Differences in the action of o,p-DDE and o,p'-DDD on hormone secretion and cell viability in JEG-3 cell line.

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Introduction

DDT (1,1,1,-trichloro-2,2-bis(p-chlorophenyl) ethane) is an insecticide with a broad spectrum of activity. It was banned in several countries in the early 1970s, because of ecological considerations, and priority to protect human health. However, it is still being used in some countries for the control of insect-transmitted diseases like typhus and malaria. It is well known that because of its lipophilic properties, DDT is present in maternal adipose tissue, maternal blood serum, umbilical cord serum, colostrums, and amniotic fluid.
In living cells DDT is metabolized to the DDE (1,1,-dichloro-2,2-bis(p-chlorophenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane). DDT and its metabolites are persistent in the environment and resistant to complete degradation.

DDT as well as its metabolites are able to cross the placenta and in this way they can interfere with fetal development. There are many epidemiological studies reporting an association between maternal blood levels and miscarriage, premature rupture of fetal membranes or pre-term birth, but surprisingly data concerning action of this chemical on hormonal status are scare. It is important to study the response of pregnancy hormones to a known reproductive toxicant under well-controlled laboratory conditions. Because such studies cannot be carried out in humans, in vitro cell culture models can be employed. JEG-3 cells (choriocarcionoma cell line) have been widely used as a trophoblast cell model to study placental function[1]. JEG-3 cells produce many peptides and steroid hormones found in normal trophoblast cells, such as hCG, GnRH and progesterone [2].

In this paper, the effect of o,p'-DDT was compared with the effect of its metabolites: o,p'-DDE, o,p'-DDD on placental secretion of progesterone, estradiol and human chorion gonadotropin (hCG).
Moreover, we checked their action on cell viability and apoptosis. Apoptosis has been demonstrated in normal placenta throughout pregnancy. Its concurrent progress with cell proliferation reflects the growth and remodeling of the placenta. Caspase-3 activation represents a critical point in transmission of the apoptotic signal.

Materials and methods

Cell culture
The JEG-3 choricarcinoma cell line was obtained from American Type Cell Culture (Rockville, MD, USA). The cells were cultured in DMEM (Sigma Chemical Co. St. Louis, MO, USA) containing 10% charcoal-stripped (and thus depleted of steroid hormones) FBS (Sigma Chemical Co. St. Louis, MO, USA) and 100uI.ml penicillin and 100μg/ml streptomycin. The cells were plated in 24-well plates at the density $5x10^4$ and cultured for 24h under the humidified 5% CO_2/95% air atmosphere at 37°C. After this time, the medium was changed for DMEM supplemented with 5% of charcoal-stripped FBS and test compounds had been added (1,10,100 ng/ml and 1μg/ml of o,p'-DDT, o,p'-DDE or o,p'-DDD, Reference Standards, EPA, Research Triangle Park, N. C, USA) for the next 24 h. At the end of the culture, media were collected and frozen at -20ºC for hormone estimation. Progesterone and hCG were measured using immunoenzyme assay (EIA) kits produced by Diametra (Italy).
The similar experiment have been performed in 96-well plates for determination of LDH and caspase-3 activity by a colorimetric method.

Lactate dehydrogenase cytotoxicity assay
Cytotoxicity detection kit (Roche Applied Science) is a colorimetric assay for quantification of cell death and cell lysis based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into supernatant. An increase in the amount of dead or plasma membrane-damaged cells results in an increase in the LDH activity in the culture supernatant. After 24-hour treatment of cells with 4μg/ml of

DDT or its metabolites, culture supernatants were collected and incubated with the reaction mixture from the kits. After 30 min, the reaction was stopped by adding 1N HCl and absorbance was measured at wavelength of 490nm with reference wavelength of 600nm in microELISA plate reader.

Caspase-3 activity

After replacing media with caspase assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 10% glycerol, and 10 mM dithiothreitol), cell lysates was incubated with caspase-3 substrate. A colorimetric assay for caspase-3 was performed using the Caspase-3 Colorimetric Assay (R&D System Inc). The amounts of colorimetric products was monitored continuously till 90 min. with a spectrophotometer (Bio-tek, Biokom) at wavelength of 405 nm.

Results and discussion

Maternal exposure to estrogenic pesticides, such as 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT) and methoxychlor (MTC), has been shown to result in reproductive disorders and/or abnormal fetal development. In humans, in the first trimester, progesterone, the hormone necessary for maintaining of pregnancy, is produced by corpus luteum, while hCG is the main hormone produced by placenta. From the second trimester till the end of pregnancy, both progesterone and hCG are produced by placenta. Since progesterone is crucial for pregnancy, its insufficient synthesis could be the reason of abortion or pre-term birth. This study has been performed to test the hypothesis that DDT as well as DDD and DDE belonging to the principal products of DDT degradation could act as endocrine disruptors in placental tissue.

Results of the presented experiments showed the decrease in progesterone secretion by JEG-3 cell line under the influence of the highest dose of o,p,-DDT and o,p-DDD but not o,p-DDE. (Fig.1).

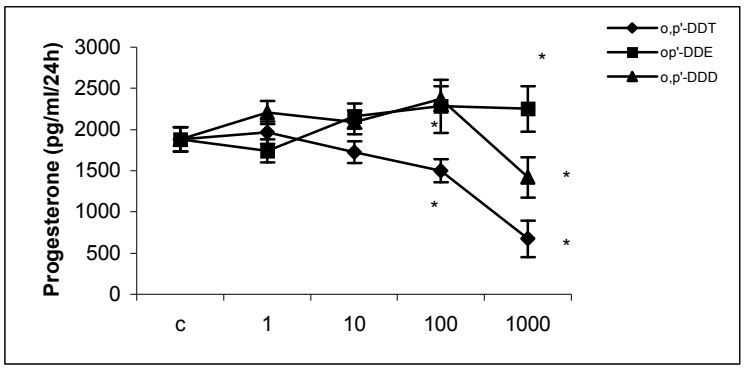

*Fig.1 The effect of o,p'-DDT, o,p'-DDE and o,p'-DDD on progesterone secretion in JEG-3 cell cultures. Data (mean±SD) are expressed relative to untreated control. *p<0.05 versus control.*

All investigated compounds inhibited hCG secretion in a dose-dependent manner (Fig.2). DDT-induced decrease in hCG and progesterone secretion could be related to an inhibition of cytochrome P450scc.

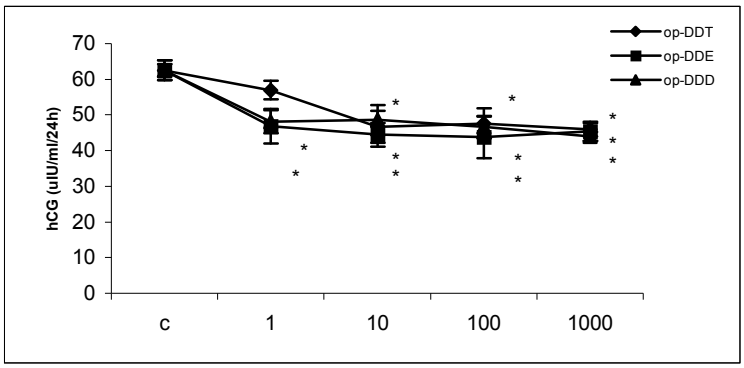

*Fig.2. The effect of o,p'-DDT, o,p'-DDE and o,p'-DDD on β-hCG secretion in JEG-3 cell cultures. Data (man±SD) are expressed relative to untreated control. *p<0.05 versus control.*

Another step of our study was devoted to the mechanisms of action of DDT and its metabolites on hormone secretion. We tried to verify if the hormonal effects of these compounds are due to their toxic action. Cell death, which was measured as the level of LDH release into the culture medium, changed only after the treatment with the high doses of o,p-DDE, thus suggesting a toxic action of this compound. Additionally, o,p-DDE induced caspase-3 activity which points to pro-apoptotic properties of this compound (Fig.4). Both o,p-DDT and o,p-DDD decreased LDH release, suggesting proliferative action of these two compounds (Fig.3). The smallest doses of o,p-DDT and o,p-DDD decreased caspase-3 activity while the higher doses had no statistically significant effect (Fig.4).

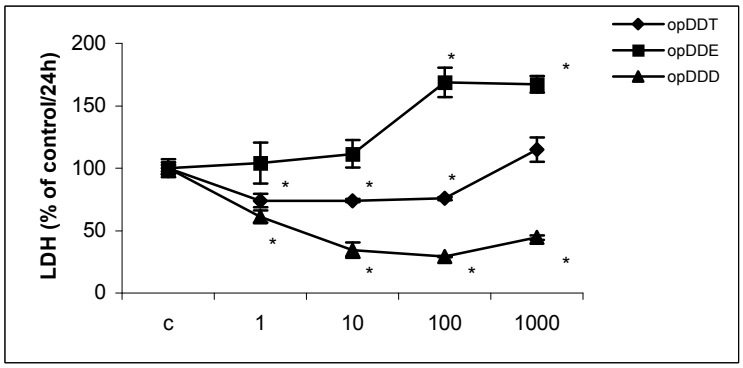

*Fig.3. The effect of o,p'-DDT, o,p'-DDE and o,p'-DDD on LDH activity in culture of JEG-3 cells. Data (mean±SD) are expressed relative to untreated control. *p<0.05 versus control.*

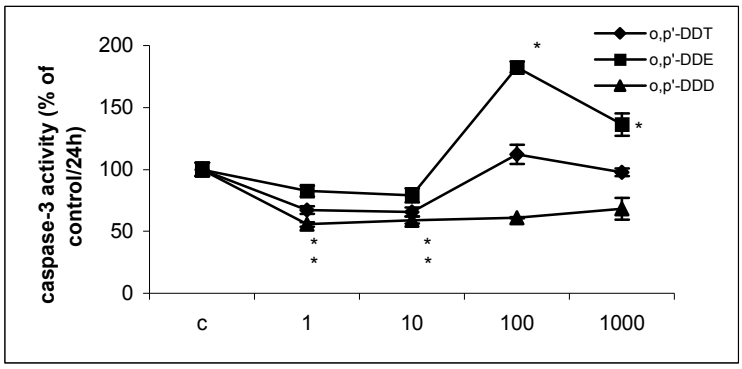

*Fig.4. The effect of o,p'-DDT, o,p'-DDE and o,p'-DDD on caspase-3 activity in culture of JEG-3 cells. Data (mean±SD) are expressed relative to untreated control. *p<0.05 versus control.*

There is a body of evidence concerning proliferative action of DDT in pig granulosa cells, Chinese hamster ovary cell line (CHO)[6], uterus[7] as well as in breast cancer cell line MCF-7 [8, 9]. Additionally, antiapoptotic action of DDT in MCF-7 cell line has been shown [8, 9]. The apoptotic action of DDT and its metabolites DDE, DDD have been shown in blood cells [11].
However, there is only one paper showing DDT effects on proliferation and apoptosis in human trophoblast [10] .
The present data have demonstrated that o,p'-DDT and o,p'-DDD have similar properties: they decrease progesterone and hCG secretion in JEG-3 cell line, but they do not affect cell viability. In contrast to them, DDE exhibits a strong toxic effect. Thus, DDE-induced decrease in hCG secretion is probably due to its action on cell viability and apoptosis, but not due to a direct action on hormone synthesis.

In conclusion the present study demonstrated the ability of o,p'-DDT, o,p'-DDE and o,p'-DDD to alter main placental hormone production and survival of trophoblast cells. These findings in turn have implication for human placental function and may have been important for explanation of observed pathological consequences in human pregnancy.

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