DDT AND ITS METABOLITE DDE INHIBIT CYP1A1 ACTIVITY IN THE HUMAN CHORIOCARCINOMA JEG-3 CELLS

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Introduction:

DDT (1,1,1,-trichloro-2,2-bis(p-chlorophenyl) ethane and its metabolite DDE (1,1,-dichloro-2,2-bis(p-chlorophenyl)ethylene) are well-known organochlorine pesticides still present in the environment. DDT has been banned from agricultural use in most countries since the 1970s, but it is still being used in limited quantities in certain countries to fight the spread of malaria. An analysis of maternal adipose tissue, maternal blood serum, umbilical cord serum, amniotic fluid and placental tissue indicated that DDT and DDE circulate through all compartments of the maternal body. Recently, it has been postulated that low doses of DDT may have epigenetic effects and cause incomplete methylation of specific gene regions in the young tissues.[1]

The placenta provides a link between the circulations of two distinct individuals. Its major functions are to transfer nutrients and oxygen from the mother to the fetus and to assist in the transfer of waste products from the fetus to the mother. Placenta plays also an important role in the synthesis of hormones, peptides and steroids that are vital for a successful pregnancy. In addition to this, placenta acts as a barrier to protect the fetus from xenobiotics delivered with maternal blood. Among cytochrome P450 (CYP) enzymes, CYP1A1 has been shown to have a significant role in placental drug detoxification. This enzyme has been detected in early and full- term placenta. Little is known, however, about its function in response to DDT and DDE in human placenta.

Therefore, the aim of the present study was to investigate the actions of p,p'-DDT and its metabolite DDE, on the activity of enzyme CYP1A1 in JEG-3 cells (choriocarcinoma cell line), which correspond to early-term human placenta. JEG-3 cells are morphologically similar to their cells of origin, i.e. to trophoblast of the normal first trimester and provide a cell model to study placental function.[2, 3] We studied both basal and TCDD-induced activities of CYP1A1. In addition, we checked if the used concentrations of DDT and DDE were cytotoxic in terms of lactate dehydrogenase (LDH)-release.

Materials and methods:

The JEG-3 choriocarcinoma cell line was obtained from American Type Cell Culture (Rockville, MD, USA). The cells were cultivated in DMEM supplemented with FBS and antibiotics in 48-well plates and initially cultured for 24h. The medium was then changed and the cells were exposed to 1μg/ml of p,p'-DDT, p,p'-DDE, o,p'-DDT or o,p'-DDE (Reference Standards, EPA, Research Triangle Park, N. C, USA) for 72 h. This concentration is in the range of concentrations of DDT and DDE reported to be present in serum of pregnant woman.[4, 5] As for CYP1A1, the activity of this enzyme was estimated using the fluorometric substrate etoxyresorufin and measuring CYP1A1-specific 7-ethoxyresorufin O-deethylase (EROD) according to the method of Kennedy et al. (1993).[6] An involvement of estrogen receptors in DDT and DDE actions was checked with a high-affinity receptor antagonist ICI 182,780 (1μM). Lactate dehydrogenase (LDH)-release was measured as previously described.[7]

Results and Discussion:

The main achievement of the study is demonstration that dichlorodiphenyltrichloroethane (DDT; 1 μg/ml) and its metabolite DDE 1,1,-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE; 1 μg/ml) inhibited CYP1A1 enzyme activity in JEG-3 cells corresponding to early-term human placenta (Fig. 1). The inhibitory actions of DDT and DDE on CYP1A were indicated in tetrachlorodibenzo-*p*-dioxin (TCDD)-treated cells, whereas the basal activity of CYP1A1 in intact JEG-3 cells remained unchanged. These data are consistent with the study of Hakkola et al. (1997) who demonstrated that CYP1A1 is highly inducible, but its constitutive level is rather low in JEG-3 cells.[8] Accordingly, in our study the basal activity of CYP1A1 was much lower than TCDD-stimulated activity of the cytochrome (Fig. 2).

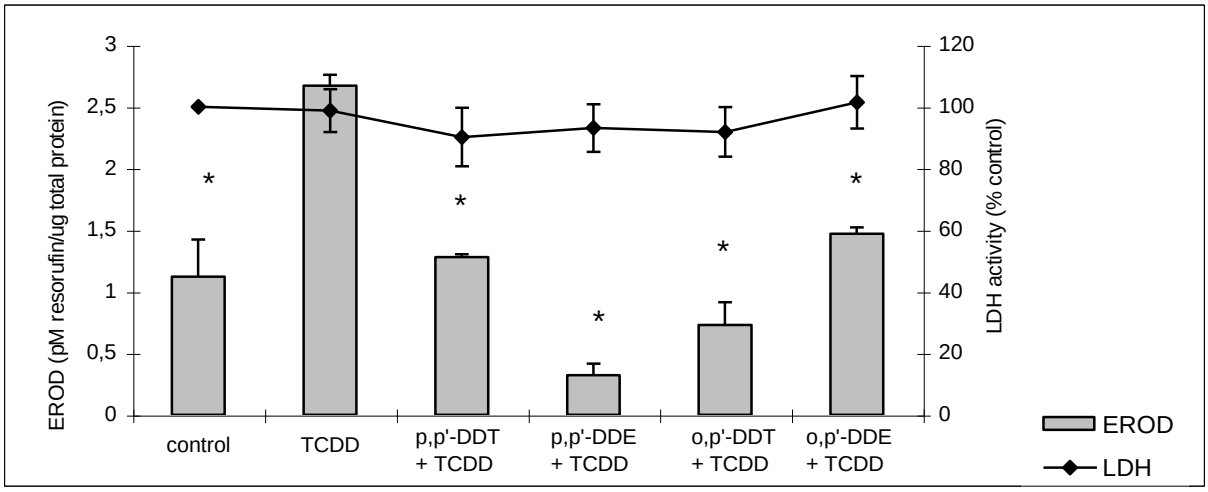

Fig. 1. Effects of 1 μg/ml of p,p-DDT, p,p-DDE, o,p'-DDT and o,p'-DDE on TCDD (3.2 ng/ml)-stimulated EROD activity and lactate dehydrogenase (LDH) in JEG-3 cells corresponding to early-term of human placenta. Cells were treated with the compounds for 72 h. The results are presented as pmoles of resorufin/μg protein or a percentage of control LDH-release. Each bar or point represents the mean of three to four independent experiments ± SEM. A number of replicates in each experiment ranged from 5 to 8. $^{*}p<0.05$ (versus control cultures).

We demonstrated for the first time that p,p'- and o,p'-isomers of DDT and DDE significantly inhibited CYP1A1 in tetrachlorodibenzo-*p*-dioxin (TCDD)-treated JEG-3 cells, thus pointing to the complex actions of all the compounds on embryonic tissues (Fig.1). We did not notice any particular isomer-specific effects of DDT and DDE in respect to CYP1A1 and LDH-release. Previously, Jeong et al. (2002) observed over 50% decrease in TCDD-stimulated EROD activity in Hepa-1c1c7 cells, but only in response to o,p'-DDT. [9]

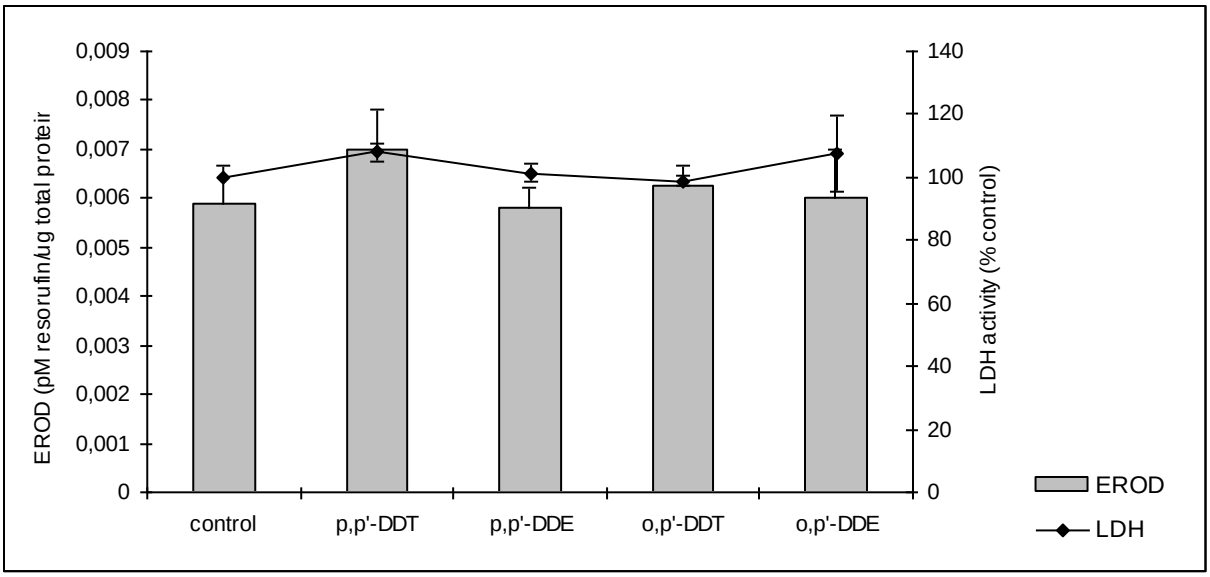

Fig. 2. Effects of 1 μg/ml of p,p-DDT, p,p-DDE, o,p'-DDT and o,p'-DDE on basal EROD activity and lactate dehydrogenase (LDH) in JEG-3 cells corresponding to early-term of human placenta. Cells were treated with the compounds for 72 h. The results are presented as pmoles of resorufin/μg protein or a percentage of control LDH-release. Each bar or point represents the mean of three to four independent experiments ± SEM. A number of replicates in each experiment ranged from 5 to 8.

This study showed that a high-affinity estrogen receptor (ER) antagonist, ICI 182,780 did not reverse the effects of DDT and its metabolite DDE (Fig. 3). Therefore, the inhibitory action of DDT and DDE against CYP1A1 activity is possibly not mediated by estrogen receptors. Similar effects were observed by Ciolino and

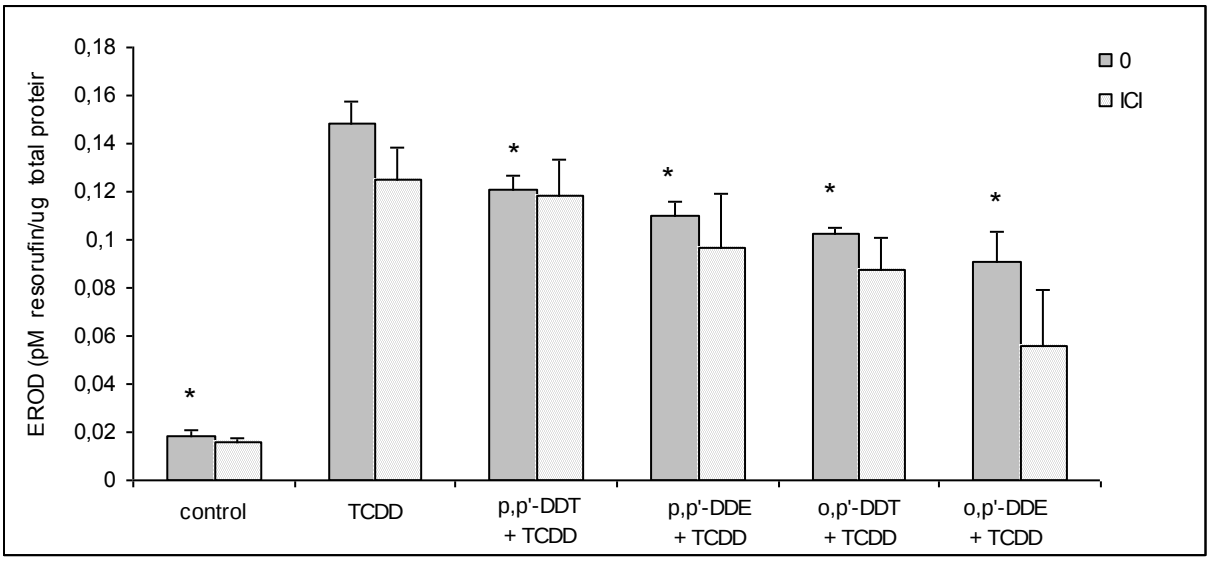

Fig. 3. Effects of a high-affinity receptor antagonist ICI 182,780 (1μM) and p,p-DDT, p,p-DDE, o,p'-DDT and o,p'-DDE (all in concentration of 1 μg/ml) on TCDD (3.2 ng/ml)-stimulated EROD activity in JEG-3 cells. Cells were treated with the compounds for 72 h. The results are presented as pmoles of resorufin/μg protein. Each bar or point represents the mean of three to four independent experiments ± SEM. A number of replicates in each experiment ranged from 5 to 8. $^{*}p<0.05$ (versus TCDD-treated cultures).

Yeh (1999), Jeong and Lee (1998), and Jeong et al. (2001) in TCDD-stimulated HepG2 and Hepa 1c1c7 cells, respectively.[10, 11, 12] In our previous study, we observed significant decrease in human chorionic gonadotropin in JEG-3 cells due to exposure to p,p'-DDT and o,p'-DDT.[13] However, the other mechanisms such as elevated expression of TNFα and AP-1-mediated gene expression through the p38 mitogen-activated protein kinase cascade, which leads to cytochrome c release from mitochondria and activation of caspase-3/7 cascade cannot be excluded.[14] Since CYP1A1 present in the human placenta is involved in the metabolism and detoxification of estrogens and xenoestrogens, the malfunction of this enzyme by DDT and its metabolite DDE disrupts the placental detoxification machinery, which may lead to increased susceptibility of the fetus to environmental toxins, and may represent a risk factor for recurrent pregnancy loss. Our data may have implication for better understanding of placental detoxification machinery at early stage of embryonic development.

Acknowledgements
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