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Effect of 3-Methylcholanthrene on Rat Uterus: Uterine Growth and Mechanism of Action of 3-Methylcholanthrene

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This study has been undertaken to examine the effect of 3-methylcholanthrene (3MC) on rat uterine growth and to understand the mechanism of action of 3MC in rat uterus. After diethylstilbesterol(DES) or tamoxifen(TAM) or 3MC or DES plus TAM or DES plus 3MC was administered into immature female rats, uterine weight of each group was measured. DES treatment resulted in 4-fold increase in uterine weight over corn oil-treated uteri. 3MC treatment had no effect on uterine weight but, DES stimulated uterine weight was inhibited by 3MC concomitant treatment. While TAM alone treatment showed slight increase in uterine weight, inhibited uterine growth stimulated by DES when it was administrated with DES concomitantly. Affinity of estradiol for estrogen receptor in the rat uterus was determined via direct binding assay with [^{3}H]estradiol and the relative binding affinities of 3MC and TAM were estimated by competetion assay. Estradiol turned out to have high affinity for rat uterine estrogen receptor (Kd=0.4 nM). The relative binding affinities of TAM and 3MC were 1% and 4.7% that of DES for rat uterine estrogen receptor, respectively. 3MC was shown to have similar affinity for rat uterine estrogen receptor to that of TAM. Effects of DES, 3MC and TAM administration in vivo on rat uterine estrogen receptor level were examined. It was confirmed that the estrogen, DES and antiestrogen, TAM decreased estrogen receptor levels from rat uterus and also 3MC decreased rat uterine estrogen receptor level when rats were treated with DES, TAM and 3MC in vivo. Data indicates that 3MC acts as an antiestrogen mediated through estrogen receptor system.

Key words: Estrogen receptor, Antiestrogen, Tamoxifen, Diethylstilbesterol, 3-Methylcholanthrene

INTRODUCTION

Arylhydrocarbons such as 3-methylcholanthrene (3MC), benzo[α]pyrene, dibenzanthracene are hazardous contaminants from automobiles, furnace, coal tar, factories, and smoking. These groups of compunds elicit a diverse group of species, strain, age, and tissue specific effects including a wasting syndrome, splenic and thymic atrophy, dermal toxicity, hepatotoxicity and porphyriia, reproductive toxicity, and teratogenecity, endocrine changes, carcinogenesis, and the induction of several enzyme systems (Cooke and Dennis, 1988; Tomatis *et al.*, 1989; Maltoni and Selikoff, 1988). It has been proposed that 3MC, and related compounds act via initial binding with intracellular arylhydrocarbon receptor. The molecular mechannism of action of 3MC as an inducer of cytochrome P450 isozymes and related monooxygenases has been investigated in labolatory animals and mammalian cells in culture (Bayad *et al.*,1991; Shichi *et al.*, 1991; Jones *et al.*, 1991; Rosenburg and Leff, 1993; Saki *et al.*, 1992). These studies have demonstrated that the induction of cytochrome P4501A1 mRNA is dependent on the interaction of the occupied receptor with specific "xenobiotic responsive elements" located in the 5′ upstream region from the cytochrome P4501A1 gene initiation site. The molecular mechanisms of other responses elicited by aromatic hydrocarbons are less well defined.

Recent studies have reported the activity of tetrachlorodibenzodioxin(TCDD) as an antiestrogen in rats, mice and MCF-7 human breast cancer cells in culture (Yao and Safe, 1989; Zachaewski *et al.*, 1991; Spink *et al.*, 1990; Romkes and Safe, 1988; Safe, 1990). For example, TCDD treatment resulted in decreased uterine weights in weanling female C57BL/6 mice and female Long Evans rats and TCDD partially blocked estrogenic effects of 17β-estradiol on uterine weights (Zachaewski *et al.*, 1992). TCDD also decreased constitutive and estradiol-induced uterine and hepatic est-

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rogen and progesterone receptor levels in the female rat (Yao and Safe, 1989) and supressed the estrogen-mediated excretion of tissue plasminogen activator activity in MCF-7 human breast cancer cell in culture. TCDD does not bind to the estrogen receptor and progesterone receptor and structure-activity studies suggested that the antiestrogenic activities of halogenated arylhydrocarbons are mediated through the arylhydrocarbon receptor (Safe, 1990; Spink *et al.*, 1990). It has been suggested that one possible mechanism for the antiestrogenic effects of TCDD may be related to the increased cytochrome P450-dependent metabolism of estradiol (Safe, 1990). TCDD and related compounds induce cytochromes P4501A1 and P4501A2 in rats and the later isozyme is an effective catalyst of estradiol 2-hydroxylase (Harris *et al.*, 1990). However, the mechanism of action of these observed uterotoxicities of arylhydrocarbons is not known. In this study, we examined the mechanism of action of 3-methylcholanthrene on rat uterine growth in order to gain the insight into the mechanism of uterotoxic effect of arylhydrocarbons.

MATERIALS AND METHODS

Materials and Animal

[2,4,6,7-^{3}H,17β]Estradiol (101 Ci/mmol, Amersham, Alrington, IL, USA), diethylstilbesterol, tamoxifen, 3-methylcholanthrene, charcoal, dextran, monothioglycerol, bovine serume albumin(Sigma Chemical Co., St. Louis, MO, USA) and Insta gel XF(R)(Packcard, Chicago, IL, USA) were used. SPF Female Sprague Dawley rats (21-23 day old, 20-25 g of body weight) were kindly donated from Yuhan Pharmaceutical Company.

Teatment of Animals

Each group of rats was treated with various doses of DES, or TAM, or 3MC (each of them dissolved in corn oil) for desired period of time via intraperitonial injection. Control rats were given 0.2ml of corn oil that was used for each injection of various doses of chemicals.

Measurement of Uterine Weight

Rats were sacrificed by cervical dislocation and uteri were isolated. After the removal of connective tissues and fat, uteri were slitted to get rid of extra water and weighed using torsion balance. Uteri were kept in the deep freezer at −70℃ until ready to be used for the experiments.

Preparation of Rat Uterine Cytosol Fraction

Six Uteri were homogenated in 1 ml of tris-EDTA-monoglycerol buffer (TEG buffer; 10 mM Tris, 1.5 mM EDTA, 10 mM monothioglycerol, pH 7.4) using polytron (set at 4) and subjected to centrifugation at 800 ×g for 30 minutes at 4℃. Taking the supernatant carried out ultracentrifuagation at 108,000×g for 45 minutes at 4℃. Supernatnats were collected and used for the determination of protein content for the further assays.

Direct Binding Analysis

Various concentrations (10^{-12}-10^{-9}M) of [^{3}H]estradiol (101 Ci/mmol) were incubated with 200 μL of cytosol fraction of rat uterine (total protein concnetration is 1.6 mg/ml) in the presence of 100 times excess unradiolabeled DES for 16 hours at 0℃. After the incubation, the unbound radiolabeled ligands were removed using 5% charcoal-dextran slurry via centrifugation at 18,000×g for 7 minutes. Bound receptors were determined by liquid scintillation counting of [^{3}H]estradiol bound using 5 ml of Insta gel XF.

Competition Assay

10^{-9}M [^{3}H]estradiol was incubated with different concentrations (10^{-10}-10^{-7}M) of DES, or different concentrations (10^{-9}-10^{-6}M) of 3MC, or different concentrations (10^{-9}-10^{-5}M) of TAM at 0℃ for 16 hours. Bound receptors were determined as described previously (Sheen *et al.*, 1985).

Exchange Assay

Cytosol fraction for exchange assay was prepared by 0.4 M Kcl extraction of nuclear preparation and incubated with 10^{-9}M [^{3}H]estradiol in the presence of 10^{-6}M DES for 4 hours at 30℃. After incubation bound receptors were measured as described earlier (Sheen *et al.*,1984).

Statistics

The statistical analysis was performed by student t-test. The significant differences between groups were evaluated with the level set at 0.05.

RESULTS AND DISCUSSION

Effect of 3-Methylcholanthrene on Rat Uterine Growth

The treatment of different doses of 3MC for 3 consecutive days have resulted in statistically insignificant changes in rat uterine weight, whereas 50 μg/Kg of DES treatment have brought about 4-fold increase in uterine weight over control. However, when 3MC was concomitantly treated with DES, 3MC decreases the uterine weight stimulated by DES (Table I). These data suggest that 3MC behaves like estrogen antagonist in

Table I. Effect of 3-methylcholanthrene on diethylstilbesterol stimulated rat uterine growth.(n=6-10)

Treatment (μg/Kg)	Uterine weight (mg) mean± S.D
Control	41.6± 8.6
Diethylstilbesterol(50)	160.0± 6.3*
3-methylcholanthrene(50)	41.8± 6.8
3-methylcholanthrene(500)	47.0± 6.0
3-methylcholanthrene(1000)	55.0± 8.0
Diethylstilbesterol(50) +3-methylcholanthrene(50)	149.0± 7.0**
Diethylstilbesterol(50) +3-methylcholanthrene(500)	147.2± 13.2**
Diethylstilbesterol(50) +3-methylcholanthrene(1000)	122.1± 15.0**

*Significant difference from control group (P<0.01)
**Significant difference from diethylstilbesterol treated group (P<0.01)

Table II. Effect of tamoxifen on diethylstilbesterol stimulated rat uterine growth.(n=6-10)

Treatment (μg/Kg)	Uterine weight (mg) mean± S.D
Control	41.6± 8.6
Diethylstilbesterol(50)	160.0± 6.3*
Tamoxifen(100)	55.0± 5.7*
Diethylstilbesterol(50)+Tamoxifen(50)	123.8± 20.7**
Diethylstilbesterol(50)+Tamoxifen(100)	113.1± 13.3**
Diethylstilbesterol(50)+Tamoxifen(500)	92.3± 14.0**

*Significant difference from control group (P<0.01)
**Significant difference from diethylstilbesterol treated group (P<0.01)

rat uterus. It was recently reported that TCDD treatment decreased uterine weights in weanling female C57BL/6 mice and female Long Evans rats and TCDD partially blocked estrogenic effects of 17β-estradiol on uterine weights (Zachaewski *et al.*, 1992).

These effects of arylhydrocarbons on uterine growth might be involved in uterotoxicities of arylhydrocarbons which was reported previously (Tomatis *et al.*, 1989).

Effect of Tamoxifen on Rat Uterine Growth

The treatment of 100 μg/Kg of TAM in rat showed increase in rat utrerine weight which is known as a partial agonist action of TAM in uterus that have not been observed in MCF-7 human breast cancer cells (Sheen *et al.*, 1985). However, when TAM was administered along with DES into rat, TAM inhibited the estrogen stimulated uterine growth (Table II). These data confirm other earlier findings (Katzellenbogen *et al.*, 1979; Roke and Katzellenbogen, 1982; Sutherland and Jordan, 1981), which indicate that antiestrogen, TAM

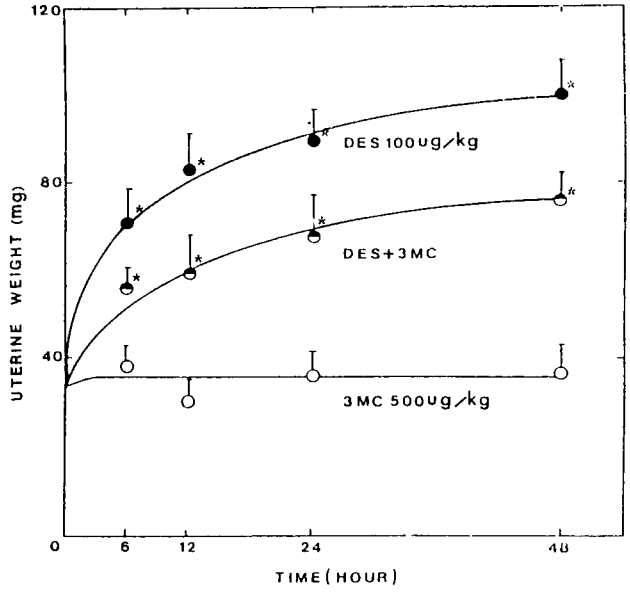

Fig. 1. Time course effect of 3-methylcholanthrene on diethylstilbesterol stimulated rat uterine growth. After the immature female rat were treated with diethylstilbesterol (DES) (100 μg/kg) or 3-methylcholanthrene (3MC) (500 μg/kg) or DES (100 μg/kg)+3MC (500 mg/kg) for various times as indicated, uterine weight was measured.
Bar represents standard deviation. (n=6-10)
*Significant difference from control group (P<0.01)
**Significant difference from DES treated group (P<0.01)

acts as an antiestrogen only when it was administered with estrogen.

Time Course Effect of 3-Methylcholanthrene on Rat Uterine Growth

As increase the time of treatment of DES in vivo, uterine weight increases. By 6 hour treatment of DES shows significant increase in rat uterine weight and after the 48 hour treatment the maximal stimulation of uterine weight was observed to be 303% that of control. However, 3MC treatment for 48 hours did not show significant difference in uterine weight changes. And 3MC inhibited the estrogen stimulated rat uterine weight increase when 3MC was concomistantly treated with DES (Fig. 1). This data suggests that 3MC may mediated through estrogen receptor system for its antiestrogenic action. Antiestrogenic activity of TCDD was reported in rats, mice and MCF-7 human breast cancer cells in culture, for example, TCDD supressed the estrogen-mediated excretion of tissue plasminogen activator activity in MCF-7 human breast cancer cell in culture (Yao and Safe, 1989; Zachaewski *et al.*, 1991; Spink *et al.*, 1990).

Determination of Affinity of Rat Uterine Estrogen Receptor with [^{3}H]estradiol

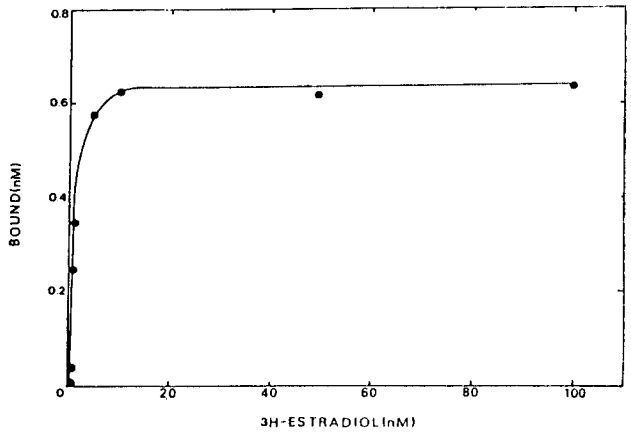

Fig. 2. Direct binding analysis of [^{3}H]estradiol to rat uterine estrogen receptor. Rat uterine cytosol (protein concentration: 1.6 mg/ml) was incubated with varying concentrations of [^{3}H]estradiol (10^{-12}-10^{-7} M) in the presence or absence of 100-fold excess of diethylstilbesterol for 20 hours at 0℃. The amount of [^{3}H]estradiol bound was then determined by charchoal-dextran adsorption method as described in "Materials and Methods".

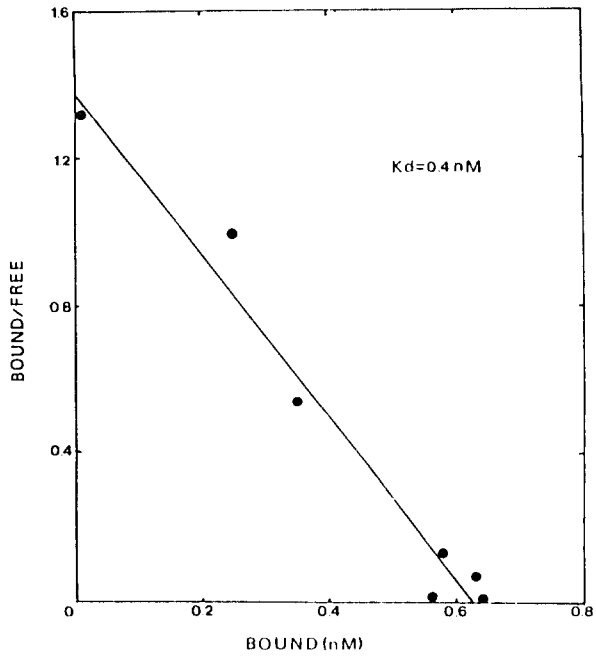

Fig. 3. Scatchard analysis of [^{3}H]estradiol to rat uterine estrogen receptor. Rat uterine cytosol(protein concentration:1.6 mg/ml)was incubated with varying concentrations of [^{3}H]estradiol (10^{-12}-10^{-7} M)in the presence or absence of 100-fold excess of diethylstilbesterol for 20 hours at 0℃. The amount of [^{3}H]estradiol bound was then determined by charchoal-dextran adsorption method as described in "Materials and Methods". [^{3}H]estradiol binding to rat uterine estrogen receptor was plotted according to the equation of Scatchard.

Direct binding analysis with rat uterine estrogen receptor have been carried out by incubating cytosol fraction of rat uterine with various concentrations (10^{-12} M-10^{-7} M) of [^{3}H]estradiol in the presence of 100 fold excess of nonradiolabeled DES at 4℃ for 16 hours. As shown in Fig. 2, as increase the concentrations of radiolabeled ligand rapid increase in receptor binding to [^{3}H]estradiol have been observed. Approximately 10^{-9} M [^{3}H]estradiol results in saturation of receptor from rat uterus preparation. This indicates high affinity of rat uterus estrogen receptor to [^{3}H]estradiol. This data shows the same profile to other studies that have been reported previously (King and Green, 1984; Welshons *et al.*, 1984; Katzenellenbogen *et al.*, 1985; Sheen *et al.*, 1984; Sudo *et al.*, 1983; Horwitz and McGuire, 1978a; 1978b).

Scatchard Analysis of [^{3}H]estradiol Binding to Rat Uterus Estrogen Receptor

Schachard plot of direct binding of [^{3}H]estradiol is shown in Fig. 3 in which the dissociation constant have been calculated from the slope of the plot to be 0.4 nM. This shows the high affinity estrogen receptor in rat uterus, and straight line indicates one class of binding site to [^{3}H]estradiol in rat uterus. The Kd value of rat uterine estrogen receptor that was calculated in this study is in the agreement to other studies that have been reported (Sheen *et al.*, 1984; Scatchard, 1949; Miller and Katzenellenbogen, 1983; Eckert and Katzenellenbogen, 1982; Sheen *et al.*, 1985; Horwitz and McGuire, 1978a).

Competition Analysis of Rat Uterus Estrogen Receptor with 3-Methylcholanthrene

After 10^{-8} M [^{3}H]estradiol was incubated with increasing concentrations of DES, or 3MC, or TAM, the specific binding of [^{3}H]estradiol was measured. The relative binding affinity of each ligand has been determined by the comparing concentration of cold ligand that results in 50% inhibition of [^{3}H]estradiol binding to estrogen receptor. When the concentrations of DES which shows 50% inhibition of radiolabeled estradiol binding to receptor was set as relative binding affinity to be 100% (Fig. 4). In other words, 4.5×10^{-9} M of DES indicates 100% relative binding affinity for estrogen receptor. Therefore, 5×10^{-7} M TAM represents 1% relative binding affinity, and 9.5×10^{-8} M 3MC represents 4.7% relative binding affinity for estrogen receptor. These show the affinity of 3MC seems to be quite similar to that of antiestrogen, TAM for the rat uterine estrogen receptor. In the case of triphenylethylene derived antiestrogens, it is known that the potency of antiestrogen correlates to the affinities of antiestrogens for the estrogen receptor (Katzellenbogen *et al.*, 1984; Sheen and Katzellenbogen, 1987; Miller *et*

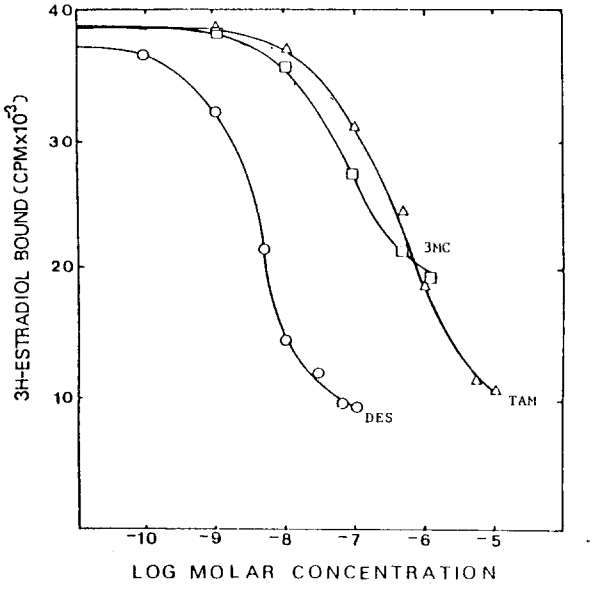

Fig. 4. Competition binding analysis of 3-methylcholanthrene. [^{3}H]estradiol (10^{-9} M) was incubated for 20 hours at 0℃ with rat uterine cytosol (protein concentration: 1.6 mg/ml) in the presence of various concentrations of diethylstilbesterol (DES, 10^{-10}-10^{-7} M) or 3-methylcholanthrene (3MC, 1 nM-1 μM) or tamoxifen (TAM, 10^{-9}-10^{-5} M). The amount of [^{3}H]estradiol bound was determined by charchoal-dextran adsorption method.

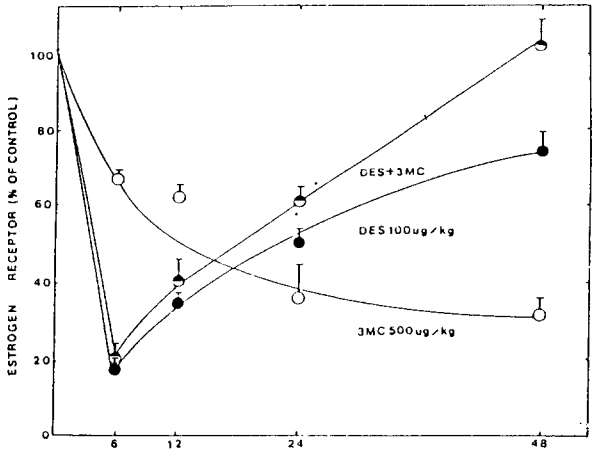

Fig. 5. Time course effect of 3-methylcholanthrene administration *in vivo* on uterine estrogen receptor level. Diethylstilbesterol (DES), 3-methylcholanthrene (3MC) or DES+3MC were injected into immature female rats for various times as indicated. Amount of uterine estrogen receptor was determined by exchange assay using [^{3}H]estradiol as described in "Materials and Methods". Bar represents standard deviation of triplicate determinations.

al., 1983). This data indicates the 3MC brings about antiestrogenic action via interation with estrogen receptor system, and its potency as an antiestrogen is similar to that of TAM. Although arylhydrocarbons showed

Table III. Effect of diethylstilbesterol, tamoxifen and 3-methylcholanthrene administration in vivo on the level of rat uterine estroegn receptor

Treatment (μg/Kg)	Estrogen receptor (fmol/mg protein)
Control	184.6± 6.2
Diethylstilbesterol(50)	42.8± 8.4
Tamoxifen(100)	67.9± 6.9
3-Methylcholanthrene(50)	92.1± 7.0
3-Methylcholanthrene(500)	62.4± 8.3
3-Methylcholanthrene(1000)	18.6± 9.6

Each value represents the mean± SD of results obtained from 6-10 rats.

Table IV. Effect of tamoxifen and ethylcholanthrene on estrogen receptor decrease by diethylstilbesterol in rat utrus uterine growth

Treatment (μg/Kg)	Estrogen receptor (fmol/mg protein)
Control	184.6± 6.2
Diethylstilbesterol(50)	42.8± 8.4
Diethylstilbesterol(50)+Tamoxifen(50)	45.5± 9.8
Diethylstilbesterol(50)+Tamoxifen(100)	37.7± 6.2
Diethylstilbesterol(50)+Tamoxifen(500)	60.9± 1.7
Diethylstilbesterol(50) +3-methylcholanthrene(50)	50.8± 3.1
Diethylstilbesterol(50) +3-methylcholanthrene(500)	53.0± 9.5
Diethylstilbesterol(50) +3-methylcholanthrene(1000)	49.1± 5.7

Each value represents the mean± SD of results obtained from 6-10 rats.

similar effects on uterine weight, 3MC appeared to be different from TCDD. Spink *et al.* reported that TCDD does not bind to the estrogen receptor and progesterone receptor and structure-activity studies suggested that the antiestrogenic activities of halogenated arylhydrocarbons are mediated through the arylhydrocarbon receptor (Spink *et al.*, 1990). It has been suggested that one possible mechanism for the antiestrogenic effects of TCDD may be related to the increased cytochrome P450-dependent metabolism of estradiol (Safe, 1990).

Time Course Effect of 3-Methylcholanthrene on the Level of Estrogen Receptor in Rat Uterus

Administration of DES (100 μg/Kg) into rat for 6 hours resulted in rapid decrease in estrogen receptor concentration and as increase the time up to 48 hours of treatment of DES brings the receptor level back up to 76% of untreated receptor level. However, the treatment of 3MC caused estrogen receptor to be decreased to 31% of control level. When est[illegible] and

3MC were treated together, the level of estrogen receptor was not different from the pattern of estrogen treated group (Fig. 5). It was reported that TCDD decreased constitutive and estradiol-induced uterine and hepatic estrogen and progesterone receptor levels in the female rat (Yao and Safe, 1989).

Effect of 3-Methylcholanthrene on the Level of Estrogen Receptor Concentration in Rat Uterus

Fifty ug/Kg of DES treatment for consecutive 3 days decreased the level of estrogen receptor to 43% of control and 100 μg/Kg of TAM treatment decreased to 68% of control level of estrogen receptor. In the case of 3MC treatment, the amount of estrogen receptor decresed as increase the concentration of 3MC as shown Table III. Concomitant treatment of DES with TAM or 3MC did not alter the effect of estrogen on the rat uterine estrogen receptor level (Table IV). Comparision of data shown in Table I and data shown in table 4 indicates that estrogen receptor level in the uterus may not be the best reflection of the level of rat uterine growth. Based on the results of this study it is clear that effects of 3MC and TAM as antiestrogens do not correlate to their effects on receptor concentration, instead their affinities for estrogen receptor are critical to bring about their antiestrogenic activities in the rat uterus.

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REFERENCES CITED

Bayad, J., Bagrel, D., Sabolovic, N., Magdalou, J. and Siest, G. T. I., Expression and regulation of drug metabolizing enzymes in an immotartalized rat hepatocyte cell line. *Biochem. Pharmacol.*, 42, 1345-1351 (1991).

Cooke, M. and Dennis, A. J.(Eds), *Polynuclear Aromatic Hydrocarbons: A Decade of Progress,* Battelle, Columbus, 1988, pp. 1-150.

Eckert, R. L. and Katzenellenbogen, B. S., Physical properties of estrogen receptor complex in MCF-7 human breast cancer cells. *J. Biol. Chem.*, 257, 8840-8846 (1982).

Harris, M., Zachaewski, T. and Safe, S., Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related compounds on the occupied nuclear estrogen receptor in MCF-7 human breast cancer cells. *Cancer Res.*, 50, 3579-3584 (1990).

Horwitz, K. B., and McGuire, W. L., Antiestrogen mechanisms of action and effects in breast cancer. In McGuire, W. L. (Eds), Breast Cancer Advances in Research and Treatment, Vol. 2, Plenum Press, New York, 1978a, pp. 155-204.

Horwitz, K. B. and McGuire, W. L., Estrogen control of progesterone receptor in human breast cancer. *J. Biol. Chem.*, 253, 2223-2228 (1978b).

Jones, S. N., Jones, P. G., Ibarguen, W., Casey, C. T. and Craigen, W. T., Induction of the cyp1a dioxin-responsive enhancer in trasgenic mice. *Nucleic Acid Res.* 19, 6547-6551 (1991).

Katzenellenbogen, B. S., Miller, M. A., Mullick, A. and Sheen, Y. Y., Antiestrogen action in breast cancer cells: Modulation of proliferation and protein synthesis and interaction with estrogen receptors and additional antiestrogen binding sites. *Cancer Res. Treat.*, 5, 231-243 (1985).

Katzenellenbogen B. S., Norman, M. J., Eckert, R. L., Peltz, S. W. and Mangle, W. F., Bioactive, estrogen receptor interations, and plasminogen activator-inducing activities of tamoxifen and hydroxytamoxifen in human breast cancer cells. *Cancer Res.* 44, 112-119 (1984).

Katzenellenbogen, B. S., Bhakoo, H. S., Fergson, E. R., Lan, N. C., Tatte, T., Tsai, T. L. and Katzenellenbogen, J. A., Estrogen and antiestrogen action in reproductive tissues and tumors. *Recent Pro. Hormon Res.*, 35, 259-300 (1979).

King, W. J. and Greene, G. J., Monoclonal antibodies localize estrogen receptor in nuclei of target cells. *Nature* (Lodon), 307, 745-747 (1984).

Maltoni, C. and Selikoff, I. J.(eds), Living in a chemical world:Occupational and environmental significance of industrial carcinogenesis. Vol. 534 *Ann. NY Acad. Sci.*, New York, pp. 1-1045 (1988).

Miller, M. A. and Katzenellenbogen, B. S., Characterization and quantitation of antiestrogen binding sites in estrogen receptor-positive and -negative human breast cancer cell lines. *Cancer Res.*, 43, 3094-3099 (1983).

Romkes, M. and Safe, S., Comparative activities of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and progesterone on antiestrogens in the female rat uterus. *Toxicol. Appl. Pharmacol.*, 92, 368-380 (1988).

Rorke, E. A. and Katzenellenbogen, B. S., Effects of estrogen and antiestrogens on estrogen receptor dynamics and the induction of progesterone receptor in MCF-7 human breast cancer cells. *Cancer Res.*, 42, 139-144 (1982).

Rosenburg, D. W. and Leff, T., Regulation of P450 in cultured human colonic cells. *Arch. Biochem. Biophys.*, 300, 186-192 (1993).

Safe, S., Polycholorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: Environmental and mechanistic consi-

derations which support the development of toxic equivalency factors (TEFs). *CRC Crit. Rev. Toxicol,* 21, 51-88 (1990).

Sakai, H., Park, S. S. and Kikkawa, Y., Differential oxidase activity of hepatic and pulmonary microsomal P450 isozyme after treatment with cytochrome P450 inducers. *Biochem. Biophys. Res. Commun.,* 187, 1262-1269 (1992).

Scatchard, G., The attractions of proteins from small molecules and ions. *Ann. N.Y. Acad. Sci.* 51, 660-672 (1949).

Sheen, Y. Y. and Katzenellenbogen, B. S., Antiestrogen stimulation of the a 37,000 molecular weight secreted protein and estrogen stimulation of the 32,000 molecular weight secreted protein in MCF-7 human breast cancer cells. *Endocrinology* 120, 1140-1151 (1987).

Sheen, Y. Y., Ruh, T. S., Mangel, W. F. and Katzenellenbogen B. S., An antiestrogenic potency and binding characteristics of the triphenylethylene H1285 in MCF-7 human breast cancer cells. *Cancer Res.,* 45, 4192-4199 (1984).

Sheen, Y. Y., Simpson, D. M. and Katzenellenbogen, B. S., An antiestrogen of the role of antiestrogen-binding sites in mediating the growth modulatory effects of antiestrogens: Studies using t-Butylphenoxyethyl diethylamine, a compound lacking affinity for the estrogen receptor. *Endocrinology,* 117, 561-564 (1985).

Shichi, H., Mahalak, S. M., Sakamoto, S. and Sugiyama, T., Immunochemical study of phenobarbital- and 3-methylcholanthrene-inducible cytochrome P450 isozymes in primary cultures of porcine ciliary epithelium. *Curr. Eye Res.* 10, 779-788 (1991).

Spink, D. C., Lincoln, D. W., Dickerman, H. W. and Gierthy, J. F., 2,3,7,8-Tetrachloro-dibenzo-p-dioxin cause as extensive alteration of 17β-estradiol metabolism an human brest cancer cells. *Proc. Natl. Acad. Sci. USA,* 87, 6717-6921 (1990).

Sudo, K., Monsma, F. J., and Katzenellenbogen, B. S., Antiestrogen binding sites distinct from the estrogen recptor:subcellular localization, ligand specificity and distribution in tissue of the rat. *Endocrinology,* 122, 425-432, (1983).

Sutherland, R. L., and Jordon, V. C. (Eds), *Antiestrogens,* New York Academic Press, New York, 1981, pp.1-273.

Tomatis, L., Aitio, A., Wilbourn, J. and Shuke, L., Human carcinogenesis so far identified. *Jpn. J. Cancer Res.,* 80, 795-807 (1989).

Welshons, W. V., Lieberman, M. F. and Gorski, J., Nuclear localization of unoccupied estrogen receptors. *Nature* (London), 307, 747-749 (1984).

Yao, C. and Safe, S., 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced porphyria in genetically inbred mice: Partial antagonism and mechanistic studies. *Toxicol. Appl. Pharmacol.,* 100, 208-216 (1989).

Zachaewski, T., Harris, M. and Safe, S., Evidence for a possible mechanism of action of the 2,3,7,8-Tetrachlorodibenzo-p-dioxin-mediated decrease of nuclear estrogen receptor levels in wild-type and mutant Hepa1c1c7 cells. *Biochem. Pharmacol.,* 41, 1931-19 39 (1991).

Zachaewski, T., Harris, M.,Biegel, S., Morrison, V., Merchant, M. and Safe, S., 6-Methyl-1,3,8-trichlorodibenzofuran(MCDF) as an antiestrogen in human and rodent cancer cell lines: Evidence for the role of the Ah receptor. *Toxicol. Appl. Pharmacol.,* 113, 311-318 (1992).