DIOXIN-LIKE (PCB 126) AND NON DIOXIN-LIKE (PCB 153) ACTION ON DNA DAMAGE AND APOPTOSIS IN GRANULOSA CELLS. PRELIMINARNY DATA.

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Introduction

Approximately 60-90% of all cancer cases are now generally believed to be due to environmental factors, to which humans are exposed by taking food, and water or inhaling air. These chemicals, including polycyclic aromatic hydrocarbons (PAHs), can be subdivided into different classes,. TCDD was classified as group 1 (documented carcinogen in humans) Benzo [a] pyrene and benzo [a] anthracene were included into group 2A (probably carcinogen in humans). PCB is not mentioned in this list. The International Agency for Research on Cancer (IARC) maintains a register of human carcinogens and suspected human carcinogens [1] . The capacity of PCBs to induce DNA strand breaks has been studied to a lesser degree and is quite controversial. DNA strand breaks are the potentially mutagenic lesions that have been proposed as a genotoxicity biomarker for the biomonitoring of environmental pollutants [2] . The mutations induced by these lesions in pollutant-exposed populations are believed to be induced by chemical carcinogens. The formation of PCB adducts has been demonstrated and measured in vitro and in vivo [3] . The capacity of PCBs to form DNA adducts depends on their metabolic rate, which is determined by the degree of chlorination [4] . The aim of the presented preliminary data was to evaluate the follicular stage-specific action of PCB 126 and PCB 153 on DNA damage and apoptosis in granulosa cells.

Material and Methods

The follicles collected from porcine ovaries were divided in three groups based on their size: small follicles (2-5 mm in diameter), medium follicles (6-8

mm in diameter) and large (8 – 12 mm in diameter) preovulatory follicles [5,6]. The separation of granulosa cells (Gc) was performed according to the technique developed in our lab [7] . The cultures were maintained at 37^0C in a humidified atmosphere of 5% CO_2. Cells were initially cultured without reagents in serum-containing (5 % calf serum) M199 for 24h at 37^o C in a humidified atmosphere of 5% CO_2 /95%0_2 to allow the cell attachment to the plates. After 24h the media were changed for the new ones and 100pg of PCB 126 or 100 ng of PCB 153 were added. The dose of PCBs was established on the basis of the dose-response curve constructed during the preliminary studies [8] . 48 hour later media were collected for steroid analysis (immunoassay ELISA kits (IBL, Hamburg) while cells were fixed for measurements of caspase –3 activity [9] or embedded in agarose for Comet Assay [10].

Results

Estradiol concentration in cultured granulosa cells collected from follicles of different sizes.

Estradiol production by granulosa cells collected from small follicles was 5.7 pg/ 100 000 cells. Secretion of estradiol increased to 13.7 pg/100 000 cells in medium follicles and 31.7 pg/100 000 cells in large follicles. Both PCBs had no effect on estradiol secretion by small- and medium-size follicles. Both congeners had statistically significant stimulatory action on estradiol secretion in the large preovulatory follicles. (Fig. 1)

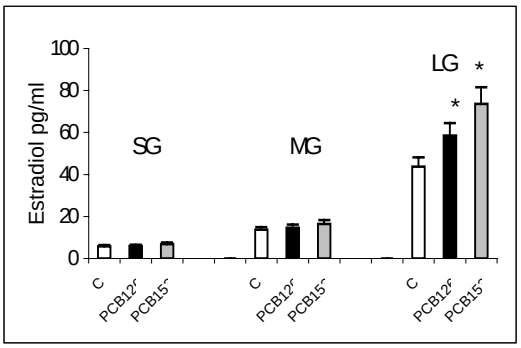

Fig.1. The effect of PCB 126 and PCB 153 on estradiol secretion by granulosa cells collected from small (SG), medium (MG) and large (LG) preovulatory follicles. *-$p<0.05$

Effect of treatment with PCB 126 and PCB 153 on caspase-3 activity in granulosa cells collected from follicles of different sizes.

In small and medium-size follicles, both PCBs had no significant action on caspase-3 activity. But, the suppression of caspase-3 activity was noted in the cells obtained from large follicles and treated with both PCBs. (Fig.2)

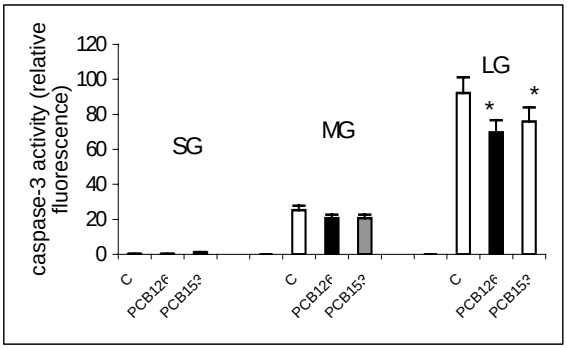

Fig. 2 The effect of PCB 126 and PCB 153 on caspase-3 activity in granulosa cells collected from small (SG), medium (MG) and large (LG) preovulatory follicles. *- $p<0.05$.

Effect of treatment with PCB 126 and PCB 153 on DNA damage in granulosa cells collected from follicles of different sizes. measured by Alkaline Single Cell Gel (SCG) Electrophoresis – Comet Assay

Both PCBs had no effect on DNA damage in granulosa cells collected from small follicles. In medium-size follicles, a decrease in DNA damage was noted only in the cells exposed to PCB 126 while in large preovulatory follicles both PCBs decreased DNA damage.

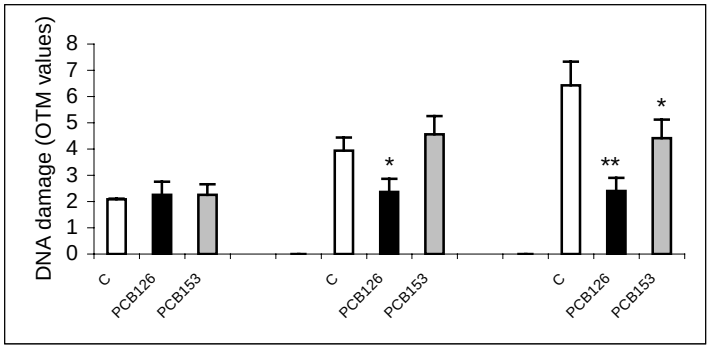

Fig. 3 The differences in the extent of DNA damage under the influence of PCB 126 and PCB 156 in granulosa cells collected from small (SG), medium (MG) and large (LG) preovulatory follicles. Values of the Olive Tail Moment (OTM) represent in this study the alkaline-labile DNA damage parameters. *-$p<0.05$; **- $p<0.01$.

Discussion

The results of the present study clearly showed that both PCBs were not genotoxic to the follicles independently of the stage of their development. However, they point to different mechanisms preventing the genotoxic action of these agents. In small follicles, no action on all three investigated parameters suggests existence of a mechanism preventing this type of follicles from the damage. In medium-size follicles, non-dioxin-like and less toxic PCB 153 had no effect on all investigated parameters, while dioxin-like PCB decreased DNA damage with no action on cell apoptosis and estradiol secretion.

These results suggested involvement of metabolic detoxification and DNA repair, diminishing carcinogenicity. DNA repair is a multifaceted process, which involves a large number of diverse enzymes [11] . Large preovulatory follicles were the most sensitive to both PCBs. An increase in estradiol secretion with parallel decrease in granulosa cells apoptosis on the one hand and a decrease in DNA damage on the other hand were observed.

It is well known that apart from DNA repair, the cells have at least two additional ways to minimize the impact of DNA damage [12, 13]. Firstly, there are DNA surveillance mechanisms, which are triggered by excessive DNA damage, and then cell cycle arrest occurs, which allows more time for DNA repair to rectify this DNA damage. From our previous data, we know that at doses used in this experiment both these congeners have no action on follicular cell viability and cell proliferation [14,15].

Secondly, a cell has a way of assessing whether it has accumulated too much DNA damage, and then it initiates a process termed apoptosis. The present data showed a decrease in apoptosis and diminution of DNA damage in large preovulatory follicles. This suggests that not apoptosis but DNA repair is probably the mechanism protecting preovulatory follicles from genotoxic action of PCBs. Further studies are necessary to confirm this hypothesis.

Acknowledgements
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