PCB 3 AND ITS METABOLITES (4-OH-PCB3 and 3,4-diOH-PCB3) HAVE NO MITOGENIC PROPERTIES IN MCF-7 CELLS LINE.

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Introduction:

Estrogen is an essential hormone that controls the normal physiology of the mammary gland and breast cancer development. The cytochrome P450 monooxygenases (CYP) is responsible for the biosyntesis and metabolism of endogenous compounds such as steroid hormones (Nelson et al., 1996). These enzymes are also involved in the metabolism of estradiol to catechol estrogens in extra hepatic tissues such as breast (Telang et al., 1991; Liehr and Ricci 1996). Human CYP 1A1 and CYP 1B1 are 17β-estradiol (E2) hydroxylases. CYP 1A1 is primarily an E2 2-hydroxylase [6] whereas CYP 1B1 is primarily an E2 4-hydroxylase with a lesser activity at C-2[7,8]. The catechols 2OH-E2 (2-hydroxyestradiol) and 4OH-E2 (4-hydroxyestradiol) can be oxidized to quinines, which are putative tumor initiators, but 4-hydroxylated form of E2 appears to be one of the most genotoxic metabolites of E2 in the breast epithelium. In contrast the influence of the 2OH-E2 is controversial; whereas some studies have established a protective effect of forms [9], others suggested that it could be the carcinogenic [10]. Thus, the ratio 2OH-E2/4OH-E2 could be a critical parameter of the carcinogenicity of E2.

Polychlorinated biphenyls (PCB) are fat – soluble compounds belong to a large group of persistent environmental contaminants which are capable of mimicking some of the biological activities of estrogens including induction of estrogen responsive enzyme activities (Li and Hansen 1996). Many of these compounds are carcinogenic after metabolic activation. Exposure to carcinogens, causes an increase in the activity of enzyme responsible for this activation. These enzymes belong to members of the cytochrome P450 (CYP) family, which are involved in the oxidative metabolism of both synthetic and natural compounds [4,5]. Thus, it is possible that PCB might be metabolize by this enzymes and/or interfere with estrogen metabolism in this extrahepatic tissues.

We have hypothesized that PCB might activate CYP 1A1 and cause that E2 will be primarily metabolized by CYP 1B1, induced more tumorgenic E2 4-hydroxylation pathways.Thus in the present study we compared the influence of E2 and PCB3 on CYP 1A1 activity and then the influence of these compounds and their metabolites on protein content as indicator of mitogenic effects. To evaluate the influence of E2 and PCB 3 on the activity of CYP 1A1, we used the EROD assay, which is specific for the CYP 1A family

Materials and Methods:

Chemicals: PCB3 and its hydroxylated metabolites, 4-OH-PCB3 and 3,4-diOH-PCB3 were synthesized using the Suzuki coupling reaction as described by Bauer et al [11]; Lehmler and Robertosn [12,13] . TCDD (Promochem, Wesel) and PCBs were solubilized in DMSO. E2, 2OH-E2, 4OH-E2 (Steraloids, Inc., Newport, RI) were dissolved in absolute ethanol. The final concentrations of DMSO and ethanol in the medium were in each case 0,1%.

Cell culture: MCF-7 human breast cancer cells (ATCC) were routinely cultured in DMEM (Sigma) supplemented with 10% heat- inactivated FBS (Sigma), 100IU/ml of penicillin and 100µg of streptomycin. Cells were grown on 75cm^2 tissue culture dishes (Nunc) in 37^{0}C incubator with a humidified mixture of 5% CO_2 and 95% air.

Forty-eight h before experiments, the medium was removed and replaced by DMEM without phenol red supplemented with 10% dextran- coated, charcoal treated FBS (10%DC-FBS). The cells were plated in the same medium and allowed to attach overnight. The next day the medium was replaced with phenol red –free DMEM supplemented with 5%DC-FBS.

Determination of EROD activity : Cells were seeded into 48-well plates at density of 60 000 cells/well. After 24h, cells were treated with 6 ng/ml E2, 6ng/ml PCB 3 and 3.2 ng/ml TCDD (positive control of CYP 1A1 induction). The kinetic conversion of ethoxyresorufin to resorufin was used as an indicator of CYP 1A1-dependent activity. EROD activity and protein determinations were measured in the same samples as described by Kennedy and Jones [14]. Analysis was conducted on a FL600 fluorescence plate reader (Bio-Tek) at 530nm/590nm for resorufin production and 400nm/460nm for fluorescamine protein determination.

Protein determination: Cells were seeded into 48-well plates at density of 20 000 cells/well. After 24h, cells were treated with: 6 ng/ml E2, 20H-E2, 4OH-E2 or with 6ng/ml PCB 3 and its metabolites (4-OH-PCB3; 3,4-diOH-PCB3) for 24h or 72 h with every day changing the medium and adding compounds in doses described

above. Protein concentration of lysates was determinated with Bradford reagent (Bio Rad Protein). The protein content was calculated from a standard curve generated using bovine serum albumine (Sigma).
Statistical analysis: All data points are expressed as means±SEM from at least three different experiments (n=3), each in triplicates. Statistical analysis was performed using STATISTICA 6.0. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison procedure. A p values less then 0.05 were considered to be statistically significant.

Results and discussion:

. Figure 1 shows that PCB3 at dose 6ng/ml induced the activity of CYP 1A1, but not as strong as TCDD used in this experiments as a positive control., while E2 had no any effect on CYP 1A1 activity. Polymorphism in the CYP 1A1 gene and expression of this gene in various tissue are being used as prognostic factors for same cancers, and this may be related, in part, to the important role of CYP 1A1 in metabolic activation of structurally divers carcinogens and toxicants.
CYP 1A1 is involved in phase I biotransformation of xenobiotics and endogenous compounds such as estrogens. The CYP enzymes can detoxify xenobiotics or bioactivities them by producing reactive intermediates. The induction of ethoxyresorufin-O-deethylation (EROD) activity through induced CYP1A1 expression in MCF-7 and MCF-10A cells by PCBs and TCDD was observed by van Duursen et al [15]. Hoivik et al. [16] showed the same effect as was presented by this data – TCDD dependent induction of CYP 1A1 without any changing in enzyme activity under the influence of E2.

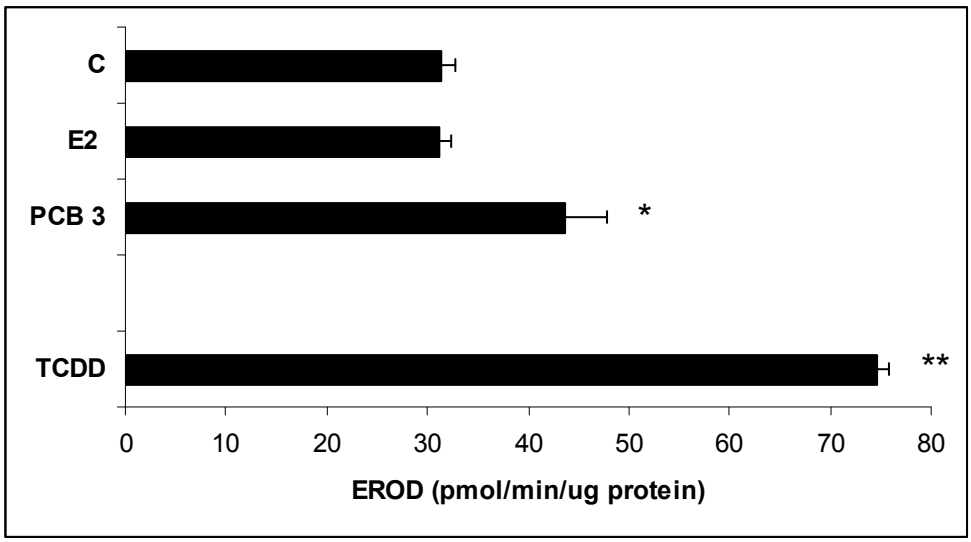

Figure 1. EROD activity in MCF-7 cells. Cells were treated with 6 ng/ml E2, 6ng/ml PCB 3 or 3.2 ng/ml TCDD for 24 h. CYP 1A1 activity was measured by the EROD assay in intact cells as described in "Materials and Methods". Data shown are the means ±SE of three independent determinations obtained in three series of experiments. *p<0,05; **p<0,01

Stimulatory action on cell proliferation measured as a protein content during both short and long exposition to investigated compound was observed under the influence of estradiole and its metabolites but not PCB3 and its metabolites. (Figs. 2, 3).

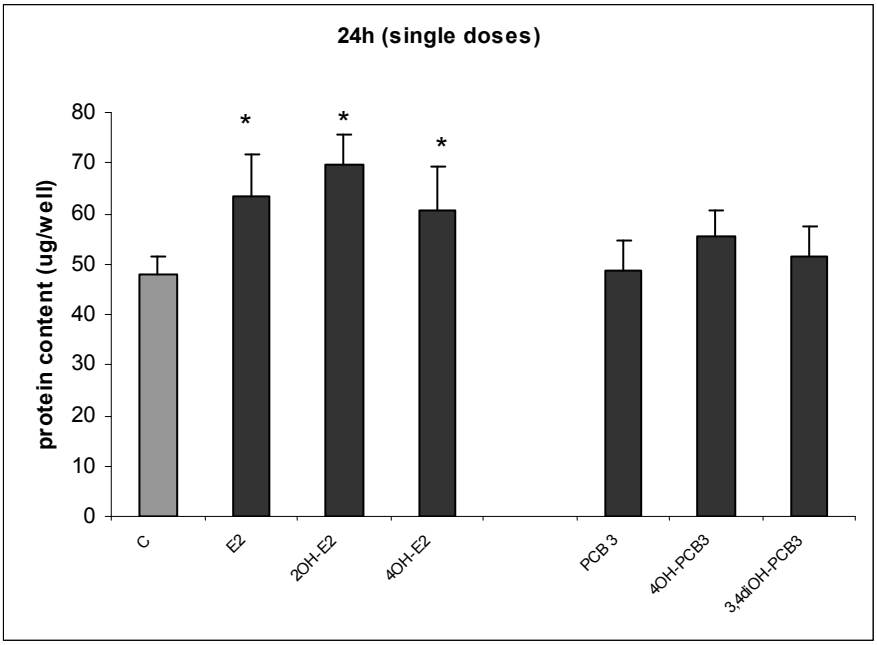

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Figure 2. The cellular protein content of MCF-7 cells treated with 6 ng/ml E2, 2OH-E2, 4OH-E2 or with 6ng/ml PCB3; 4 -OH-PCB3; 3,4-diOH-PCB3 for 24h. Data shown are the means ±SE of three independent determinations obtained in three series of experiments. *p<0,05.

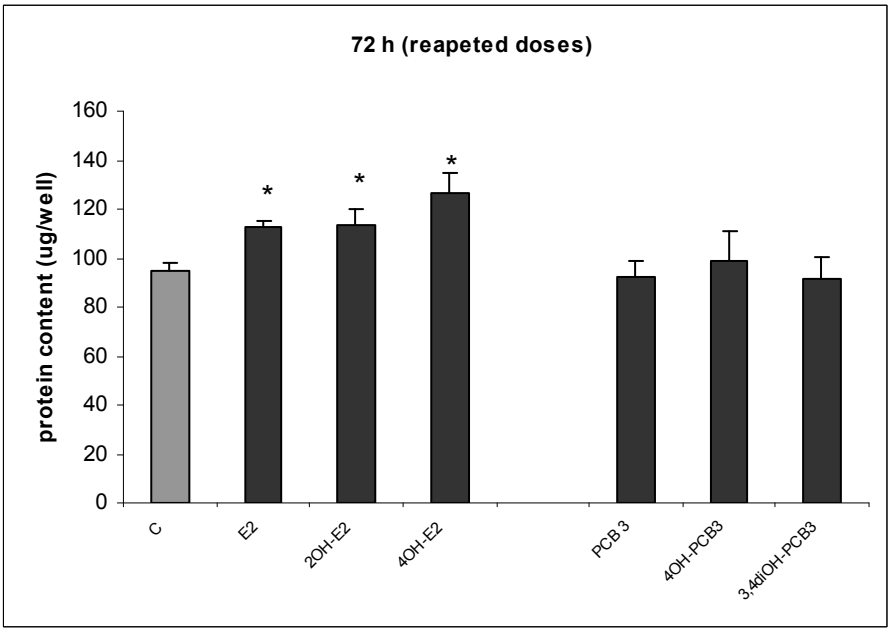

Figure 3. The cellular protein content of MCF-7 cells treated with 6 ng/ml E2, 2OH-E2, 4OH-E2 or with 6ng/ml PCB3; 4 -OH-PCB3; 3,4-diOH-PCB3 for 72h. The medium was changed every 24h with adding compounds. Data shown are the means ±SE of three independent determinations obtained in three series of experiments. *p<0,05.

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Results of the presented data are in agreement with observation made by Schutze et al.[17] who showed that incubation of ER-positive MCF-7 human breast cancer cells with 2OH-E2 and 4OH-E2 results in tight nuclear binding of the estrogen receptor and increase proliferation of MCF-7 cells [17]. Data concerning action of lower chlorinated and rapidly metabolised PCBs, are scarce.

.**Conclusion.** We hypothesis that PCB3 and even its both metabolites are not potent mitogen in breast cancer cell line, but though activation of CYP1A1 could modify the metabolism of estradiol into a more toxic pathway (4OH-E2), which have strong mitogenic activity.

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