2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ACTION ON METABOLISM OF CHOLESTEROL AND TESTOSTERONE BY FOLLICULAR CELLS IN CULTURE

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Introduction

From animal studies, TCDD was postulated to alter oestrous cyclicity and ovulation (1). In some species certain effects of TCDD are mediated by several other factors, especially female sex hormones. Although some of the effects of TCDD are mediated via the hypothalamic-pituitary axis, direct effects on the ovary have been observed (2). Estrogenic and antiestrogenic effects of TCDD have been described in many tissues (3,4,5). In our previous data (6,7,8) it has been shown that TCDD given to the culture medium of ovarian follicular cells accumulates in the tissue and alters steroids secretion. However, the mechanism of this action still is unclear. In granulosa and theca cells co-culture (GT) an increase in E2 secretion was observed only after 48 h of treatment with TCDD. A long term exposure to TCDD caused a decrease of E2 secretion. It may suggest that the decrease of E2 secretion by GT cultures is probably due to reduction in the activity of the enzymes converting cholesterol to pregnenolon (cytochrome $P450_{scc}$) or by inhibition of P450arom.

The aims of the present study were to confirm this hypothesis.

Material and Methods

Reagents

Parker medium M199, trypsin, and calf serum were purchased from Laboratory of Sera and Vaccines, Lublin, Poland. Antibiotic antimycotic solution (100x), testosterone, 25-hydroxycholesterol, aminoglutethimide were obtained from Sigma Chemical Co. St. Louis, MO, USA.

2,3,7,8-TCDD DMSO solutions were prepared by dissolving of the residue from evaporation of the concentrated toluene standard obtained from Wellington Laboratories (Product No: DD-2378-S). The TCDD concentrations were adjusted by dilution and confirmed by double fragmentation GC-MS/MS analysis.

Cell cultures

Porcine ovaries were obtained from a local abattoir. Large follicles were obtained from ovaries collected in days16-18 of oestrus cycle. Granulosa cells (Gc) and theca interna layers (Tc) subsequently prepared according to the technique described by Stokosowa et al.(9). After collection, granulosa and theca cells were suspended in M-199 medium supplemented with 5% calf serum and cultured in basal condition or with the addition of 3.2 ng/ml TCDD. The cultures were maintained at 37° C in humidified atmosphere of 5% CO_2.

Experiments

Exp.1 TCDD action on cell viability was measured using Alamar Blue assay. This assay is based on detection of metabolic activity. The absorbance was measured at wave of 570 and 600 nm in micro ELISA plate reader.

Exp.2. TCDD action on the activity of P450scc was measured by the conversion of 25-0H to progesterone. Additionally aminoglutethimide, the inhibitor of P450scc in a dose 20 μg/ml, was used to confirm the effect of TCDD on these enzymes.

Exp.3. To show if the inhibitory effect of TCDD on estradiol secretion is due to an increase of P450arom activity, cells were cultured with testosterone in a dose of 10^{-7}M as a substrate for estradiol production. Aromatase inhibitor (CGS 16949A; 4,5,6,7,8-tetrahydroimidazo [1,4-alpydrin-5-yl] benzonitrile monohydrochloride was used to confirm the action of TCDD on these enzymes. At the end of the culture estradiol and testosterone levels were determined radioimmunologically using Spectra kits (Orion, Diagnica, Finland), supplied by Polatom (wierk, Poland).

Results

The effect of TCDD on granulosa and theca cells toxicity

TCDD in a dose of 10nM (3.2 ng/ml) did not decrease theca and granulosa cells viability during time course of our study.

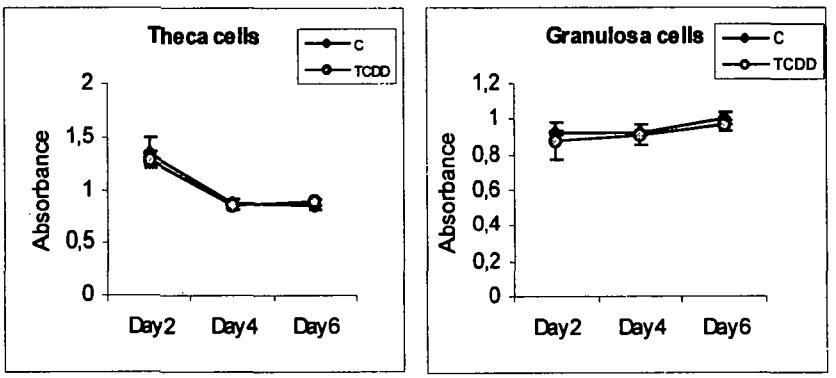

Fig 1 The effect of TCDD on granulosa and theca cells toxicity

The effect of aminoglutethimide the inhibitor of P450scc on testosterone and estradiol secretion by basal and TCDD-treated cells .

TCDD added to the culture medium decreased both 25-0H-stimulated testosterone and estradiol secretion (26% and 26% respectively) (Fig 3). Aminoglutethimide added to culture media decreased both basal (84%) and TCDD-treated (80%) estradiol secretion.

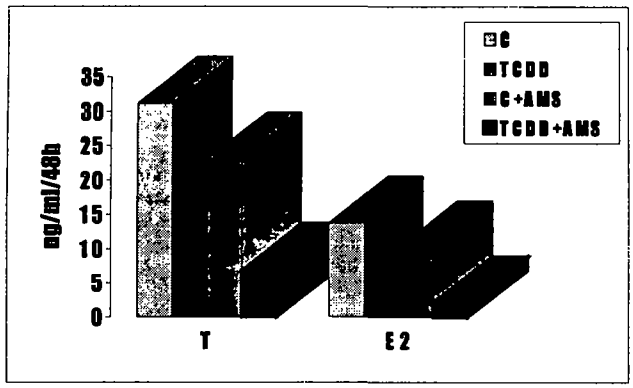

Fig. 2 The effect of Aminoglutethimide the inhibitor of P450scc on testosterone and estradiol secretion by basal and TCDD-treated cells.

The effect of CGS 16949A an inhibitor of P450arom on testosterone and estradiol secretion by basal and TCDD-treated cells.

TCDD added to the testosterone-treated co-culture of granulosa and theca cells increased significantly testosterone secretion (31% vs testosterone alone treated cells). CGS 16949A improve testosterone secretion to 14% in TCDD treated cells. In the same culture condition, the decrease of estradiol secretion in TCDD treated cells and additional inhibitory effect after the addition of CGS 16949A was noted.

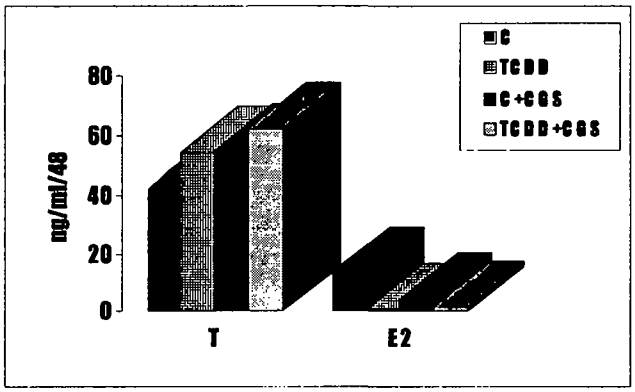

Fig 3 The effect of CGS 16949A an inhibitor of P450arom on testosterone and estradiol secretion by basal and TCDD-treated cells

Discussion

In the presented data we observed TCDD reduction of testosterone and estradiol secretion in follicular cells culture supported by exogenous 25-OH cholesterol, a substrate to pregnenolone formation. This effect was intensifying after the addition of TCDD with aminoglutehimide the inhibitor of P450scc. It is in agree with Moor et al.(10) showed that testosterone synthesis was decreased in TCDD treated rats because pregnenolone production by the testis was inhibited. They concluded that TCDD inhibited testicular steroidogenesis predominantly by inhibition the mobilization of cholesterol to cytochrome P450scc. It was also showed that TCDD added to the testosterone treated cells increased testosterone secretion and in this same culture condition decreased of estradiol secretion in TCDD treated cells
Additional inhibitory effect on estradiol secretion and increase of testosterone secretion after the addition of CGS 16949A was noted suggesting inhibition of P450arom activity.

Conclusion

THESE DATA CONFIRM HYPOTHESIS THAT TCDD ACTS RATHER AS AN ENDOCRINE DISRUPTOR THAN A TOXIC CHEMICAL.

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