in the uteri of immature female rats treated with E2 plus tetramethrin



(A) Northern blot analysis of CaBP-9k mRNA in the uteri of immature female rats treated with E2 plus tetramethrin. 18-day old female SD rats were treated with E2 plus tetramethrin by subcutaneous injection for 3 days. Total RNA was individually isolated from the three uteri per group. Lower panel indicates 18S rRNA used as an indicator for quality of total RNA. (B) Schematic representation of CaBP-9k mRNA expression by E2 plus tetramethrin.

Figure 6 : Uterine ERα protein expression in immature female rats treated with tetramethrin



Data are presented as mean ± SD. Significantly different from control, *P<0.01.