**Pregnane X Receptor-Dependent Induction of the CYP3A4 Gene by o,p′-1,1,1-Trichloro-2,2-Bis (p-Chlorophenyl)ethane**

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**ABSTRACT:**

CYP3A4, the predominant cytochrome P450 (P450) expressed in human liver and intestine, contributes to the metabolism of approximately half the drugs in clinical use today. CYP3A4 catalyzes the 6β-hydroxylation of a number of steroid hormones and is involved in the bioactivation of environmental procarcinogens. The expression of CYP3A4 is affected by several stimuli, including environmental factors such as insecticides and pesticides. The o,p′-1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane (DDT) isomer of DDT comprises approximately 20% of technical grade DDT, which is an organochloride pesticide. We have recently shown that o,p′-DDT exposure increases CYP3A4 mRNA levels in HepG2 cells. To determine the mechanism by which o,p′-DDT induces CYP3A4 expression, transactivation and electrophoretic mobility shift assays were carried out, revealing that o,p′-DDT activates the CYP3A4 gene promoter through the pregnane X receptor (PXR).

The cytochrome P450 (P450) gene superfamily encodes a group of heme-containing monoxygenases, many of which metabolize compounds used as therapeutic drugs. P450s also play an important role in the oxidation of toxic chemicals and carcinogens, as well as endobiotics such as steroids, prostaglandins, and fatty acids (Nelson et al., 1996). The human CYP3A subfamily contains four members: CYP3A4, CYP3A5, the predominantly fetal form CYP3A7, and the relatively poorly understood CYP3A43 (Nelson et al., 1996; Westlind et al., 2001). CYP3A4 is the most abundant P450 expressed in human liver and small intestine, where it comprises between 50 and 60% of the total P450, respectively (Kolars et al., 1994; Shimada et al., 1994).

Importantly, CYP3A4 plays a significant role in the metabolism of approximately half the drugs in use today (Shimada et al., 1994). In addition, CYP3A4 catalyzes 6β-hydroxylation of a number of steroid hormones, including testosterone, cortisol, and progesterone (Yamazaki and Shimada, 1997). It is also involved in the bioactivation of environmental procarcinogens, such as aflatoxin B1 (Forrester et al., 1990) and benzo[a]pyrene (Li et al., 1995).

Interindividual variations of up to 40- and 17-fold in the expression levels of CYP3A have been observed among human liver and small intestinal specimens, respectively (Shimada et al., 1994; Paine et al., 2006), and a 10-fold variation in metabolism of CYP3A4 substrates in vivo has been reported (Thummel and Wilkinson, 1998). This variability is the product of both genetic and environmental factors. CYP3A4 is highly inducible by synthetic glucocorticoids, macrolide antibiotics, phenobarbital, and environmental contaminants through activation of the estrogen receptor (ER), glucocorticoid receptor, constitutive androstane receptor, and pregnane X receptor (PXR) (Raunio et al., 2005). Therefore, abnormal CYP3A4 gene expression can result in severe toxicity or therapeutic failure, as well as disruption of hormone homeostasis.

Pesticides warrant particular attention because the general human population may be exposed to such chemicals through many routes.
The present study was to evaluate the effects of or both. Because CYP3A4 induction could be a key determinant of gene expression.

The ability of the o,p'-DDT isomer to act as a ligand to the ER (Nelson, 1974; Forster et al., 1975) suggests that its influence on transcriptional activity may be mediated by this nuclear receptor. On the other hand, recent studies have shown that some organochlorine pesticides, including o,p'-DDT, can activate the PXR (Coumoul et al., 2002; Lemaire et al., 2004, 2006). Therefore, the effects of o,p'-DDT on CYP3A4 gene induction may be exerted through either PXR or ER, or both. Because CYP3A4 induction could be a key determinant of variations in susceptibility to chemical toxicants, it is important to establish whether o,p'-DDT alters CYP3A4 expression. The aim of the present study was to evaluate the effects of o,p'-DDT in vivo on CYP3A4 expression, as well as to deepen our knowledge of the molecular mechanism by which this pesticide might alter CYP3A4 gene expression.

Materials and Methods

Materials. HepG2 cells were obtained from American Type Culture Collection (Manassas, VA). The o,p'-DDT, trypan blue, rifampicin, 17β-estradiol, ethanol, and dimethyl sulfoxide were obtained from Sigma-Aldrich (St. Louis, MO). [4-14C]Testosterone was obtained from DuPont NEN (Boston, MA).

Animals and Treatments. Transgenic mice with intestinal expression of CYP3A4 (Granvil et al., 2003) were housed in a pathogen-free facility and fed with autoclaved Purina (St. Louis, MO) rodent chow with water available ad libitum. All the animal manipulations were conducted in accordance with the Mexican Official Norm NOM-062-ZOO-1999 and the Guide for the Care and Use of Laboratory Animals as adopted by the U.S. National Institutes of Health. Animals were randomly distributed into five treatment groups (n = 5). Each mouse received daily gavage doses of corn oil vehicle (control), 50 mg/kg body weight pregnenolone 16a-carbonitride (PCN) for 5 consecutive days, or 1, 2, and 100 mg/kg body weight o,p'-DDT for 1 day. Animals were sacrificed by cervical dislocation. The small intestines were removed, frozen in liquid nitrogen, and stored at −70°C.

Cell Culture. HepG2 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), penicillin (100 U/ml), streptomycin (100 μg/ml), 1% l-glutamine, and 1% nonessential amino acids (Invitrogen). Cell cultures were maintained in 75-cm² flasks at 37°C in a humidified incubator with a 5% CO₂ atmosphere.

Isolation of Total RNA. Total RNA was prepared from cultured HepG2 cells or mouse duodenum using the TRIzol reagent according to manufacturer’s instructions (Invitrogen). RNA was quantified spectrophotometrically at 260 nm, and the purity was assessed by measuring O.D.₂₆₀/O.D.₂₃₀ ratio. RNA integrity was evaluated by electrophoresis of RNA samples on 1% agarose gels. cDNA was prepared from 4 μg of total RNA using the SuperScript Premplification System for First Strand Synthesis (Invitrogen) and oligo(dT) according to manufacturer’s instructions.

Real-Time Quantitative Polymerase Chain Reaction Detection. All the polymerase chain reaction (PCR) reagents were purchased from Applied Biosystems (Foster City, CA). Quantitative real-time PCR assay (qPCR) of the transcripts was performed with gene-specific fluorescent labeled probes in a 7000 Sequence Detector (Applied Biosystems). The probes were labeled with 6-carboxyfluorescein and VIC as the 5’-fluorescent reporter. Nonfluorescent quenchers at the 3’-end of the probes were designed using Primer Express software (Applied Biosystems) and are listed in Table 1. The specificity of the CYP3A4 primers and probe was verified by the lack of amplification from genomic DNA. A standard curve was constructed by serial dilutions of the cDNA of interest as the template. Endogenous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S, validated for stable expression in HepG2 cells and mouse duodenum, was used for normalization of the mRNA data. The PCR reaction mixture contained 4 μl of cDNA, 1× TaqMan Universal PCR Master Mix (Applied Biosystems), and 0.9 and 0.25 μM primers and probe, respectively. PCR reactions were performed with an ABI PRISM 7000 sequence detector system in the following cycling protocol: 1 cycle of 50°C for 2 min, 1 cycle of 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Each sample was analyzed twice in three independent experiments. The results were analyzed using the comparative C méthod as described previously (Livak and Schmittgen, 2001).

Reverse Transcription-PCR Analysis. Total RNA from mouse duodenum was isolated by homogenizing tissue in a guanidine/phenol solution (Invitrogen). Reverse transcription was performed using OneStep reverse transcription-PCR (RT-PCR) kit, according to the manufacturer’s instructions (Qiagen, Valencia, CA). For interleukin 6 (IL-6) and GAPDH the following program was used: denaturation at 94°C for 4 min and 35 cycles of PCR consisting of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. Forward and reverse primers were as follows: IL-6, 5'-TGTGCTG- GCAACAACAGCCG-3' and 5'-GTACTCCAGAAGACAGAGG-3' (product size 298 bp), respectively, and GAPDH, 5'-GAGGGGGCATTCCA- CAGTCCTC-3' and 5'-CATCACCATTTTCCAGGACC-3' (product size 340 bp), respectively. The PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide to visualize PCR amplification products. Relative intensity was determined using Sigma Gel program (Jandel Scientific software, Sigma).

Testosterone Hydroxylation. HepG2 cells were exposed to 10 μM o,p'-DDT for 24 or 48 h. Then, 3.6 nmol of [14C]testosterone dissolved in ethanol was added to cultures. Six hours later, the medium was collected for analysis of testosterone metabolites, and cells were rinsed twice with phosphate-buffered saline (PBS) and scraped with a rubber policeman for protein analysis. Testosterone metabolites were assayed as described previously (Waxman et al., 1991) with some modifications. In brief, 1 ml of ethyl acetate was added to a 0.5-ml aliquot of culture medium, and the mixture was vortexed for 1 min. The layers were separated by centrifugation at 1600g for 5 min, and the organic layer was transferred into a clean test tube. After a second extraction, the organic extracts were combined, and the solvent was evaporated under N₂ at room temperature. The products were separated by thin-layer chromatography using two solvent systems (A: dichloromethane/acetone, 4:1; B: chloroform/ethyl acetate/ethanol, 4:1:0.7) and identified by comparison with unlabeled standards. Radioactivity of each metabolite was indirectly determined using densitometric analysis of bands printed on the plate-sensitive Typhoon 4000 (Applied Biosystems, Billerica, MA). Protein concentrations were determined by using the Bradford reaction (Bio-Rad Laboratories, Hercules, CA).

Plasmids. The reporter plasmid p3A4-1200 was constructed as follows: a 1200/+98 fragment was generated by PCR amplification using a bacterial artificial chromosome clone (Sata et al., 2000) containing the CYP3A4 gene and oligonucleotides (5’-CTCTTGAGCTCGAATCTTCGCCTGCCTGTT- GCTTCTCC-3’ and 5’-CTCAAGCTTCTCCTCTCTGACGCTTG- TCAGGC-3’), which introduced XhoI and HindIII restriction sites. The fragment was then cloned into the XhoI/HindIII site of a pGL3-Basic vector

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Sequence (5’-3’)</th>
<th>Fragment Length</th>
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<tbody>
<tr>
<td>CYP3A4</td>
<td>Forward: CCTGCTGCTGCTGCTGTTATTAGGA</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Reverse: GGTGTTGCTGCTGCTGTTATTGGT</td>
<td>83</td>
</tr>
<tr>
<td>Cypba1</td>
<td>Forward: GGCAGGAAATCTCCTGTTCTTAPAA</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Reverse: CATCAAGCATGTCATTCTCACTTCG</td>
<td>83</td>
</tr>
</tbody>
</table>
driving a firefly luciferase gene (Promega, Madison, WI). The p3A4-1200 construct encloses three estrogen response elements (ERE) and one PXR response element (PXRE) (Hashimoto et al., 1993; Goodwin et al., 1999). The integrity of the construction was verified by enzyme digestion and confirmed by sequencing. The p3A4-362(7836/7208 ins) reporter construct [carrying the xenobiotic responsive enhancer module (XREM) region, the proximal promoter (−362/+53), and the luciferase reporter gene] was provided by Christopher Liddle (Goodwin et al., 1999). The p3A4-362(7836/7208 ins) vector encloses one PXRE and one ERE motif (Hashimoto et al., 1993; Goodwin et al., 1999). ERα and hPXR expression vectors were provided by Pierre Chambon from the Institut de Génétique et de Biologie Moléculaire et Cellulaire and Ronald Evans from The Salk Institute, respectively.

**Transient Transfection.** HepG2 cells were cultured in DMEM as described above. The transfection of HepG2 cells was performed using an Eppendorf Multiporator with hypo-osmolar buffer (Eppendorf, Westbury, NY). Each sample (5 × 10⁶ cells) was resuspended in 800 µl of hypo-osmolar buffer containing 4 µg of the p3A4-362(7836/7208 ins) or the p3A4-1200 vectors. A mixture of 100 ng of pRL-CMV as an internal control and 4 µg of the PXR or ERα expression vector was transferred to 4-mm cuvettes. A single 1200-V, 100-µs pulse was delivered to each sample. After electroporation, the cuvettes were incubated at room temperature for 1 min, and then the cells were transferred into OPTIMEM medium. The medium was replaced after 3 h with fresh DMEM containing 10% fetal bovine serum. Twenty-four hours after transfection, the cells were treated by adding o,p'-DDT, rifampicin, or 17β-estradiol to the culture medium. After 24 h of incubation with the applied treatment, the cells were homogenized for enzymatic assays with phosphate lysis buffer (Promega). Luciferase activity was performed using the Dual-Glo Luciferase Assay System (Promega) and a DT 20/20 luminometer (Turner Biosystems, Sunnyvale, CA). Blanks were obtained by conducting luciferase activity assays in mock-transfected cells. Firefly luciferase activity levels were measured and normalized for transfection efficiency. Mean luciferase activity values ± S.D. of triplicate determinations are shown. ∗, p < 0.05 versus control (C).

**Results**

**o,p'-DDT Activates the CYP3A4 Gene Promoter through PXR and ERα.** A reporter construct (CYP3A4-1200) containing 1200 bp of the human CYP3A4 gene promoter sequence (carrying three ERE) linked to a luciferase gene was used to delineate the mechanism by which o,p'-DDT influences CYP3A4 gene expression in HepG2 cells. o,p'-DDT at 10 µM increased luciferase activity by about 22%. In comparison, 50 µM rifampicin, a known PXR ligand, increased luciferase activity by about 30% (Fig. 1).

To assess whether the o,p'-DDT effects on CYP3A4 gene expression were mediated by the PXR and/or ERα, HepG2 cells were cotransfected with PXR or ERα expression vectors and CYP3A4-1200 reporter construct. In the absence of exogenous ligands, increased levels of PXR or ERα did not result in an increase of luciferase activity (Fig. 2, columns 2 and 5). However, addition of o,p'-DDT or rifampicin to cell cultures cotransfected with the PXR expression vector resulted in an increase of luciferase activity by about 40 and 80%, respectively (Fig. 2, columns 3 and 4). Cells cotransfected with the ERα expression vector and treated with o,p'-DDT or rifampicin showed the highest luciferase activities (Fig. 2, columns 6 and 7).

**Electrophoretic Mobility Shift Assays.** For electrophoretic mobility shift assays (EMSA), 20 µg of nuclear extracts was mixed with 10 µl of protein buffer (1×) (24 mM HEPES, 1 mM EDTA, 8 mM MgCl₂, 120 mM KCl, 20% glycerol, and 1 mM DTT) containing 2 µg of poly(dI-dC), 1 mM MgCl₂, and 1 mM Spermidine. After a 10-min incubation at 4°C, 2 µl of 32P-labeled double-strand oligonucleotides (CYP3A4ER5: 5'-ATATGAACTCAAGAGGGTCAGTGA-3' or CYP3A4ER6-mut: 5'-ATATGGAACCTCAGAGGGTCAGTA-3'; mutations are italicized) was added. After incubating for an additional 10 min at 4°C, the samples were run on a 6% polyacrylamide gel with 0.5× Tris borate-EDTA buffer. The gel was dried and exposed to X-film autoradiographs.

**Statistical Analysis.** The results are presented as mean ± S.D. Statistically significant comparisons were detected by Student’s t test or the Mann-Whitney test as appropriate. The criterion for significance was p < 0.05 in all the cases.

![Graph](image-url)
DDT or 17β-estradiol resulted in a 70% increase in the luciferase activity (Fig. 2, columns 6 and 7). These data establish that o,p′-DDT effects, at least in part, are mediated by PXR and ERα.

To determine the role of the XREM that regulates the CYP3A4 gene through PXR activation, a reporter construct containing the XREM region, the CYP3A4 proximal promoter, and the luciferase gene through PXR activation, a reporter construct containing the o,p′-DDT treatment, increased levels of 6β-hydroxytestosterone and andro-
stenedione metabolites were observed. After the 48-h \textit{o,p'-DDT} treatment, increased levels of $\beta_\beta$, $16\beta_\beta$, and 2$\alpha$-hydroxytestosterone and 6-dihydroxytestosterone metabolites were observed.

To examine whether \textit{o,p'-DDT} exposure alters CYP3A4 mRNA expression in vivo, young adult male CYP3A4-transgenic mice were exposed to 1, 10, and 100 mg/kg \textit{o,p'-DDT}, and the CYP3A4 and Cyp3a11 mRNA levels in the duodenum of these mice were determined by rtPCR. CYP3A4 mRNA expression was higher in mice exposed to \textit{o,p'-DDT} or PCN than in the control animals (Fig. 6A). Indeed, duodenum CYP3A4 mRNA expression was 32-fold higher in the 1 mg/kg \textit{o,p'-DDT}-treated animals than in the controls. Similar results were obtained when mice were treated with 50 mg/kg PCN. However, after 10 mg/kg \textit{o,p'-DDT} treatment, CYP3A4 mRNA levels were increased by 13-fold (although a substantially lower increase than that of the 1 mg/kg dose), and no difference was observed when mice were exposed to 100 mg/kg \textit{o,p'-DDT}. A similar pattern of results was observed for Cyp3a11 mRNA expression (Fig. 6B).

It was previously reported that IL-6 strongly represses CYP3A4 transcription (Muntane-Relat et al., 1995). Others reported that DDT is able to increase the IL-6 levels (Kim et al., 2004). Therefore, IL-6 mRNA levels were examined in duodenum from mice treated with \textit{o,p'-DDT} (Fig. 7). Treatment with \textit{o,p'-DDT} resulted in a moderate increase of IL-6 mRNA level, with a maximum induction of 1.2-fold relative to the control value. These results may explain, at least in part, the observed decrease in CYP3A4 and Cyp3a11 induction.

**Discussion**

During the past decade, expression of the CYP3A4 gene was found to be induced by an array of structurally diverse compounds (Luo et al., 2004). The interaction of some of these compounds with classic steroid hormone receptors suggests that multiple signal transduction pathways may be involved. Given the importance of CYP3A4 in the metabolism of a variety of drugs, an understanding of this phenomenon is crucial to minimize the potential for undesirable drug interactions.

We recently found that \textit{o,p'-DDT} treatment increases CYP3A4 mRNA levels in HepG2 cells (Medina-Diaz and Elizondo, 2005). This pesticide is an agonist for ER$\alpha$ and is thus considered an endocrine disruptor chemical with estrogenic activity (Kuiper et al., 1998). Other organochloride pesticides, such as dieldrin and chlordane, have the potential to activate PXR (Coumoul et al., 2002). Both nuclear receptors, ER$\alpha$ and PXR, regulate CYP3A4 expression (Goodwin et al., 1999; Tsuchiya et al., 2005). To determine whether \textit{o,p'-DDT} acts through ER$\alpha$ and/or PXR, two reporter constructs were used: the CYP3A4-1200 reporter vector containing one PXRE and three ERE, and the p3A4-362(7836/7208 ins) reporter construct containing two PXRE and one ERE. Transactivation studies revealed that 10 $\mu$M \textit{o,p'-DDT} treatment only mildly activated the CYP3A4-1200 construct, resulting in a maximum 1.22-fold induction over that observed in vehicle-treated cells. A 1.5-fold increase in induction was observed when cells were cotransfected with the PXR or ER$\alpha$ expression vectors. On the other hand, treatment with 10 $\mu$M \textit{o,p'-DDT} induced CYP3A4-1200 activity as efficiently as 10 nM estradiol. This result is in agreement with the fact that the hormone has 1000 times greater affinity for ER$\alpha$ than the pesticide (Nelson, 1974).

When cells were transfected with the p3A4-362(7836/7208 ins) reporter vector and treated with \textit{o,p'-DDT}, dose-dependent promoter activation was observed; the maximum activation produced a 2.8-fold greater induction than in vehicle-treated cells. Cotransfection of the PXR expression vector increased the induction effects to 4-fold greater than controls and resulted in a 16-fold increase in CYP3A4 mRNA levels. These results are in agreement with previous observations where \textit{o,p'-DDT} increases CYP3A4 promoter activity by 6-fold (Lemaire et al., 2004). Although ERE and ER$\alpha$ located at the proximal promoter play a role in CYP3A4 induction, our findings indicate that the \textit{o,p'-DDT} exposure increases CYP3A4 mRNA levels mainly through the XREM region, probably via activation of PXR. However, the XREM region is a complex array of transcription factor-binding sites that include a response element highly homologous to the recognition sequence for the orphan retinoic acid receptor-related receptor $\alpha$-1, a site with identity to a binding motif recognized by hepatocyte nuclear factor-4, a putative binding site for CAAT/enhancer binding protein and two potential glucocorticoid receptor response element half-sites (Goodwin et al., 1999). Therefore, the CYP3A4 promoter activation by \textit{o,p'-DDT} through these transcription factors cannot be ruled out. EMSA studies showed that treatment with 10 $\mu$M \textit{o,p'-DDT} increased protein-ER$\alpha$ complex formation; no complex...
formation was observed with an ER6 mutant or unlabeled ER6 probes. Together these results suggest that the pesticide activates the PXR and thereby increases binding to the ER6 response element. Interestingly, ER6-protein complex formation is greater when cells were treated with 10 \( \mu \)M \( \alpha,p'\)-DDT than with 50 \( \mu \)M rifampicin. These data differed from the transactivation studies in which rifampicin had greater promoter activation potency than the pesticide. It was reported that regions of the PXR ligand binding domain (LBD) are mobile and can change position when bound by distinctly shaped ligands (Orans et al., 2005). These ligand-dependent conformational changes may modify co-repressor or coactivator binding, and these changes may underlie the discrepancy observed between the amount of ER6-protein complex formed and the transactivation response.

The promoter activation mediated by \( \alpha,p'\)-DDT resulted in an increase in CYP3A4 activity measured by the cell capacity to metabolize testosterone. After 48 h of treatment, a significant increase of 6\( \alpha\)-, 16\( \beta\)-, and 2\( \alpha\)-testosterone and 6-dihydrotestosterone was observed. Other P450s may be involved in testosterone metabolism, particularly CYP2C9 and CYP2C19. It has been reported that hydroxylation activities at the 16\( \beta\) position are higher in CYP2C9 and CYP2C19 than in CYP3A4. However, 15\( \beta\)-, 6\( \beta\)-, and 2\( \beta\)-hydroxylation of testosterone are catalyzed most actively by CYP3A4 (Yamazaki and Shimada, 1997). Because CYP2C9 and CYP2C19 genes are regulated by PXR, the contribution of these enzymes on testosterone 16\( \beta\)-hydroxylation has to be considered. Besides the potential to interact with androgen receptors and ER, these results clearly indicate that this pesticide has the capacity to alter testosterone levels by increasing the CYP3A4 activity and testosterone catabolism. Therefore, it is probable that levels of other endobiotics metabolized by this enzyme are also modified. Moreover, it was shown in humans that exposure to DDT increased estradiol metabolism (Poland et al., 1970; Nhachi and Loewenson, 1989). This is of particular interest because estradiol metabolites, such as 16-hydroxyestradiol, are ER agonists associated with increased breast cancer incidence (Kabat et al., 1997). In accordance, a positive correlation between CYP3A4 activity and breast cancer also has been observed (Huang et al., 2003).

Species differences were found among the several animal models that have been proposed to assess xenobiotic effects on P450 regulation. Because CYP3A4 is expressed only in humans and few in vivo models are available, a transgenic mouse model expressing the human CYP3A4 gene was used. Oral treatment with 1 mg/kg \( \alpha,p'\)-DDT increased CYP3A4 mRNA levels by more than 30-fold relative to controls. Similar effects were observed in the experiments examining mouse Cyp3a11 mRNA levels. It is relevant to mention that adipose tissue samples from nonoccupationally exposed subjects were found to have a concentration of 1.79 mg/kg \( \alpha,p'\)-DDT and 17.45 mg/kg total DDT (Waliszewski et al., 2004). Thus, modulation of CYP3A4 gene transcription by this pesticide as observed in the mouse model may be occurring in human populations and could result in drug interactions and therapeutic failures or toxicities.

![Graph showing the effects of \( \alpha,p'\)-DDT treatment and PXR overexpression on CYP3A4 mRNA levels. The graph shows a significant increase in CYP3A4 expression after 24 h of treatment with \( \alpha,p'\)-DDT, with or without PXR overexpression.](image-url)

**FIG. 5.** Effects of \( \alpha,p'\)-DDT treatment and PXR overexpression on CYP3A4 mRNA levels. HepG2 cells were transfected with 4 \( \mu \)g of PXR expression vector and treated with 10 \( \mu \)M \( \alpha,p'\)-DDT for 24 h. Relative expression of CYP3A4 mRNA was determined by qPCR. All the measurements were normalized to a calibrated standard sample. Data represent mean values ± S.D. from two independent experiments for triplicate. *p < 0.05 versus control.

**TABLE 2**

Effects of \( \alpha,p'\)-DDT on testosterone metabolism in HepG2 cells

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>24-h Treatment</th>
<th>48-h Treatment</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>( \alpha,p')-DDT</td>
</tr>
<tr>
<td>15( \alpha)-OHT</td>
<td>0.036 ± 0.010</td>
<td>0.048 ± 0.009</td>
</tr>
<tr>
<td>6-( \alpha)-OHT</td>
<td>0.093 ± 0.017</td>
<td>0.093 ± 0.002</td>
</tr>
<tr>
<td>6( \beta)-OHT</td>
<td>0.125 ± 0.004</td>
<td>0.143 ± 0.002*</td>
</tr>
<tr>
<td>16( \beta)-OHT</td>
<td>0.056 ± 0.009</td>
<td>0.051 ± 0.008</td>
</tr>
<tr>
<td>5( \alpha)-OHT</td>
<td>0.089 ± 0.005</td>
<td>0.098 ± 0.005</td>
</tr>
<tr>
<td>6-DHT</td>
<td>0.086 ± 0.011</td>
<td>0.083 ± 0.009</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.137 ± 0.002</td>
<td>0.151 ± 0.017*</td>
</tr>
<tr>
<td>NI-1</td>
<td>0.169 ± 0.019</td>
<td>0.163 ± 0.012</td>
</tr>
<tr>
<td>NI-2</td>
<td>0.025 ± 0.003</td>
<td>0.036 ± 0.004*</td>
</tr>
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</table>

*\( p < 0.05\) versus nontreated cultures.

Data are presented as nmol/mg protein, mean ± S.D. (n = 3).
The levels of CYP3A4 induced by the pesticide treatment decreased by about one-half after 10 mg/kg \( \text{o,p'-DDT} \) treatment and was lost at 100 mg/kg. In humans, DDT is metabolized by CYP3A4 into the 1,1-dichloro-2,2-bis(\( p \)-chlorophenyl)ethylene (DDE) and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene metabolites (Kitamura et al., 2002). However, the sustained inductive capacity of the DDE metabolite was shown in studies reporting that rats treated with DDE present an 11-fold increase in CYP3A1 mRNA levels and a 5-fold induction of a promoter activity driven by PXR response elements (Wyde et al., 2003). Although species differences on CYP3A regulation have been described, metabolic inactivation of \( \text{o,p'-DDT} \) seems not to be responsible for the observed decrease in CYP3A4 induction.

It remains a possibility that the decreased CYP3A4 induction at the higher doses was caused by an effect of \( \text{o,p'-DDT} \) treatments on PXR. It has long been known that hepatic metabolism is reduced during inflammation as a result of a cytokine-regulated decrease in P450 levels (Muntane-Relat et al., 1995). On the other hand, others found that \( \text{o,p'-DDT} \) induces the production of several cytokines, including IL-6 in macrophages (Kim et al., 2004). Moreover, IL-6 negatively regulates the expression of PXR and constitutive androstane receptor, as well as of CYP3A4 mRNA in primary human hepatocytes (Passcussi et al., 2000). In the present study, a moderate increase in IL-6 mRNA level was observed after \( \text{o,p'-DDT} \) treatment in the mouse intestine. These results explain, at least in part, the decrease in CYP3A4 and Cyp3a11 induction produced by the pesticide. The mechanism by which this cytokine acts is still unknown. However, Jun/activator protein-1, a transcription factor up-regulated by IL-6, was shown to interact and inhibit the transcriptional activity of 9-cis retinoic acid receptor \( \alpha \), a heterodimerization partner of PXR (Zhou et al., 1999).

The sequence differences in the LBD of PXR account for most of the variations in CYP3A induction across species. Compounds that are known to differentially induce CYP3A expression in mouse, rat, rabbit, or human activate only the corresponding PXR. For example, rifampicin activates human but not mouse PXR, whereas PCN is a weak activator of human and a much more potent activator of mouse PXR (Kocarek et al., 1995). Our results indicate that \( \text{o,p'-DDT} \) activates both receptors, suggesting that the differences in the LBD between human and mouse PXR do not affect the pesticide capacity to induce expression of CYP3A P450s. It is also noteworthy that it was shown in transgenic mouse studies that the mouse PXR can activate CYP3A4.

In conclusion, the present study revealed that the pesticide \( \text{o,p'-DDT} \) induces the CYP3A4 gene promoter through PXR, resulting in an increase in CYP3A4 mRNA and activity levels. We also showed that \( \text{o,p'-DDT} \) was able to increase CYP3A4 and Cyp3a11 mRNA levels in vivo. Finally, \( \text{o,p'-DDT} \) may induce other genes that are under PXR regulation, such as other P450s (e.g., CYP3A7, CYP2C8, CYP2C9, CYP2C19, and CYP2B6), phase II enzymes (UDP-glucuronosyltransferases and glutathione-S-transferases), efflux pumps (multidrug resistance proteins 1 and 2), and genes involved in bile
References


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