EXPOSURE OF HUMAN JEG-3 CELL LINE TO TCDD ALTERS PROGESTERONE SECRETION BUT DOES NOT ACT ON THEIR VIABILITY AND APOPTOSIS.

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Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related compounds are lipophilic and difficult to metabolize. Any environmental exposure of living organisms to these congeners results in their accumulation in fat tissue and bioconcentration in humans via the food chain. TCDD acts as an endocrine disrupter to alter differentiation and function of the reproductive system (1,2,3). Therefore, these compounds represent a serious health risk, especially to the fetus and infants, whose enzymatic and metabolic systems are not yet mature. Our previous data showed high accumulation of TCDD in cultured human placental tissue which caused a decrease in hormone secretion (4). However, the mechanism of this action is still unclear.
JEG-3 cell line from malignant placental tissue has been used as an in vitro model for investigation of the effects of xenobiotics on placenta toxicity (5,6). These cells are morphologically similar to their origin, the trophoblast of the normal first trimester placenta (7), and produce many peptides and steroid hormones found in normal trophoblast cells, such as hCG, GhRH, progesterone (8).
The aim of the present study was firstly, to show dose- and time-dependent effects of TCDD on progesterone production by JEG-3 cells and secondly, to examine mechanism of its action on cell viability and apoptosis.

Materials and Methods

TCDD: TCDD (Promochem, Wesel) was dissolved in DMSO (Sigma) and prepared as the 1000-fold concentrated stock solutions. Cells were treated with TCDD in 0.1% DMSO or only with 0.1% DMSO as a control.
Cell culture: The human choriocarcinoma JEG-3 cell line was obtained from ATCC and cultured in minimum essential medium (BioMed, Lublin) supplemented with 10% FBS (Sigma), 100 IU/ml of penicillin and 100 μg/ml of streptomycin .Cells were cultured under standard conditions at 37^0C, 5%CO_2 and saturated humidity.
JEG-3 cells were seeded in 96-well plates (Nunc) at a density of 3×10^3 cells/well. After incubation for 24h, the medium was replaced and cells were cultured for another 24h, 48h and 72h without changing the medium in order to investigate time-dependent action of increasing doses of TCDD (0.016ng/ml; 0.032ng/ml; 0.16ng/ml; 0.32ng/ml; 1.6ng/ml; 3.2ng/ml; 16ng/ml and 32ng/ml).

Cytotoxicity Detection Kit (Roche Applied Science) was used for measurement of cell viability. Caspase-3 activity was assayed as a marker of apoptosis according to Nicholson et al., 1995 (10). Progesterone (P4) in the culture medium was assayed by using a direct enzyme immunoassay (EIA) as described earlier (11). The standard curve range was from 0.39 to 100 ng/ml and effective dose for 50% inhibition (ID50) of the assay was 4.5 ng/ml. The intra- and inter-assay coefficients of variation were 6.6 % and 9.2%, respectively. Each treatment was repeated three times in quadruplicates and thus the total number of replicates was 12. Since the variations between the experiments were small, those 12 results were averaged and analyzed by analysis of variance followed by Duncan's new multiple range test. The results are expressed as a mean ± SEM (n = 12).

Results and discussion

The present study demonstrates that TCDD inhibits progesterone secretion by JEG-3 cells without changing viability or induction of caspase-3 activity. This indicates that TCDD acts directly on steroid production and does not cause necrosis or apoptosis of cells.

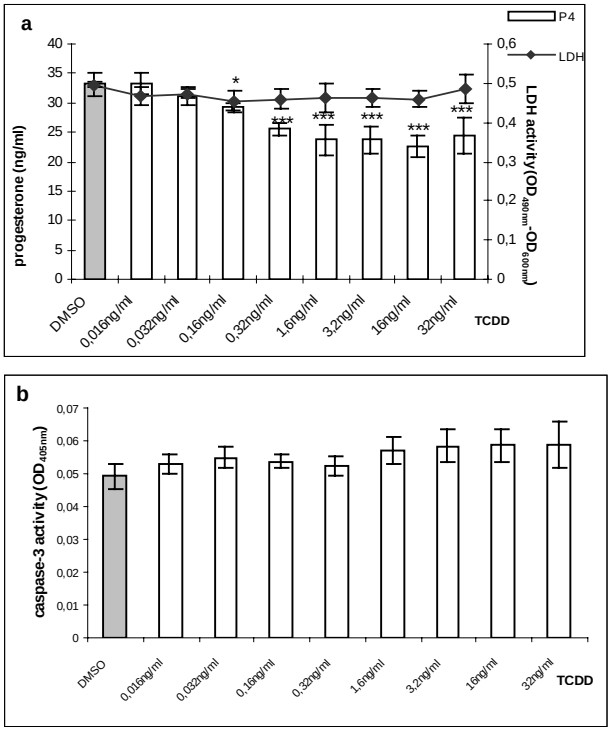

Figure 1. Dose-dependent effect of TCDD on progesterone secretion, viability of JEG-3 cells (a) and caspase-3 activity (b) after 24 hours of treatment. (* $p<0.05$; **$p<0.01$; ***$p<0.001$)

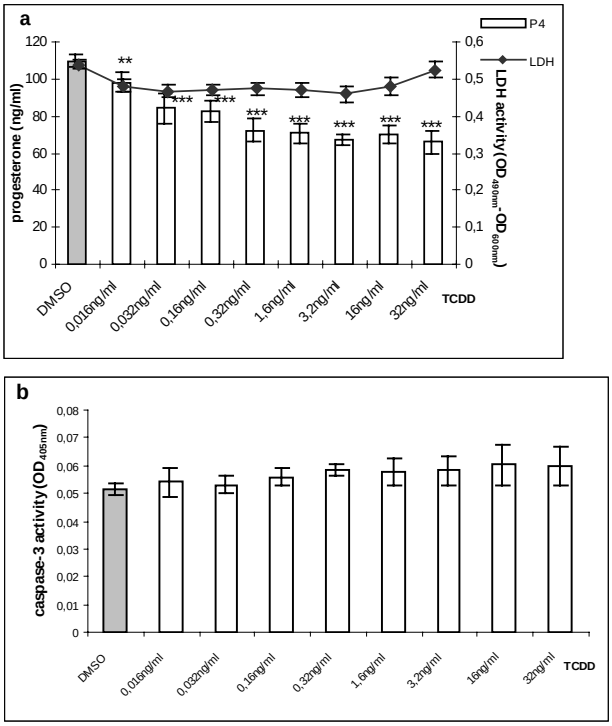

Figure 2. Dose-dependent effect of TCDD on progesterone secretion, viability of JEG-3 cells (a) and caspase-3 activity (b) after 48 hours of treatment. (* $p<0.05$; **$p<0.01$; ***$p<0.001$)

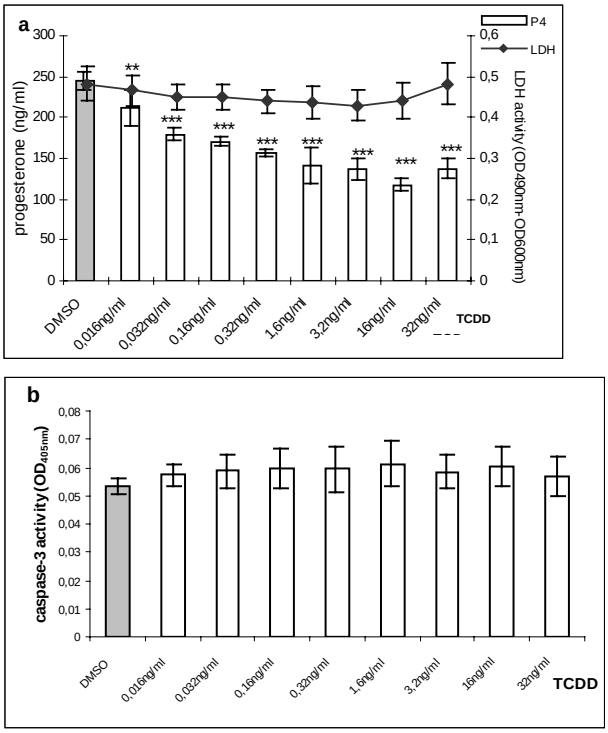

Figure 3. Dose-dependent effect of TCDD on progesterone secretion, viability of JEG-3 cells (a) and caspase-3 activity (b) after 72 hours of treatment. (* $p<0.05$; **$p<0.01$; ***$p<0.001$)

The placenta is a unique organ constituting the feto-maternal unit. It not only anchors the fetus physically to the uterus and co-ordinates the dialogue between the embryo and maternal body, but also acts as an endocrine unit for the production of the hormones needed by the maternal body and the fetal organs. The placenta becomes the primary site of estrogen and progesterone biosynthesis during pregnancy.

The results of the present study clearly show that the strongly decreased progesterone release from TCDD-treated cells in culture is not due to cytotoxicity. This is in agreement with data of Letcher et al (14) who showed no action of TCDD on protein level, DNA content and percent lactate dehydrogenase (LDH) leakage in JEG-3 cells.

Instead, TCDD may influence placental steroidogenesis, possibly by reduction in the activity of cytochrome P450scc or 3 beta-hydroxysteroid dehydrogenase, thereby causing the decreased progesterone secretion into the medium. This mechanism was suggested before for placental cells isolated from normal placenta (11) and luteal cells (12) cultured *in vitro* as primary cell culture. The observed decrease in progesterone secretion was reversed by addition of a substrate (25-0H or pregnenolone) to the culture media. The other possibility is that TCDD acts on progesterone metabolism and in the consequence increases non-active cholesterol metabolites.

The data presented by Zhang at al (15) could be a confirmation of direct action of TCDD on steroidogenic pathways. These authors compared the effects of benzo(a)pyrene (BaP) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on proliferation the human placental choriocarcinoma JEG-3 cell line. BaP significantly inhibited [3H]thymidine incorporation, whereas no effect of TCDD was observed over a 7-day period. Inappropriate progesterone secretion can be the cause of adverse pregnancy outcomes. The decreased progesterone level increases the susceptibility of uterine muscle to contractile stimuli, what may lead to preterm labor. It is known that human exposure to halogen aromatic hydrocarbons is associated with intrauterine growth retardation and developmental deficits (16, 17). Conversely, progesterone has effects on 17 β-HSD oxidoreductase and thus the metabolism of estradiol.

Altered placental function may be among the most sensitive end points for the assessment of developmental risk from human exposure to TCDD and related compounds, and may underlie a number of the developmental toxicities observed following *in utero* exposure.

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Reference

1. Murray FJ, Smith FA, Nitschke KD, Humiston CG, Kociba RJ, Schwetz BA. Toxicol Appl Pharmacol. 1979; 50:241-52.
2. Gray LE Jr, Kelce WR, Monosson E, Ostby JS, Birnbaum LS. Toxicol Appl Pharmacol. 1995; 131:108-18.
3. Gregoraszczuk EL, Zabielny E, Ochwat D. J Physiol Pharmacol 2001; 52: 303-311
4. Augustowska K, Gregoraszczuk EL, Grochowalski A, Milewicz T, Mika M, Krzysiek J, Chrząszcz R. Reproduction 2003; 126: 681-687.
5. Drenth HJ, Bouwman CA, Seinen W, Van den Berg M. Toxicol Appl Pharmacol. 1998; 148:50-5.
6. Letcher RJ, van Holsteijn I, Drenth HJ, Norstrom RJ, Bergman A, Safe S, Pieters R, van den Berg M. Toxicol Appl Pharmacol. 1999;160:10-20.
7. Patillo RA, Gey GO, Kohler PO. Ann NY Acad Sci 1971; 172: 228
8. Bahn RS, Worsham A, Speeg KV Jr, Ascoli M, Rabin D. J Clin Endocrinol Metab. 1981;52:447-50.
9. Nicholson DW, Ali A, Thorberry NA, Vaillancourt JP, Ding CK, Gallant M, Gareau Y, Griffin PR, Labelle M, Lazebnik YA, Munday NA, Raju SM, Smulson ME, Yamin TT, Yu VL, Miller DK. Nature. 1995; 375: 37-43.
10. Skarzynski DJ, Jaroszewski JJ, Bah MM, Deptula KM, Barszczewska B, Gawronska B, Hansel W. Biol Reprod. 2003;68:1674-81.
11. Augustowska K, Gregoraszczuk EL, Milewicz T, Krzysiek J, Grochowalski A, Chrzaszcz R. Endocr Regul. 2003;37:11-9.
12. Gregoraszczuk EL, Zabielny E, Ochwat D. J Physiol Pharmacol. 2001; 52:303-11.
13. Hassoun EA, Bagchi D, Stohs SJ. Toxicol Letters 1995; 76:245-250.
14. Letcher RJ, van Holsteijn I, Drenth HJ, Norstrom RJ, Bergman A, Safe S, Pieters R, van den Berg M. Toxicol Appl Pharmacol. 1999; 160:10-20.
15. Zhang L, Shiverick KT. Biochem Biophys Res Commun. 1997; 231:117-20.

16. Chen YC, Guo YL, Hsu CC, Rogan WJ. JAMA. 1992; 268:3213-8.
17. Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY, Taylor JS, Wu YC, Yang D, Ragan NB, Hsu CC. Science. 1988; 15; 241: 334-6.