EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON LUTEAL CELL FUNCTION. TISSUE CULTURE APPROACH.

E.L. Gregoraszczuk., E.Zabielny., R. Piekło., A. Grochowalski* ., A.Wójtowicz., M. Mika **

Department of Animal Physiology, Institute of Zoology, Jagiellonian University, Kraków, Poland.
* Department of Chemistry, Cracow University of Technology, Kraków, Poland
**Department of Animal Physiology, Academy of Agriculture, Kraków, Poland.

Introduction

Steroid hormones play an essential role in the control of ovarian cyclicity and the patterns of episodic release are remarkably conserved across species with respect to the preovulatory rise in estrogen followed by progesterone secretion during the luteal phase.

The environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent disrupter of vertebrate endocrine systems. At a single oral dose TCDD irregulates cycles, characterized mainly by prolonged periods of diestrus[1]. The direct action of TCDD on ovarian steroidogenesis is unknown and the evidence is really scarce.

One study[2] showed a decrease in progesterone production by luteinized granulosa cells after 24h exposure, in another study[3], TCDD was shown to reduce estradiol production by luteinized granulosa cells without an effect on progesterone production. Prior to this study, there is no other studies concerning the effect of TCDD on luteal cell function.

Corpus luteum is the compartment of the ovary which life span is very strictly limited. The regular sexual cycle is dependent on the precision of the life span of corpus luteum. One of the endocrine functions of corpora lutea is the production of progesterone.

In the present study, we set out to determine whether exposure to TCDD affects the luteal cell steroidogenesis and whether its actions on the corpora lutea may increase the apoptotic cell death of the steroid-producing cells.

Experimental

Chemicals

2,3,7,8-TCDD solutions were prepared by dilution of evaporated, concentrated toluene standard (Promochem) in DMSO. The concentrations of TCDD DMSO solutions were adjusted and confirmed by GC-MS/MS analysis. Medium M199, Penicillin, Trypsin, and Calf Serum (Laboratory of Vaccines, Lublin, Poland). Acridine Orange (Sigma).

Animals and cell isolation

Ovaries were obtained from Large White sows from a local slaughterhouse immediately after slaughter, placed in ice-cold PBS and transported to the laboratory. The phase of the oestrous cycle was determined according to the established morphological criteria[4]. Dissected corpora lutea from each animal were enzymatically dissociated according to the technique of Gregoraszczuk[5]. Luteal cells were obtained from pools of freshly existed mature corpora lutea (8-10 days after ovulation) from three animals in order to produce the luteal pool used in any given replicate to minimise the variation possibly existing among corpora lutea in the same ovaries and between ovaries in the some animals and among animals.

Cells were suspended in medium M199 supplemented with 5% of calf serum at a concentration of 3.5x 10^5 cells/ml medium. Cell viability measured using the trypan blue exclusion test was 85%.

Cells were grown in multiwell plates (Nunc) in a humidified atmosphere with 5% CO_2 in the air. At least three different experiments (n=3), each in triplicate have been done.
To perform the dose response curve cells were cultured with 0.1, 1.0, 10 nM of TCDD for 48h. The influence of permanent treatment of 10 nM TCDD was investigate in culture for 96 h. After incubation, all cultures were terminated and the media were frozen until further steroid analysis. Progesterone concentration was determined radioimmunologically using Spectria kits (Orion, Diagnostica, Finland), supplied by Polatom (Świerk, Poland).
Cells growning on glasses were stained with oil red O[6] to visualize lipid droplets within cells or submitted to a histochemical test[7] to demonstrate activity of 3β̃hydroxysteroid dehydrogenase, an enzyme which promotes progesterone synthesis.
Cytotoxic effect was tested using Acridine Orange: after staining the cells were examined under light microscopy, classified and counted. Cells were classified as follows: a) viable with bright green nucleus with intact structure, b) early apoptopic with bright green nucleus showing condensation of chromatin as dense green areas in the nucleus, c) necrotic with orange nucleus with intact structure.

Results and Discussion

Steroid secretion

Fig.1 shows the dose response curve of progesterone secretion under the influence of 0, 0.1, 1 and 10nM TCDD. The most inhibitory effect we observed at the presence of 10 nM of TCDD.

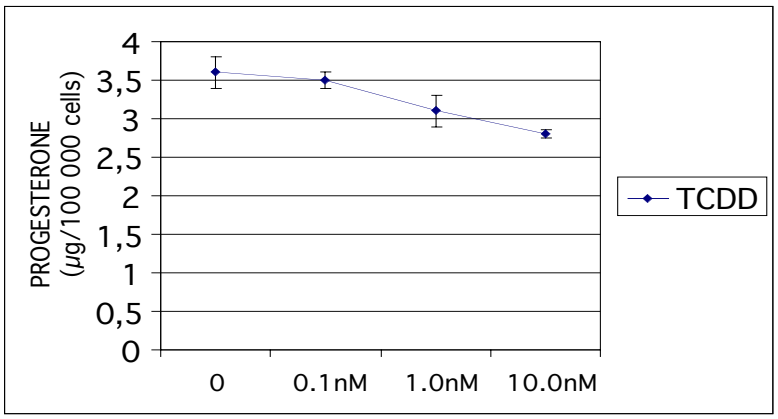
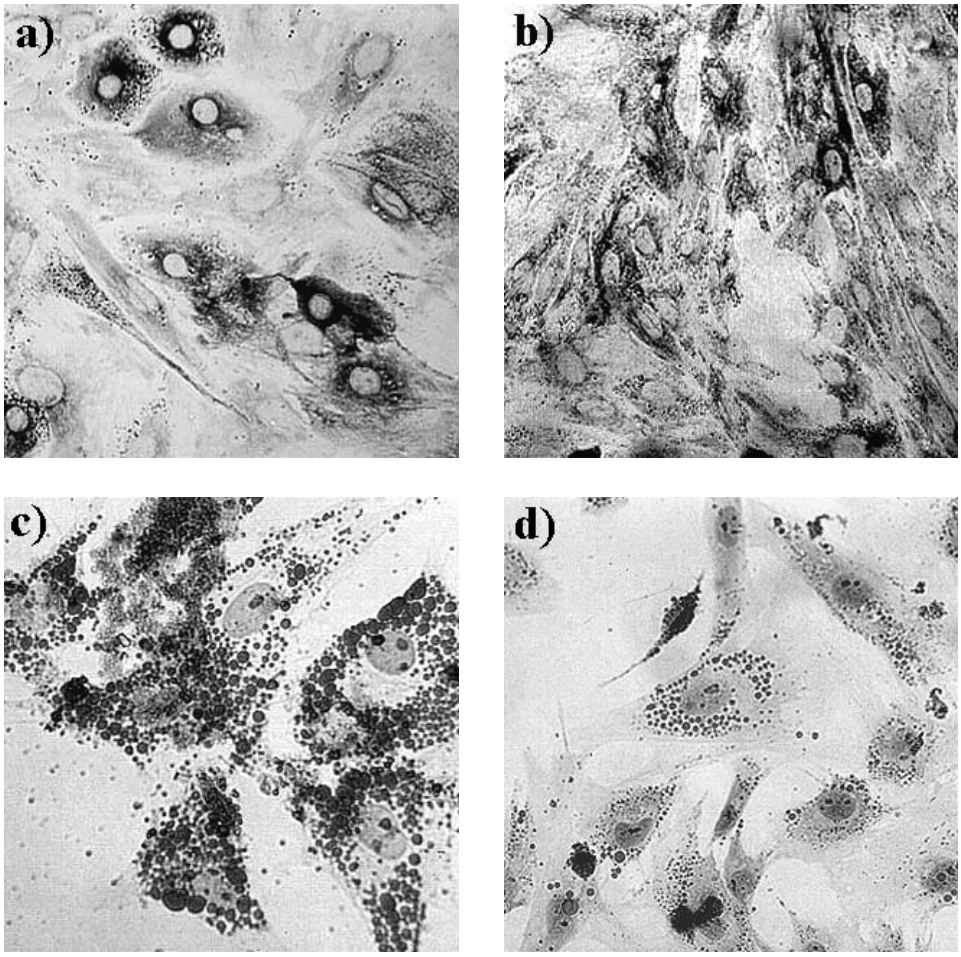


Fig.1. Dose response curve of progesterone secretion under the influence of 0, 0.1, 1and 10nM of TCDD.

Morfological observation

There are observed the decrease of lipid droplets and activity of 3β-HSD in cells grown in the permanent treatment with TCDD.

Fig.2. Four-day monolayer of luteal cell culture: a) monolayer of control luteal cells culture tested for the activity of 3β-HSD, b) corresponding cell culture in medium containing 10nM of TCDD.
c) control culture stained with ORO (×320), d) similar luteal cell culture in medium containing 10nM TCDD



The permanent treatment of luteal cells with10 nM TCDD for 96 hrs inhibited progesterone secretion by luteal cells from 2.6±0.16 ng in control to 1.61±0.17 ng/10^5 cells ($p<0.05$)

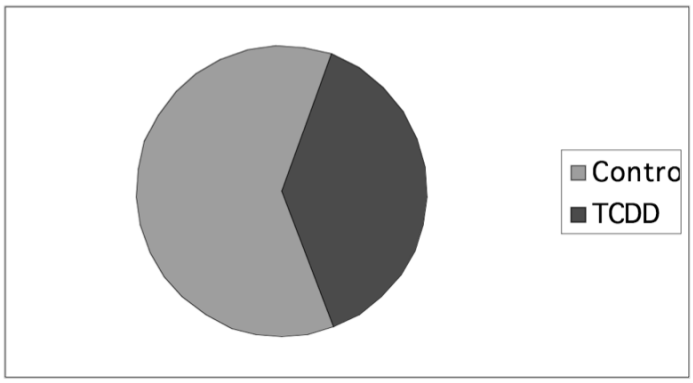


Fig.3. Effects of permanent tratment of 10 nM TCDD on progesterone secretion by luteal cells. Data are presented as mean SEM ($p<0.05$).

Cytotoxic Effect

In the control culture 86% of cells were classified as viable cells, while permanent treatment of 10 nM of TCDD decreased that number to 45%. Moreover, addition of TCDD at that doses increased the number of apoptotic (control = 9%, 10nM TCDD=22%) and necrotic (control = 5%, 10nM TCDD = 33%) luteal cells after 96h in culture, compared with controls.(Fig.4.)

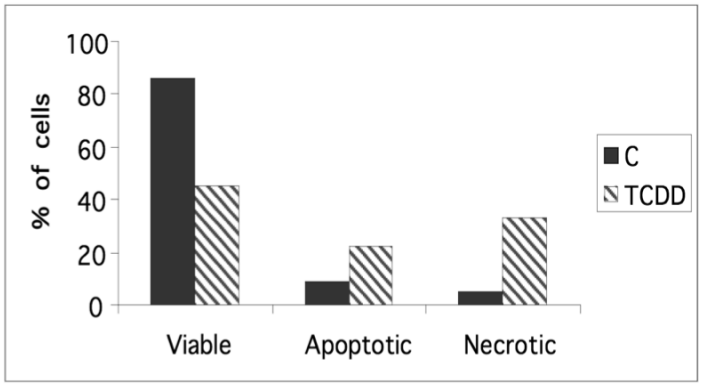


Fig. 4. The influence of TCDD on amount of viable, apoptotic and necrotic luteal cells.

Conclusion

These data suggest that TCDD alters steroidogenic function in luteal cells by acting on steroidogenic pathway and is capable of influencing apoptopic cell death by a mechanism that is stil unclear.

Further studies are required to elucidate loci of TCDD action on ovarian steroidogenesis.

Acknowledgements

This work was partly supported by BW/IŻ/98 and partly by The First Polish-American Bank S.A. Kraków.

References

1. Li X., Johnson DC., Rozman KK. *Toxicol. Lett.* **1995** 78, 219
2. Enan E., Moran F., VandeVoort CA., Stewart DR., Overstreet JW., Lasley BL. *Reporod. Toxicol.* **1996** 10, 497
3. Moran FM, Enan E, Vndervood CA, Steward DR, Conley AJ, Overstreet JW, Lasley BL. *Soccity for the Study of Reproduction*, Portland, OR, **1997** [Suppl 1] 56
4. Gregoraszczuk EL. *Endocrine Regulation*, **1992** 26,189
5. Gregoraszczuk EL.. *Endocr. Exp*. **1983**,17, 59
6. Casselman WGB. Histochemical Technique. Methuen et Co. LTD, London and John Willey and Sons, Inc., NY. **1959**, 64
7. Fisher TV and Kahn RH. Histochemical studies of rat ovarian follicular cells in vitro. In Vitro **1972,** 7, 201.
8. Gorman A., McCarthy J., Finucane D., Reville W. And Cotter T.G. Morfological assesment of apoptosis. In: Techniques in Apoptosis.A users guide. Ed: T.G. Cotter and S J Martin, Portlan Press Ltd. London **1996**, 1-20.