COMPARISON OF DELOR 103 AND DELOR 106 ACTION ON HUMAN PLACENTAL STEROIDOGENESIS - IN VITRO STUDY

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Introduction

Polychlorinated biphenyls and related compounds elicit a diverse spectrum of toxic responses. They are able to pass through the human placenta [1]. Taking into account fat solubility of these compounds and the maternal origin of 10 to 20% of fetal fatty acids, PCBs, and hexachlorobenzene (HCB) may impair fetal development[2]. Human exposures to halogenated aromatic hydrocarbons such as polychlorinated biphenyls are known to be associated with adverse pregnancy outcomes including intrauterine growth retardation (IUGR), congenital structural anomalies and cognitive developmental deficits. Chen and Rogan observed fetal development impairment in women exposed to PCB and PCDD/F [3,4]. Therefore, they represent a serious health risk, especially for the fetus and infants, since their enzymatic and metabolic systems are not yet mature. The human placenta expresses high levels of aromatase and thus regulates the balance of estrogens in the uterus.

Our studies involving the primary culture of placental cells isolated from placental cotyledons harvested immediately after expulsion showed a discrepancy between the action of pure TCDD and dioxin mixture on placental steroids secretion [5]. This was possibly due to an additional effect of pentachlorodibenzo-p-dioxin (PeCDD) and pentachlorodibenzofuran (PeCDF), which covered >50 % of the total toxic equivalents (TEQ) present in this mixture.

The aim of the presented data was to compare the action of low-chlorinated and high-chlorinated biphenyls on placental steroidogenesis. The cumulation or metabolisation of isomers present in used mixtures was studied in the second step. The commercial mixtures used in this study were produced in Czechoslovakia till 1984 as DELOR 103 or DELOR 106. The 60% of production was exported to Eastern Europe; rest 40% (approximately 10000 tons) was distributed in Czechoslovakia. DELOR 103 and DELOTHERM (Tri to TetraCBs) contribute 77% to total produced PCB.

Material and methods

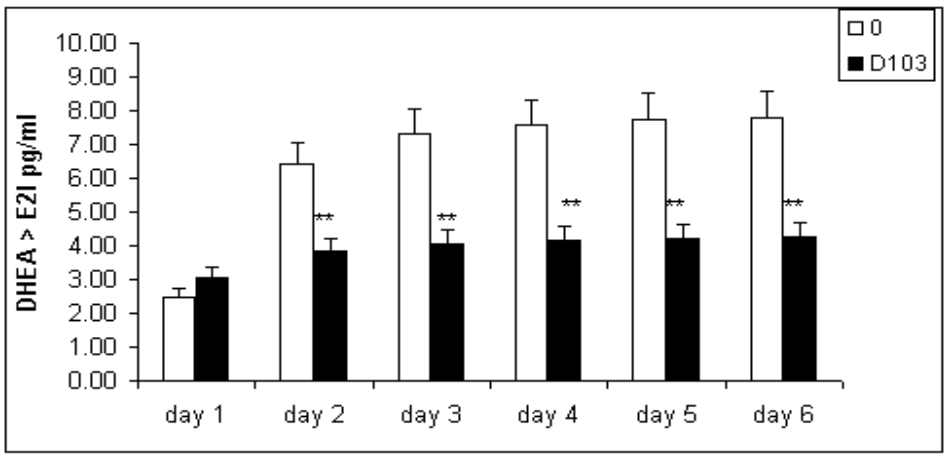
Placental tissue was collected in a gynecological hospital in Krakow, Poland where the clinical information on pregnancy outcomes was gathered. Collection of placentas and gathering of clinical histories followed previously established protocols. Placental cotyledons were harvested immediately after expulsion of the placenta, placed in ice-cold PBS and transported to the laboratory. Placental tissue was cut into small pieces and incubated in Erlenmeyer flasks containing 3 ml of M199 medium according to Gregoraszczuk [6]. The flasks were incubated at 37^{o} C with constant shaking at 70 rpm for 5 days.

In the control culture placental tissue was cultured in Parker medium (M199) supplemented with 5% of calf serum; in experimental groups solution of DELOR 103 or DELOR 106 (prepared from standard solutions by dilution to EtOH) were added daily for 5 days at a dose of 200 pg from day 0 till day 6 of culture. The media were changed every day, collected and frozen for further steroid analysis. Total dose was 1200 pg. 24h after the last treatment the culture medium was frozen for steroids concentration analysis. Dehydroepiandrosterone (DHEA; 1ng/ml), a natural substrate for estradiol synthesis in the placental tissue was used to measure the effect of PCB mixtures on estradiol production. E2 were determined radioimmunologically using Spectra kits (Orion, Diagnica, Finland).

Results

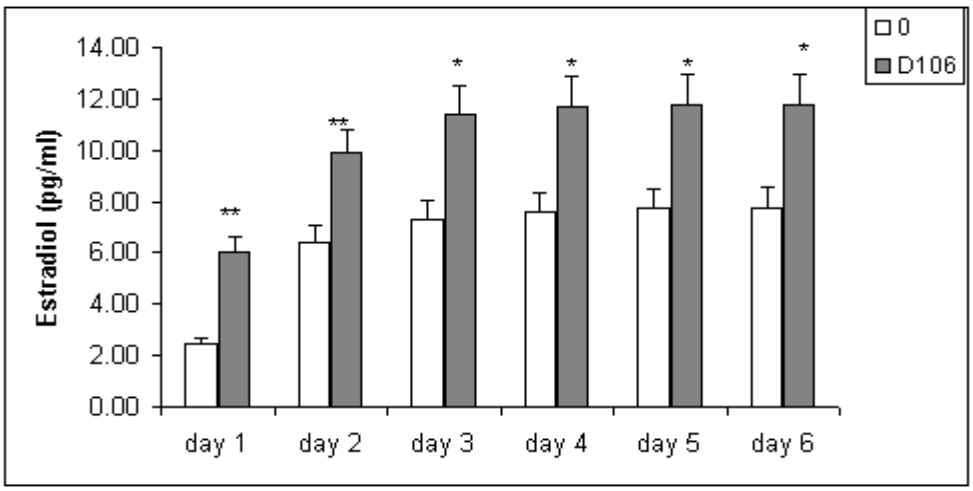
During the first day of culture DELOR 103 had no effect on the conversion of dehydroepiandrosterone (DHEA) to estradiol (E2). The situation changed with longer exposure to DELOR 103 mixture. The estradiol secretion decreased to 50% compared to control sample in days 2 to 5.

Fig. 1 The efffects of DELOR 103 (200pg/ml) on conversion of dehydroepiandrosterone (DHEA; 1ng/ml) to estradiol. **p<0,01



Statistically significant increase in estradiol secretion was noted in all days of exposure to 200 pg/ml DELOR 106.

Fig. 2 The effects of DELOR 106 (200pg/ml) on conversion of dehydroepiandrosterone (DHEA; 1ng/ml) to estradiol. *p<0,05; **p<0,01



Discussion

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During late pregnancy the placenta is the major source of estrogens, deriving its substrate from the fetal adrenal due to the inability of the placenta to synthesise androgen. Fetal adrenal DHAS(dehydroepiandrosterone sulfate) can be converted to estrone and estradiol in the placenta. In the present experiments we noted differences in the action of DELOR 103 and DELOR 106 on estradiol secretion. We noted an estrogenic action of DELOR 103 starting from 2 days of exposure and antiestrogenic action of DELOR106 at all times of exposition. The human syncytial trophoblast is known to serve several roles in pregnancy. It mediates the transport of nutrients and immunoglobulins from the maternal to fetal circulation and also functions as an endocrine organ, secreting steroid and protein hormones[7]. The human placenta expresses high levels of aromatase activity and thus regulates the balance of estrogens in the uterus. An alteration in aromatase function in the uterus has been shown to permanently affect human embryo[8]. The TCDD and dioxin-like PCB 126 were shown to decrease aromatase activity in a concentration dependent manner and inhibit cell proliferation in JEG-3 cells choriocarcinoma cell cultures of the cytotrophoblast from the malignant placental tissue [9, 10]. On the other hand humans are daily exposed to mixtures of chemicals, rather than to individual chemicals. From a public health point of view, it is most relevant to answer the question of whether or not the components in a mixture interact in a way that results in an increase in their overall effect compared with the sum of the effects of the individual components.

The presented data are the first showing effects of repeated exposure to low-chlorinated or high- chlorinated biphenyls mixture on placental steroidogenesis. Contrasting to the action of low-chlorinated DELOR 103, which showing antiestrogen action, high-chlorinated DELOR 106 showed strong estrogenic action.

The estrogenic action of the DELOR 106 on placental tissue observed in the presented study should be taken into consideration in view of the data of Chen[11], who examined the transfer of polychlorinated dibenzo-p-dioxins, dibenzofurans and non-orto biphenyls to offspring and placenta. They showed a dose-dependent increase in hepatic sequestration with TCDD, PeCDD, and PeCDF, OCDF. TCDD and three PCBs reached equilibration between the fetus and placenta.
Since estriol may affect uterine CAP (cystyl aminopeptidase) gene expression[12], it could contribute to the progressive increase in uterine responsiveness to xenobiotics in primate pregnancy during the third trimester of gestation, and its measurements may be of predictive value in delineating patients at risk of premature delivery[12,13].
In conclusion, taking into consideration the biphasic dose-dependent effect of estradiol on pregnenolone-progesterone conversion suggested by Genti-Raimondi [14] both antiestrogen action of tri- to pentaCB mixture (DELOR 103) and estrogenic action of penta-to octaCB mixture (DELOR 106) can alter fetal placental steroidogenesis and in the consequence abnormal pregnancy outcomes. However, since information concerning mechanisms of PCBs mixture action depending on the chlorination degree on placental cells is scarce, these preliminary experiments are of pioneering character.

Acknowledgements
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