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Effects of two isomers of DDT and their metabolite DDE on CYP1A1 and AhR function in human placental cells

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Abstract:
The aim of this study was to investigate the actions of two isomers of DDT (*p,p'*-DDT, *o,p'*-DDT) and DDE (*p,p'*-DDE, *o,p'*-DDE) on the human placenta. We studied the effects of DDT and its metabolite DDE on CYP1A1 activity and on CYP1A1 and aryl hydrocarbon receptor (AhR) protein expression in placental cells. We used explants from third-trimester human placental tissue and JEG-3 cells, which are first-trimester human placenta cells. The main finding of this study was that the activity of CYP1A1 in the human placenta, measured in terms of ethoxyresorufin-O-deethylase (EROD) activity, was suppressed by treatment of 1, 10, and 100 ng/ml *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *o,p'*-DDE. Immunoblot analyses indicated that both isomers of DDT and DDE inhibited the expression of CYP1A1 most effectively at 48 h and/or 72 h after the treatment. Because CYP1A1 activity is mediated by AhR, we evaluated the expression of AhR in placental tissue exposed to DDT and DDE for 1 h to 72 h. Our data showed that DDT and DDE gradually decreased the level of AhR protein, starting at 3 h or 24 h after the start of the experiment. Our results strongly support the involvement of the AhR/CYP1A1 signaling pathway in the mechanism of action of DDT and DDE in the human placenta.

Key words:
human placental explant, JEG-3 cells, pesticide, EROD, AhR protein expression, CYP1A1 protein expression

Introduction

1,1,1-Trichloro-2,2-di(4-chlorophenyl)ethane (DDT) and its metabolites are characterized by their relatively low toxicity, but they have the ability to accumulate in fatty tissues of humans and animals [4, 27]. Many epidemiological studies have shown the presence of DDT and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (DDE) in maternal adipose tissue, maternal blood serum, umbilical cord serum, mature milk and amniotic fluid [5, 12, 13, 22]. Moreover, DDT and its metabolites are able not only to accumulate in placental tissue but also to cross the placenta and enter the developing fetus [2]. There are many epidemiological reports documenting the association between the levels of these compounds in maternal blood and miscarriage or preterm birth rates [6, 8, 16].

The human placenta is responsible not only for the production of a number of hormones and proteins necessary for normal fetal development and pregnancy maintenance but also for the metabolism of many compounds present in the body of the mother. The placenta metabolizes many endogenous substances, foreign chemicals and drugs and environ-

mental xenobiotics. The important players in this process are monooxygenases, which are enzymes belonging to the cytochrome P450 (CYP) superfamily. CYP1A1 is the most commonly and consistently expressed isoform in the early- and full-term placenta [1, 9]. This enzyme catalyzes the metabolism and detoxification of many environmental contaminants, such as polycyclic aromatic hydrocarbons (PAHs), and is also involved in the metabolism of endogenous substrates, like estradiol [11, 29]. CYP1A1 expression and activity are regulated by the aryl hydrocarbon receptor (AhR), which is present in many tissues, including the placenta. AhR is a ligand-dependent transcription factor that activates the transcription of target genes, such as CYP1A1, CYP1A2, CYP1B1, and oncogenes [17]. Surprisingly, there is no data on CYP1A1 function in response to DDT and DDE in the human placenta.

The aim of the present study was to investigate the actions of *p,p'*-DDT and *o,p'*-DDT and their metabolite DDE on the expression of AhR and CYP1A1 in placental cells following 1 to 72 h of exposure. We also measured the activity of CYP1A1 in placental cells. We used explants from third-trimester human placental tissue and cells from the choriocarcinoma JEG-3 cell line, which are cells from a first-trimester human placenta. Because JEG-3 cells have a very low basal expression of the CYP1A1 gene, we used the well-known AhR agonist 2,3,7,8-tetrachlorodibenzo- *p*-dioxin (TCDD) to induce ethoxyresorufin-O-deethylase (EROD) activity [9].

Materials and Methods

Reagents

Phenol red-free DMEM, trypsin, charcoal/dextran-treated fetal bovine serum (FBS), penicillin, streptomycin, TRIS, ethoxyresorufin-O-deethylase, resorufin, fluorescamine, NADPH, acetonitrile, HEPES, CHAPS, DTT, EDTA, Tween 20, bromophenol blue and DMSO were purchased from Sigma (Chemical Co., St. Louis, MO, USA). The cytotoxicity lactate dehydrogenase (LDH) detection kit was purchased from Roche Applied Science, Germany. TCDD and the DDT compounds (*p,p'*- DDT, *o,p'*-DDT, *p,p'*-DDE and *o,p'*-DDE) were purchased from Reference Standards, EPA (Research Triangle Park, NC, USA). Stock solutions of these test compounds in DMSO were prepared and added to M199 supplemented with 5% FBS. The final concentration of DMSO in the culture medium was always 0.1%.

JEG-3 cells culture

The choriocarcinoma JEG-3 cell line was obtained from American Type Cell Culture (Rockville, MD, USA). The cells were cultured in DMEM without phenol red (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), 100 UI/ml penicillin and 100 µg/ml of streptomycin.

For the experiments, the cells were plated in 48-well plates (NUNC) at a density of 5×10^4 (for the 24 h treatment), 4×10^4 (for the 48 h treatment) or 3×10^4 (for the 72 h treatment) and initially cultured for 24 h under a humidified 5% CO_2/95% air atmosphere at 37°C. After this initial incubation, the medium was changed to DMEM supplemented with 5% charcoal-stripped FBS in the presence of 1, 10 or 100 ng/ml *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT or *o,p'*-DDE. These amounts cover the range of concentrations of DDT and DDE reported to be present in the serum of pregnant women [20, 22]. All inducers were dissolved in DMSO, resulting in a final vehicle concentration of 1% (v/v). Control (no vehicle) and DMSO-treated wells were included in the experimental design to determine the effect of DMSO on CYP1A1 activity and expression (results not shown).

To study the effect of both isomers of DDT and DDE on TCDD-stimulated CYP1A1 activity, cells were co-treated with 3.2 ng/ml TCDD and 1, 10 or 100 ng/ml *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT or *o,p'*-DDE. After 72 h of culture, 100 µl of medium was collected for the LDH analysis, and the cells were collected and frozen at –70°C for the EROD assay. The choice of the exposure of 72 h was based on preliminary experiments, in which JEG-3 cells were treated with DDT and DDE for 24 h, 48 h, and 72 h. Exposures to DDT or DDE for less than 72 h did not affect CYP1A1 activity.

Placental explant cultures

Placentas were collected at the obstetrical teaching hospital in Kraków, Poland, where the clinical information on the pregnancy outcome was gathered. Each placental explant originated from a normal pregnancy

ending with the delivery of healthy child. Placenta collection followed previously established and published protocols that were approved by the local institutional review board. Full-term placentas were obtained from nonsmoking women undergoing elective cesarean section at weeks 37–41 of gestation. Placental cotyledons were harvested at the time of cesarean section under sterile operating theater conditions, immediately placed in ice-cold phosphate buffered saline (PBS) and transported to the laboratory within 30 min of delivery. Placental tissues were rinsed three times with PBS containing 100 UI/ml penicillin and 100 μg/ml streptomycin. Decidual tissue and blood vessels were removed from the villous placenta by blunt dissection, and the tissue was minced into 2–3 mm pieces. Explants with a total wet weight of approximately 20–30 mg were dispersed into wells in 12-well plates (NUNC) containing 1.5 ml of DMEM supplemented with 5% charcoal-stripped FBS and penicillin/streptomycin. The explants were incubated in triplicate at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

To investigate the action of both DDT isomers and their metabolites on the activity of CYP1A1, placental explants were cultured in DMEM supplemented with 5% FBS and in the presence of 1, 10, 100 and 1000 ng/ml of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT or *o,p'*-DDE for 24 h, 48 h and 72 h. All inducers were dissolved in DMSO, resulting in a final vehicle concentration of 1% (v/v). Control (no vehicle) and DMSO-treated wells were included in the experimental design to determine the effect of DMSO on CYP1A1 activity and expression (results not shown).

Additionally, in separate experiments, cells were cultured in the presence of 10 μM α-naphthoflavone, a well-known inhibitor of CYP1A1 activity. In the final preparatory step, the medium was removed, and the tissues were frozen at –70°C for CYP1A1 activity measurement.

To study the expression of AhR and CYP1A1, placental explants were cultured in DMEM supplemented with 5% FBS and in the presence of 100 ng/ml of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT and *o,p'*-DDE. After 1, 3, 6, 14, 24, 48 and 72 h of culture, the tissues were frozen in liquid nitrogen and stored at –70°C for western blot analysis of AhR and CYP1A1 protein expression.

LDH cytotoxicity assay

The cytotoxicity detection kit was used in a colorimetric assay for the quantification of cell death and cell lysis based on the measurement of LDH activity released from the cytosol of damaged cells into the 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 the JEG-3 cells were treated for 72 h with 1, 10 or 100 ng/ml of DDT isomers or their metabolite DDE, 100 μl of culture supernatant was collected and incubated with the reaction mixture from the kits. After 30 min, the reaction was stopped by addition of 1 M HCl, and the absorbance was measured at a wavelength of 490 nm with a reference wavelength of 600 nm in a microELISA plate reader (Bio-Tek Instruments, Biokom).

EROD assay

We estimated the activity of the CYP1A1 enzyme using the fluorometric substrate EROD. The fluorescent EROD assay of CYP1A1 activity was performed in 48-well plates according to the method of Kennedy et al. [19]. The total protein concentration in each well was measured using fluorescamine according to the method of Kennedy and Jones [18].

The EROD assays were carried out in multiwell plates, and the fluorescent product, resorufin, and the total amount of protein were quantified within the same wells with a fluorescence plate reader (Bio-Tek Instruments, Biokom). The ethoxyresorufin metabolite, resorufin, was measured using an excitation wavelength of 530 nm and an emission wavelength of 590 nm.

Protein concentration was measured using fluorescamine at an excitation wavelength of 400 nm and an emission wavelength of 460 nm.

Western blot analysis

For immunoblotting, the tissues were homogenized twice in 20 μl of ice-cold lysis buffer containing 50 mM HEPES, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 10% glycerol and 10 mM DTT. For the measurement of CYP1A1 activity, the tissues were homogenized twice in 20 μl of ice-cold PBS. The homogenates were clarified by centrifugation at 15,000 × g at 4°C for 30 min, and total protein concentration was determined in the supernatants with the Bradford reagent (Bio-Rad Protein Assay; Bio-Rad Laboratories, Munchen, Germany) using bovine serum albumin (BSA) as the standard. From the whole explant

lysate, 100 μg of total protein was reconstituted in the appropriate amount of sample buffer consisting of 125 nM Tris, pH 6.8, 4% SDS, 25% glycerol, 4 mM EDTA, 20 mM DTT and 0.01% bromophenol blue. The samples were separated by 7.5% SDS-polyacrylamide gel electrophoresis in a Bio-Rad Mini-Protean II Electrophoresis Cell, and then the proteins were transferred to nitrocellulose membranes using a Bio-Rad Mini Trans-Blot apparatus. Following the transfer, the membranes were washed, and nonspecific binding sites were blocked with 5% dried milk and 0.2% Tween 20 in 0.02 M TBS for 2 h. The membranes were then incubated overnight with the Ah receptor antibody (goat anti-human polyclonal antibody, Santa Cruz Biotechnology, Inc.) diluted at 1:500 or with anti-CYP1A1 (goat anti-human polyclonal antibody, Santa Cruz Biotechnology, Inc.) diluted 1:500 in TBS/Tween at 4°C. After incubation with the primary antibody, the membranes were washed with TBS and 0.02% Tween 20 and incubated for 2 h with horseradish peroxidase-conjugated secondary antibody (donkey anti-goat IgG, Santa Cruz Biotechnology, Inc.) diluted at 1:1000 in TBS/Tween. To control for the amounts of protein that were loaded onto the gel, the membranes were stripped and reprobed with an anti-β-actin antibody (Sigma Chemical Co., St. Louis, MO, USA). Signals were detected by electrogenerated chemiluminescence (ECL) using the Western Blotting Luminol Reagent (Santa Cruz Biotechnology, Inc.) and visualized with the use of a PhosphorImager FujiLas 1000. All data bands visualized by chemiluminescence were quantified using a densitometer and the Image Gauge 4.0 software (Fuji Film, Dueseldorf, Germany).

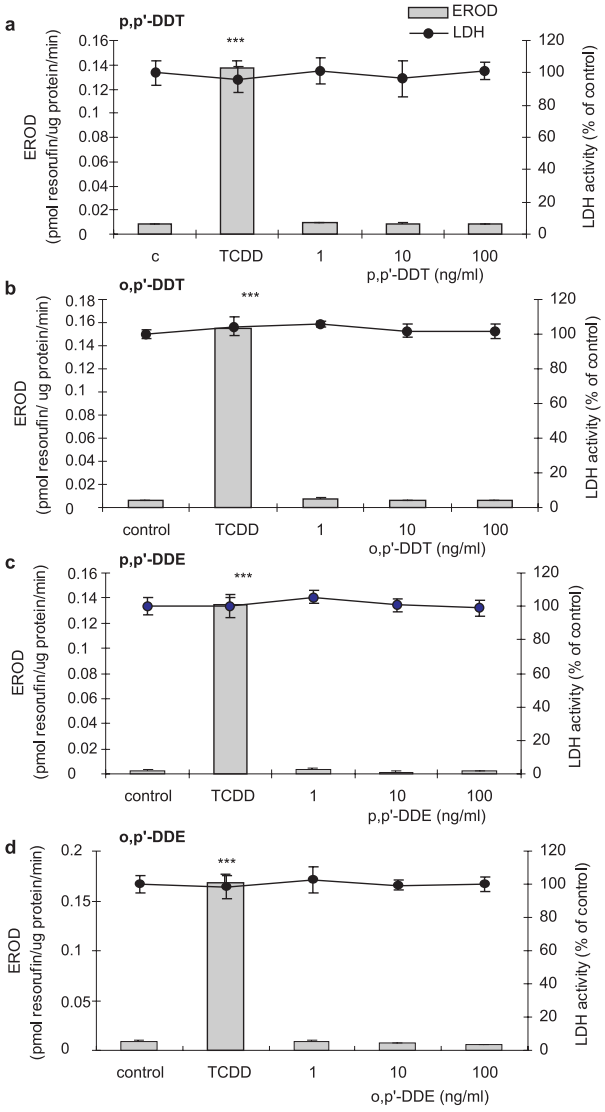

Fig. 1. Effects of increasing concentrations of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDE (1, 10, 100 ng/ml) on CYP1A1 and LDH activity in cultured JEG-3 cells after 72 h of exposure. The data are the means ± SEM of three independent experiments, each of which consisted of four replicates per treatment group. *** $p < 0.001$ *vs.* control cultures

Statistical analysis

The data are presented as the means ± SEM of three independent experiments. Each treatment was repeated three times (n = 3) in quadruplicate, resulting in a total number of 12 replicates. The average of the quadruplets was used for the statistical calculations. The data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison procedure.

Results

Effects of the DDT and DDE isomers on JEG-3 cells

Effects of the DDT and DDE isomers on CYP1A1 activity in JEG-3 cells

The basal activity of CYP1A1 in DDT (*p,p'*-DDT, *o,p'*-DDT)- and DDE (*p,p'*-DDE, *o,p'*-DDE)-exposed JEG-3 cells remained unchanged, i.e., it stayed at the control level 72 h after treatment (Figs. 1a–d). Each

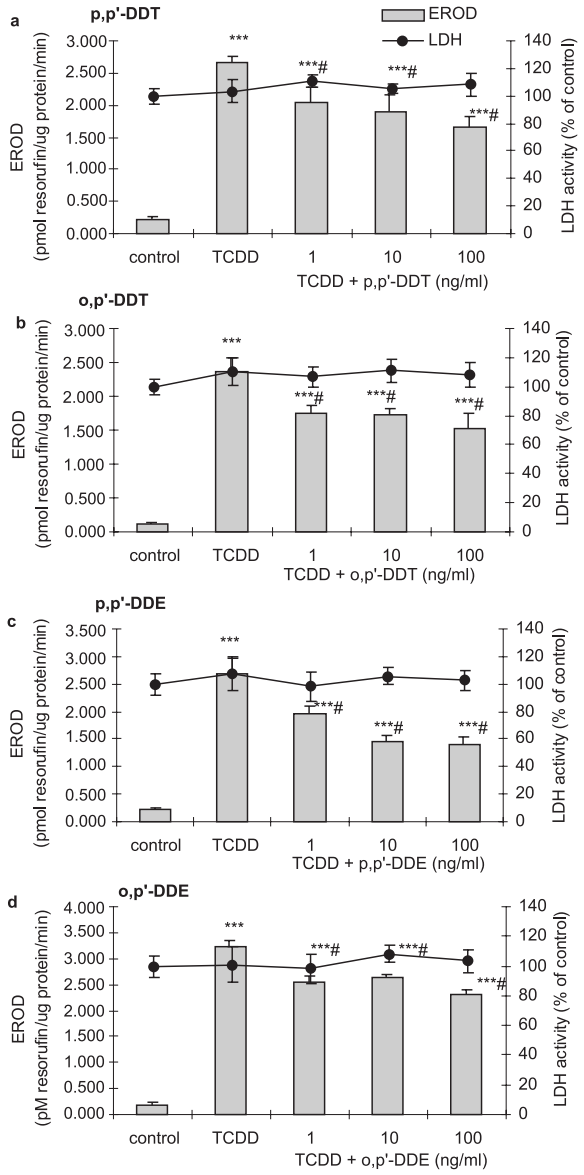

Fig. 2. Effects of increasing concentrations of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDE (1, 10, 100 ng/ml) on TCDD (3.2 ng/ml)-stimulated CYP1A1 and LDH activity in cultured JEG-3 cells after 72 h of exposure. Each point represents the mean ± SEM of three independent experiments, each of which consisted of four replicates per treatment group. *** $p < 0.001$ *vs.* control cultures; # $p < 0.05$ *vs.* the cultures exposed to TCDD

compound was used at concentrations of 1, 10, and 100 ng/ml. With DDT, the activity of CYP1A1 ranged from 0.0048 to 0.009962 pM resorufin/μg protein, whereas with DDE, it ranged from 0.001488 to 0.008568 pM resorufin/μg protein. TCDD (3.2 ng/ml) was used as an internal positive control, and it stimulated EROD activity in all of the experimental systems at levels of over 0.1475 pM resorufin/μg protein.

Effects of DDT and DDE isomers on TCDD-stimulated CYP1A1 activity in JEG-3 cells

The TCCD (3.2 ng/ml)-stimulated activity of CYP1A1 was inhibited by DDT and its metabolite DDE at all of the concentrations tested (1, 10, and 100 ng/ml) (Figs. 2a–d). Both DDT isomers, *p,p'*-DDT and *o,p'*-DDT, decreased the TCDD-stimulated EROD activity to the same extent, i.e., by 54.6–44.2% of the TCDD-induced activity (Figs. 2a, b). CYP1A1 was inhibited more efficiently by *p,p'*-DDE than by *o,p'*-DDE; thus, an isomer-specific effect was observed. *p,p'*-DDE inhibited CYP1A1 activity by 49.3–63.4% of the TCDD-stimulated value, whereas *o,p'*-DDE reduced CYP1A1 activity by only 17.9–28.4% of the TCDD-stimulated value (Figs. 2c, d). The inhibitory actions of DDT and DDE were dose dependent, and the most effective dose was 100 ng/ml.

Effects of the DDT and DDE isomers on LDH activity in intact and TCDD-treated JEG-3 cells

The compounds tested at concentrations ranging from 1 to 100 ng/ml were not toxic to the intact and TCDD-treated JEG-3 cells at 72 h of exposure. The level of LDH activity in DDT- or DDE-treated cells did not exceed the control value (Figs. 1a–d; 2a–d).

Effects of the DDT and DDE isomers on human placental explants

Effects of the DDT and DDE isomers on CYP1A1 activity in human placental explants

Both isomers of DDT (*p,p'*-DDT, *o,p'*-DDT) and DDE (*p,p'*-DDE, *o,p'*-DDE) used at a concentration

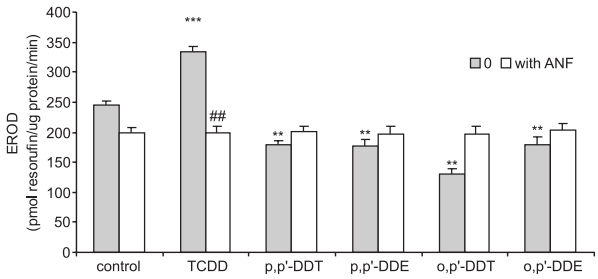

Fig. 3. Effect of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE (100 ng/ml), TCDD (3.2 ng/ml), and α-naphthoflavone (10 μM) on CYP1A1 protein level in placental explant cultures after 72 h of exposure. The data are the means ± SEM of three independent experiments, each of which consisted of four replicates per treatment group. *** $p < 0.001$, and ** $p < 0.01$ *vs.* control group; ## $p < 0.01$ *vs.* the TCDD-stimulated group

of 100 ng/ml inhibited the activity of CYP1A1 in human placental explants, as observed after 72 h of exposure. The strongest inhibitory effect was observed in *o,p'*-DDT-treated human explants because the activity was decreased by more than 114 pM resorufin/μg protein below the control (Fig. 3). Additionally, TCDD (3.2 ng/ml) was used as an internal positive control, and it stimulated EROD activity with over 330 pM of resorufin/μg protein. α-Naphthoflavone reversed the effect of TCDD on EROD activity, but it did not influence the effects of DDT and DDE on EROD activity in human placental explants.

Effects of the DDT and DDE isomers on the expression of CYP1A1 in human placental explants

Immunoblot analyses showed the expression of CYP1A1 protein in human placental explants following 1, 3, 6,

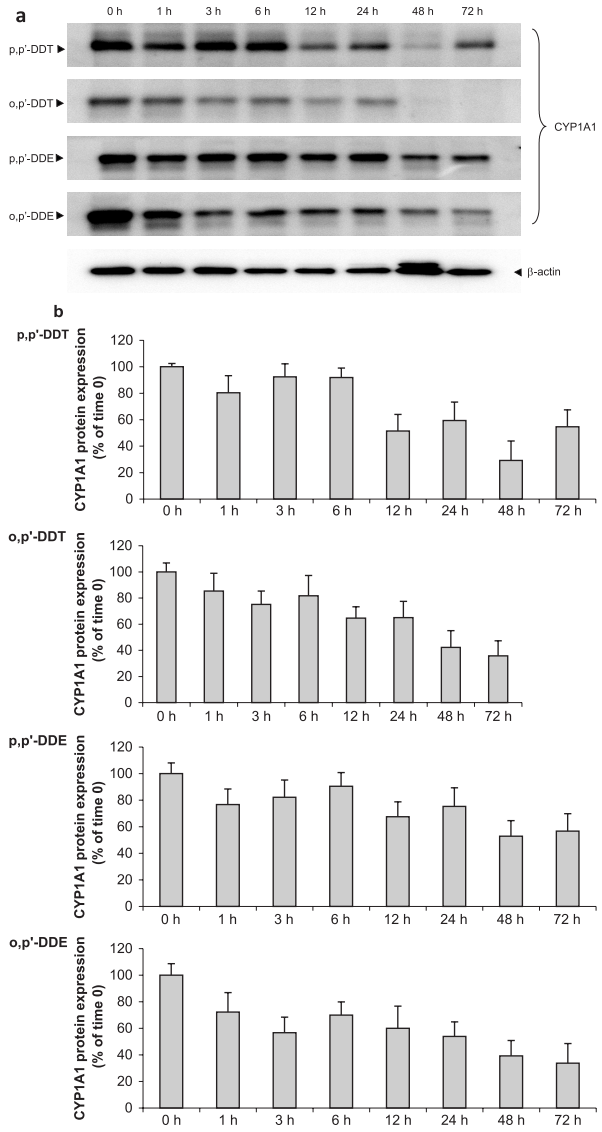

Fig. 4. Representative western blot of CYP1A1 protein levels in placental tissue homogenates treated with 100 ng/ml of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDE for 0, 1, 3, 6, 12, 24, 48 and 72 h. The blots were stripped and reprobed with an anti-β-actin antibody to control for the amounts of protein loaded onto the gel (**a**). CYP1A1 bands were quantified by densitometry. The results are shown as the percentage of CYP1A1 protein relative to the control (time 0) data point. Each point is the mean ± SEM range of two samples (**b**)

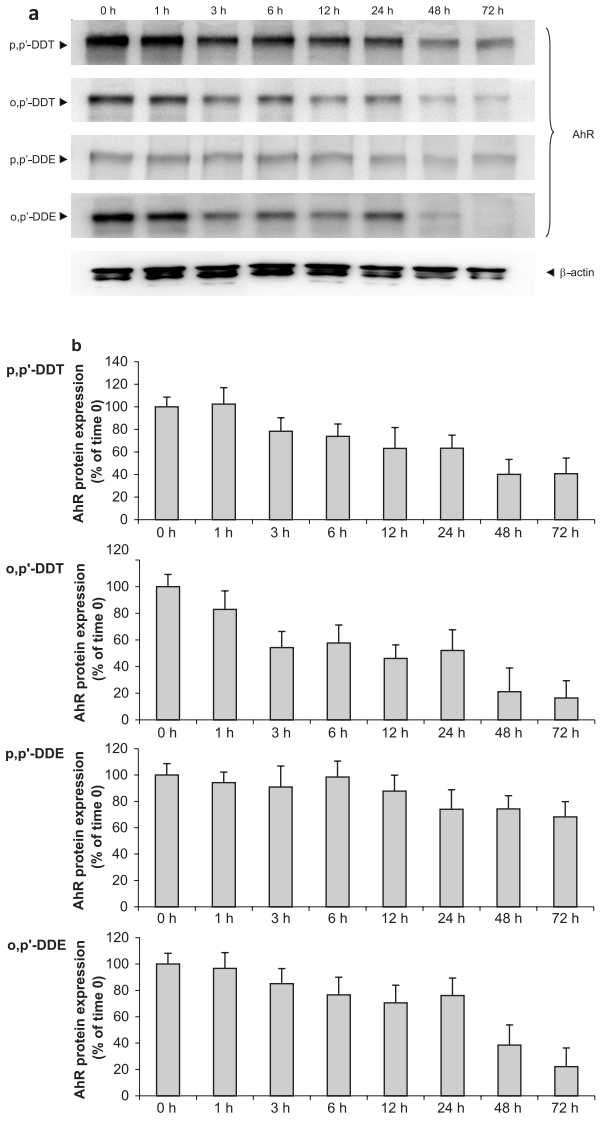

Fig. 5. Representative western blots of AhR protein levels in placental tissue homogenates treated with 100 ng/ml of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDE for 0, 1, 3, 6, 12, 24, 48 and 72 h. The blots were cut, and the appropriate bands were probed with an anti-β-actin antibody to control for the amounts of protein loaded onto the gel (**a**). AhR bands were quantified by densitometry. The results are shown as the percentage of AhR protein relative to the control (time 0) data point. Each point is the mean ± SEM range of two samples (**b**)

12, 24, 48, and 72 h of exposure to DDT and DDE. Both isomers of DDT and DDE used at a concentration of 100 ng/ml inhibited the expression of CYP1A1, which was most pronounced at 48 and/or 72 h after treatment (Fig. 4).

The equivalent loading of the samples was verified with the expression of β-actin.

Effects of the DDT and DDE isomers on the expression of AhR in human placental explants

Immunoblots revealed the expression of the AhR protein in human explants after 1, 3, 6, 12, 24, 48, and 72 h of exposure to DDT and DDE. Treatment with 100 ng/ml *p,p'*-DDT, *o,p'*-DDT or *p,p'*-DDE gradually decreased the level of AhR protein starting at 3 h of exposure (Fig. 5). However, in the case of *o,p'*-DDE, the decrease in the AhR protein level started at 24 h.

Equal concentrations of protein in all samples were verified with the expression of β-actin (loading control).

Discussion

CYP1A1 is involved in the biotransformation of many environmental contaminants, but its role in the metabolism of DDT and DDE in the human placenta had not been demonstrated. CYP1A1 is the most commonly expressed cytochrome in the human placenta, and its function depends on the stage of gestation. This study demonstrated for the first time the ability of *p,p'*-DDT and *o,p'*-DDT and their DDE metabolites to affect the activity of CYP1A1 and the expression of CYP1A1 and AhR proteins in choriocarcinoma JEG-3 cells, corresponding to first-trimester human placenta cells, and in explant cells from third-trimester human placentas. CYP1A1 has been shown to have a significant role in placental drug detoxification and has been detected in early and full-term placentas. According to our data, the basal activity of CYP1A1 in DDT (*p,p'*-DDT, *o,p'*-DDT)- and DDE (*p,p'*-DDE, *o,p'*-DDE)-exposed JEG-3 cells remained unchanged and stayed at a very low level through 72 h of treatment. Such a low CYP1A1 activity is possibly due to the low expression of CYP1A1 mRNA in these cells, as indicated by Hakkola et al. [9]. However, our experiments performed on TCDD-treated JEG-3 cells revealed the inhibitory actions of *p,p'*-DDT and *o,p'*-DDT and their DDE metabolites on the activity of CYP1A1. Furthermore, we demonstrated that both first-trimester JEG-3 cells and in third-trimester human placental explants responded to DDT and DDE by a decrease in CYP1A1 activity. The activity of CYP1A1 was suppressed by all concentrations of DDT and DDE. A similar inhibitory effect of *o,p'*-DDT in Hepa cells was reported by Jeong and Kim [15]. Isomer-specific effects were observed with DDE, which might be due to the opposite agonist/antagonist actions on estrogen receptors, as suggested by the study by Di Lorenzo et al. [7] on the response of liver tissues to *p,p'*-DDT and *o,p'*-DDT.

DDT and its metabolite DDE are known to exhibit hormonal activity in various tissues, including the placenta [30, 31]. There are several mechanisms by which DDT and DDE could act as endocrine disruptors: through the steroidogenic pathway, receptor-mediated changes in protein synthesis or their antiandrogenic and estrogenic actions. However, most of the endocrine effects of DDT and DDE result from their ability to mimic estradiol-17β. Our study provides strong evidence of an inhibitory action of DDT/DDE on CYP1A1 in the human placenta. Because the data on the effect of DDT/DDE on CYP1A1 are scarce, we can only discuss our results in relation to other factors exhibiting estrogenic activity and the ability to down-regulate CYP1A1, such as estradiol, estriol, 4-nonylphenol, methoxychlor, di-*ortho*-substituted polychlorinated biphenyls and resveratrol [3, 10, 14, 21, 28]. According to these studies, estrogens and estrogen-like compounds suppressed CYP1A1 activity and/or CYP1A1 mRNA expression in Hepa cells, hepatocytes and MCF-7 cells [25], thus acting in a similar way as DDT/DDE did in our study. The only study to show an opposite effect of estrogen on CYP1A1 activity was conducted with mouse ovarian cancer cells treated with TCDD and estradiol, and this effect was possibly due to the high endogenous concentration of estrogen produced by cancer ovarian cells [28].

According to our data for the JEG-3 cells and human placental explants, CYP1A1 could be the target of DDT and DDE in the human placenta. Thus, we decided to investigate the expression of CYP1A1 in DDT- and DDE-treated human placental tissue. Immunoblot analyses indicated that both isomers of DDT (*p,p'*-DDT, *o,p'*-DDT) and DDE (*p,p'*-DDE, *o,p'*-DDE) inhibited the expression of CYP1A1, which was most pronounced following 48 and/or 72 h

of treatment. Because the action of CYP1A1 is mediated by AhR, we hypothesized that the expression of AhR would be altered in the placental tissue exposed to DDT and DDE for 1 to 72 h. Our data showed that *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE gradually decreased the level of the AhR protein, starting at 3 h of exposure. However, in the case of *o,p'*-DDE, the decrease in the AhR protein level started only after 24 h of exposure. Our results suggest an involvement of the AhR/CYP1A1 signaling pathway in the action of DDT and DDE in the human placenta. The interaction between DDT/DDE and the transcriptional activity of AhR was previously proposed by Jeong and Kim [15], who demonstrated an impairment of the dioxin-response element (DRE) binding to DNA in *o,p'*-DDT-treated Hepa cells. Thus, it seems that the inhibitory action of DDT/DDE on CYP1A1 is universal across tissues and may depend on AhR. Our data strongly support an involvement of AhR in this action because AhR expression in the human placenta was significantly decreased 24–48 h after DDT/DDE treatment and possibly caused an impairment of AhR-mediated transcription. However, the mechanisms of this action are still controversial and need to be clarified. The only other data on the mechanisms of CYP1A1 down-regulation concern the action of estrogen-like compounds and estrogen receptor interaction with AhR [24]. According to these data, activated ER associates with the xenobiotic response element (XRE) and may either potentiate or repress AhR-mediated transcription [26].

In conclusion, our study demonstrated, for the first time, that JEG-3 cells and explants from third-trimester human placentas respond to *p,p'*-DDT and *o,p'*-DDT and their DDE metabolites with a decrease in CYP1A1 activity and decreases in CYP1A1 and AhR protein expression. Our results strongly support the involvement of the AhR/CYP1A1 signaling pathway in DDT and DDE action in both JEG-3 cells and placental explants. Because CYP1A1 is involved in metabolism and detoxification in the human placenta, any malfunction of this enzyme caused by DDT and its metabolite DDE disrupts the placental detoxification machinery, which may lead to an increased susceptibility of the fetus to environmental toxins and may be a risk factor for recurrent pregnancy loss.

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