Differential Regulation of Cytochrome P450 1A1 and 1B1 by a Combination of Dioxin and Pesticides in the Breast Tumor Cell Line MCF-7

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ABSTRACT

Dioxin and pesticides with xenoestrogenic activity are environmental contaminants that are suspected of promoting human diseases such as cancers. However, few studies have addressed the molecular consequences of a combination of these contaminants, a situation that is likely to occur in the environment. We investigated the effects of natural and xenostrogens on basal and 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced cytochrome P450 (CYP) 1A1 and 1B1. The CYP1B1/1A1 ratio is a critical determinant of the metabolism and toxicity of estradiol in mammary cells. Here we show that in MCF-7 cells, 17β-estradiol and α-endoosulfan can repress whole cell ethoxyresorufin-O-deethylase activity, lowering CYP1A1 mRNA levels as well as promoter activity as assessed by transient transfection assays. These negative effects are observed at both the basal and tetrachlorodibenzo-p-dioxin-induced levels. Under the same conditions, CYP1B1 mRNA levels and promoter activity are not affected. The effects on mRNA-induced levels are also observed in another mammary cell line, T47D, but not in mammary cell lines that do not express aryl hydrocarbon receptor and estrogen receptor (ER). Moreover, the use of ER antagonists shows that these effects are ER dependent in MCF-7 cells. In human hepatoma HepG2 cells, which lack functional ER, α-endoosulfan, but not 17β-estradiol, displays a repressive effect on CYP1A1 through a different mechanism. These results show that xenoestrogens, by altering the ratio of CYP1B1/CYP1A1, could redirect estradiol metabolism in a more toxic pathway in the breast cell line MCF-7.

INTRODUCTION

Several synthetic compounds, such as pesticides, herbicides, or industrial byproducts, display xenoestrogenic activity. These compounds tend to accumulate in the environment and can be found in products consumed by human, particularly food; they are thus considered major environmental contaminants in addition to dioxins (known as polychlorinated dibenzodioxin), furans (known as polychlorinated dibenzofuran), polyaromatic hydrocarbons, and heavy metals. It is assumed that these compounds, alone or in combination, may promote human diseases (1).

Xenoestrogens bind to and activate the ER, thus increasing the estrogen-dependent transcription of target genes and promoting undesirable estrogenic effects. They are suspected to play a role in decreasing the quantity and quality of human and animal semen during the last 50 years and in the increasing incidence of testicular cancer in men and breast cancer in women in industrialized countries (2–4).

Breast carcinogenesis depends on several parameters, such as the absence or presence of ER, exposure to xenobiotics, and/or genetic factors (5–7). Genotoxic molecules can be exogenous or endogenous. Estrogen catechols produced by the breast epithelium are an example of genotoxic molecules derived from the hydroxylation of the natural estrogens estrone, E2, and estriol, respectively. The predominant forms are 2OH-E2 and 4OH-E2. Another hydroxylated metabolite is 16α-hydroxy-estrone (8). The catechols 2OH-E2 and 4OH-E2 can be oxidized to quinones, which are putative tumor initiators (9, 10). Quinones derived from 2OH-E2 react with DNA to form stable adducts but do not seem to generate mutations. In contrast, the 3,4-catechol estradiol quinone, derived from 4OH-E2, forms depurinating adducts and readily leads to mutation events (10–12). Thus, this 4-hydroxylated form of E2 appears to be one of the most genotoxic metabolites of E2 in the breast epithelium. In contrast, the influence of the 2OH-E2 is controversial; whereas some studies have established a protective effect of this form (13), others have suggested that it could be to carcinogenic (14). Moreover, 2OH-E2 could inhibit the genotoxicity of 4OH-E2 via its 2-methoxy form (15, 16). Thus, the ratio 2OH-E2/4OH-E2 could be a critical parameter of the carcinogenicity of E2 (17–20).

In breast epithelium, the production of estrogen catechols depends on the activity of monooxygenases, particularly cytochromes CYP1A1 and -1B1 (21, 22). CYPs are drug-metabolizing enzymes that play a major role in the metabolism of hydrophobic xenobiotics and endogenous compounds (23). CYP1A1 displays hydroxylase activity at the C2 position, but also at the C6α and C15α positions of E2 (24), whereas CYP1B1 displays its primary activity on the C4 position (25). CYP1A1 produces primarily 2OH-E2, whereas CYP1B1 produces 4OH-E2 (9). As in the case of the catechol ratio, the relative expression of these genes and the CYP1A1/CYP1B1 ratio could then be critical in terms of carcinogenesis.

The expression of CYP1A1, -1A2, and -1B1 depends on the activity of the AhR. The AhR belongs to the basic helix loop helix/per-ARNT-sim protein family (26). It is expressed in many tissues, including breast epithelium (27). Activation of the cytoplasmic complex containing the AhR depends on the binding of a ligand. As a consequence, the AhR enters the nucleus and forms an active heterodimer with a nuclear protein called ARNT, which also belongs to the basic helix loop helix/per-ARNT-sim family (28). The AhR-ARNT complex binds to specific responsive elements, xenobiotic responsive elements, located in the promoters and enhancers of target genes and activates their transcription (26). Polyaromatic hydrocarbons or halogenated aromatic hydrocarbons can be ligands of the AhR. Among these molecules, the best characterized activator is TCDD, which belongs to the family of molecules called dioxins (29). Studies using animal models have revealed that exposure to TCDD leads to many toxicological symptoms, including increased frequency of cancers (30). Epidemiological studies on highly exposed human populations revealed a more moderate toxicity, but recent evidence confirmed a significant increase in cancer and other pathologies in these populations (30).

A confusing factor of population studies is the influence of other contaminating compounds. In this report, we address this question by...
studying the effect of a combination of dioxin and pesticides on the expression of the CYP1A1 and -1B1 genes in different cell lines. The mammary gland epithelial cells express significant levels of AhR and ER (31). In the MCF-7 and T47D cell lines TCDD increases the expression of both cytochromes (32). However, the addition of xenoestrogens repressed CYP1A1 but not CYP1B1, thus leading to a decrease in the CYP1A1/CYP1B1 ratio. As shown by the use of antiestrogens and AhR/ER− mammary cell lines, this effect is the result of a selective ER-dependent repressive effect of the xenoestrogen pesticides on CYP1A1 expression in MCF-7 cells. This negative effect is also observed in HepG2 cells but through a different mechanism.

**MATERIALS AND METHODS**

**Chemicals.** TCDD was used from a 0.155 mM stock solution (Promochem, Molsheim, France). α-Endosulfan was obtained from Promochem (Molsheim, France). ICI 172,780 was a generous gift of Dr. Redeuilh (INSERM, St Antoine Hospital, Paris, France). Other chemicals were obtained from Sigma Chemical Co. unless otherwise stated, and oligonucleotides were from Genset (Paris, France).

**Cell Culture.** The human hepatoma cell line HepG2, the human mammary tumor cell lines MCF-7, T47D, and MDA-MB-435s, and the human transformed mammary cell line HBL100 were maintained in DMEM (Life Technologies) with phenol red and supplemented with 10% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin (all from Life Technologies, Diamant, Puteaux, France), and 0.5 μg/ml fungizone (Squibb, Princeton, NJ). Forty-eight h before the experiments, the medium was removed and replaced by DMEM without phenol red (Life Technologies) supplemented with 10% charcoal-treated calf serum (from which steroids had been removed), and the same concentrations of penicillin, streptomycin, and fungizone as described above. The T47D cell line was maintained with 10−7 M insulin.

**Northern Blots.** Total RNA was prepared using the RNA Easy Midi Kit (Qiagen). Northern blots were performed as described previously (33). Probes were synthesized from cDNAs with the Megaprime DNA labeling kit (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Results were quantified with a Phosphorimager and ImageQuant software (Molecular Dynamics).

**Plasmids.** The FL expression plasmid (pGL3 basic vector) was purchased from Promega. The p glob-RL was a gift from Dr. Y. Morel (DGA, La Bouchet, France) and was used as a control in transfection experiments because it displays stable activity under the treatments used in this study. Recombinant plasmids were constructed as follows. The plasmid pA1-FL (constructed by Dr. Y. Morel; Ref. 34), containing the 5′ region of the human CYP1A1 gene (positions −1566 to +73) upstream of the FL coding sequence, and the p glob-RL plasmid have been described previously (34). The EcoRI-digested pGL3 basic vector was obtained by the following procedure (constructed by C. Tomkiewicz, INSERM U490, Paris, France). The pGL3 basic vector was double digested by KpnI and HindIII, and a double-stranded oligomer (linker containing a EcoRI site and KpnI/HindIII extremities) was then ligated. Oligonucleotides used to synthesize the linker were: 5′-CGAGCTCCGAATTCGCGA′-3′ and 5′-AGCTTTGAGAATTCTGGACGTCG-3′ (bold indicates EcoRI restriction site). Two oligonucleotides were used to amplify the promoter of the human CYP1B1 (positions −1635 to +588) by PCR: 5′-TAAGGGAGGACAGAAGGA-3′ and 5′-GGAAAGAAAGGGGGCGTT-3′. The PCR product was subcloned in pCR2.1 from the TA Cloning Kit (Invitrogen). Both pGL3 EcoRI basic vector and pCYP1B1- TA cloning vector were restricted by EcoRI. The EcoRI-restricted pCYP1B1 product was then subcloned into the EcoRI-digested pGL3 basic vector upstream of the FL reporter gene to yield pB1-FL.

Plasmids were checked by DNA sequencing analysis and amplified using the Qiagen Maxiprep Kit.

**Transfection Experiments.** Transfection experiments were performed in HepG2 cells as described previously. Briefly, 1 day prior to the transfection, cells (0.3 × 106 cells/6-cm dish) were seeded into DMEM without phenol red, 10% charcoal-treated FCS, penicillin, streptomycin, and fungizone (see section on cell culture for details). The pFL (CYP1A1 or CYP1B1; 1 μg of DNA) and p glob-RL (1 μg of DNA) were introduced into the cells by the calcium phosphate coprecipitation technique followed by a 2-min glycerol shock. Twenty-four h after transfection, cells were treated by adding various agents to the culture medium. After an overnight incubation, cells were homogenized for enzymatic assays with phosphate lysis buffer (Promega). A dual luciferase assay (firefly and Renilla) was performed with a Promega kit and a luminometer. The RL assay was used to normalize the transfection efficiency in all culture dishes. Blanks were obtained by assaying luciferase activity in mock-transfected cells.

MCF-7 cells were seeded, treated, and homogenized exactly the same as for HepG2 cells. Transfection was performed as follow: medium was removed and replaced with 4 ml of DMEM without phenol red, penicillin, streptomycin, and fungizone.

Fig. 1. EROD activity in whole cell assays using MCF-7 (A) or HepG2 cells (B). Cells were treated with 10 nM TCDD, 10 nM E2, 10 μM α-endosulfan, or a combination of these compounds. CYP1A1 activity was measured by the EROD assay in intact cells as described in “Materials and Methods.” Data shown are the means ± SE (bars) of three independent determinations obtained in three series of experiments. Induction by TCDD is significant (P < 0.0001). Significant differences from basal or TCDD-induced levels: ****, P < 0.0001; ***, P < 0.001; **, P < 0.01; *, P < 0.05. NS, nonsignificant difference.
fungizone. Each dish was transfected with 500 µl of medium containing 6 µl of DAC-30 solution (Eurogentec) mixed with 1 µg of pFL (CYP1A1 or CYP1B1) and 1 µg of pglob-RL. After 4 h, the medium was removed and replaced by fresh medium.

**EROD Activity in Intact HepG2 and MCF-7 Cells.** HepG2 and MCF-7 cells in 24-well plates were cultured until confluence. The medium was removed, and the cells were washed once with PBS and then cultured in DMEM without phenol red, charcoal-treated FCS, penicillin, streptomycin, and fungizone as described for the cell culture experiments. EROD activity was then measured using the technique described by Ciolino et al. (35). The activity was measured in MCF-7 and HepG2 cells; both cell lines express the AhR. However, only the MCF-7 cell line is able to respond to physiological concentrations of E2 (1–10 nM).

**Statistics.** Student’s two-tailed t tests were performed using ANOVA software (PLC Inc.).

**RESULTS**

**Endosulfan Inhibits EROD Enzyme Activity in Intact Cells.** To evaluate the influence of estrogens on the activity of CYP1A1, we used the EROD assay, which is specific for the CYP1 family (35, 36). The activity was measured in MCF-7 and HepG2 cells; both cell lines express the AhR. However, only the MCF-7 cell line is able to respond to physiological concentrations of E2 (1–10 nM). α-Endosulfan was used at a concentration of 10 µM, which is not toxic for either cell line (37, 38). Fig. 1 shows that 10 nM TCDD induced the activity of CYP1A1 in both cell types with induction higher in HepG2 cells (30- and 6-fold, respectively). The effects of E2 were then evaluated on both the basal and TCDD-induced activities. Fig. 1A shows that E2 significantly reduced both basal and TCDD-induced activity in MCF-7 cells (45 and 66% decrease, respectively).
The same pattern was observed with endosulfan (50 and 55% decrease, respectively). To evaluate the importance of the ER signaling pathway, we studied in parallel the E2 effect in HepG2 cells (38). The effect of E2 was considerably reduced (Fig. 1B): no significant effect was observed on basal activity. A limited but significant decrease of the TCDD-induced activity could be detected (15% decrease). This was observed on basal activity. A limited but significant decrease of EROD activity in 

CYP1B1 mRNA Levels. The observed decrease of EROD activity in

TCDD-induced CYP1B1 mRNA levels. Fig. 2, A and D, show that both natural and xenoestrogens did not significantly modify those levels (108 and 82% of basal control levels, respectively; 88 and 93% of TCDD-induced control levels, respectively). These results showed that both types of estrogens significantly affect the basal and TCDD-induced mRNA levels of human CYP1A1 in MCF-7 cells without affecting those of CYP1B1.

To confirm these results, we used other mammary cell lines. In the T47D cell line, which expresses functional AhR and ER, we found the same regulation of CYP1A1 and CYP1B1 mRNAs as described for the MCF-7 cell line (Fig. 2E). E2 and α-endosulfan inhibited the TCDD-induced CYP1A1 mRNA levels by ~48 and 62%, respectively. We used other mammary cell lines, MDA-MB-435s and HBL100, which do not express AhR and ER. In these cell lines, CYP1A1 mRNA levels were undetectable, whereas CYP1B1 mRNA levels were unchanged in the presence of TCDD, E2, and/or α-endosulfan (data not shown).

In the human hepatoma HepG2 cells (Fig. 2B), basal levels of CYP1A1 and CYP1B1 mRNA could not be detected even after long exposure of the Northern blots. TCDD increased the CYP1A1 mRNA level without any effect on CYP1B1. E2 treatment did not modify the TCDD-induced mRNA levels, whereas α-endosulfan decreased the TCDD-induced mRNA, in accordance with the results found in the EROD experiments.

Endosulfan Inhibits Human CYP1A1 Promoter Activity without Affecting CYP1B1 Promoter Activity. The effects of estrogens on the CYP1A1 and CYP1B1 promoter activities were studied by transfection assays using the p1A1-FL and p1B1-FL plasmids. As shown in Fig. 3, TCDD significantly induced the expression of the reporter gene in MCF-7 cells (~3-fold increase). The effects of E2

The same pattern was observed with endosulfan (50 and 55% decrease, respectively). To evaluate the importance of the ER signaling pathway, we studied in parallel the E2 effect in HepG2 cells (38). The effect of E2 was considerably reduced (Fig. 1B): no significant effect was observed on basal activity. A limited but significant decrease of the TCDD-induced activity could be detected (15% decrease). This observation confirmed that the HepG2 cells are less sensitive to E2 than the MCF-7 cells (probably because of the lack of functional ER). In contrast, α-endosulfan significantly decreased both the basal and TCDD-induced levels (12 and 34% decrease, respectively). This cell-specific effect may be attributable to the activation of other molecular signaling pathways (see below).

Endosulfan Inhibits CYP1A1 mRNA Levels without Affecting CYP1B1 mRNA Levels. The observed decrease of EROD activity in the presence of estrogens led us to study the mRNA levels of various CYPs in the MCF-7 cell line. Both CYP1A1 and -1B1 display EROD activity with different kinetics ($V_{max}$ (1B1)/$V_{max}$ (1A1) = 0.1; Ref. 36). Basal CYP1A1 mRNA levels were very low in control MCF-7 cells but could be detected in Northern blot after prolonged exposure (data not shown). E2 and α-endosulfan lowered the basal mRNA levels (35 and 49% decrease, respectively; data not shown). As shown in Fig. 2, A and C, treatment of MCF-7 cells with 10 nm TCDD caused an ~3.5-fold increase in human CYP1A1 gene expression compared with control. Treatment of MCF-7 cells with E2 or α-endosulfan decreased TCDD-induced CYP1A1 mRNA levels (~60 and 40% decrease, respectively; Fig. 2C).

In the MCF-7 cells, the human CYP1B1 gene was expressed at significant levels and was also induced by TCDD (2.8-fold increase). We studied the influence of E2 and α-endosulfan on the basal and
Altogether, these results show that xenestrogens and natural estrogens inhibit both the basal and TCDD-induced CYP1A1 promoter activities without affecting the CYP1B1 transcription and that different mechanisms may account for these effects in different cells.

**DISCUSSION**

The implication of estradiol in breast tumorigenesis is widely documented (5, 6). Several mechanisms have been described for the well-known mitogenic effect of this hormone on mammary epithelial cells. Several xenohormones bind and activate the ER in a manner similar to that of estradiol (38). Thus, they could mimic the deleterious effects of estradiol on these cells. An alternative mechanism of estradiol carcinogenicity stems from the metabolism of this hormone, which generates several catechol derivatives from monohydroxylation reactions. Some of these compounds display a strong genotoxicity (10, 17). This is in particular the case of 4OH-E2, which is generated by the metabolism of estradiol.

To confirm the differential regulation of CYP1A1 and CYP1B1 observed in Northern blots, we studied the CYP1B1 promoter activity in MCF-7 cells. Fig. 5 shows that both the basal and TCDD-induced CYP1B1 promoter activities were not significantly modified by E2 or \( \alpha \)-endosulfan.

In addition, we tested the effect of ICI 172,780, a pure ER antagonist. Fig. 3 shows that ICI 172,780 did not influence either the basal or the TCDD-induced levels of CYP1A1 promoter-driven transcription. Treatment of cells with ICI 172,780 limited the inhibitory effects of E2 and \( \alpha \)-endosulfan.

To further characterize the endosulfan effect and to evaluate the contribution of its estrogenic properties, two other xenestrogens, dieldrin and chlordane, were tested on basal and TCDD-induced human CYP1A1 promoter-driven transcription. Fig. 4 shows that chlordane and dieldrin significantly affected the basal levels of the human CYP1A1 promoter (\( \sim \)40 and 50% decrease, respectively). The same effect was observed for TCDD-induced levels (\( \sim \)60 and 50% decrease, respectively).

To confirm the differential regulation of CYP1A1 and CYP1B1 observed in Northern blots, we studied the CYP1B1 promoter activity in MCF-7 cells. Fig. 5 shows that both the basal and TCDD-induced CYP1B1 promoter activities were not significantly modified by E2 or \( \alpha \)-endosulfan.

To assess the cell specificities and the role of ER in the mechanisms, the same transfection experiments were carried out in HepG2 cells. In the presence of TCDD, only a weak negative effect of the natural estrogen, E2, was observed (Fig. 6). A significant inhibitory effect of \( \alpha \)-endosulfan on the TCDD-induced promoter activity was observed in HepG2 cells. ICI 172,780 had no effect on the \( \alpha \)-endosulfan inhibition, suggesting that \( \alpha \)-endosulfan does not use the ER pathway. Recent studies suggest that organochlorine pesticides are ligands of the hepatic nuclear receptor PXR, which can be activated by rifampicin (39). Treatment of the HepG2 cells with rifampicin did not affect the basal activity of CYP1A1 promoter but mimicked the \( \alpha \)-endosulfan effect (Fig. 5). Thus, this result suggests that the PXR rather than the ER could be implicated in the endosulfan effects in HepG2.

Fig. 6. CYP1A1 promoter activity in HepG2 cells. Transient transfection experiments were performed as described in “Materials and Methods,” using a FL reporter vector containing the human CYP1A1 promoter. The amount of FL transcription (F.Luc) was normalized to the transcription of a RL control reporter vector (R.Luc). Cells were treated with 10 nM TCDD, 10 nM E2, 10 \( \mu \)M \( \alpha \)-endosulfan, or combinations of these compounds. Data shown are the means \( \pm \) SE (bars) of four independent experiments performed in triplicate. Induction by TCDD is significant (\( P < 0.0001 \)). NS, nonsignificant difference.

Fig. 5. CYP1B1 promoter activity in MCF-7 cells. Transient transfection experiments were performed as described in “Materials and Methods,” using a FL reporter vector containing the human CYP1B1 promoter. The amount of FL transcription (F.Luc) was normalized to the transcription of a RL control reporter vector (R.Luc). Cells were treated with 10 nM TCDD, 10 nM E2, 10 \( \mu \)M \( \alpha \)-endosulfan, or combinations of these compounds. Data shown are the means \( \pm \) SE (bars) of three independent experiments performed in triplicate. Induction by TCDD is significant (\( P < 0.0001 \)). NS, nonsignificant difference.
mainly by CYP1B1. On the other hand, CYP1A1 generates primarily 2OH-E2, which is less toxic and has been considered as protective (9, 10, 13, 14). In various studies, the 4OH-E2/2OH-E2 ratio has been shown to be a tumor marker. The relative activities of CYP1B1 and CYP1A1 are critical determinants of this ratio. This study therefore assessed the effect of a combination of carcinogenic xenobiots on the expression of these CYP genes.

The mammary gland epithelium expresses significant levels of AhR and ER (31). One of the best model to study the effects of AhR and ER ligands on mammary cells is the MCF-7 carcinoma cell line, which express both receptors. In this cell line, basal expression of CYP1B1 and CYP1A1 was low but still detectable. Both genes were induced by the dioxin TCDD (CYP1A1 > CYP1B1). In fact, the increases in the activities of both CYPs contributed to the increase of E2 metabolism and constitute one mechanism for the well-established antiestrogenic activity of TCDD. The induction of CYP1B1 may lead to an increase in the amount of 4OH-E2, but this could be compensated by the concomitant increase in the amount of 2OH-E2 (15, 16).

It is well known that humans usually are contaminated by a combination of several xenobiots. It is likely that the interaction between these contaminants, synergy or antagonism, will be relevant for the final toxic effect. We show here that in MCF-7 cells, pesticides displaying xenoestrogenic activities specifically repress CYP1A1 activity and its expression, particularly when it is induced by TCDD. In contrast, CYP1B1 gene expression is not altered by the same xenoestrogenic compounds or by estradiol. Thus, the mRNA ratio of CYP1A1 to CYP1B1 is decreased in cells treated with TCDD and estrogenic compounds when compared with the ratio in cells treated by TCDD alone. This effect was also observed in T47D mammary cells, which express both AhR and ER, but not in other, receptor-deficient cell lines. These results suggest that this modulation could modify the metabolism of estradiol and redirect it into a more toxic pathway (i.e., increase in 4OH-E2). Our data suggest that the regulation of the genes encoding E2-metabolizing enzymes may constitute an important aspect of the toxicity of a combination of dioxins and pesticides. Such a combination of contaminants may not be uncommon because dioxins are produced during the synthesis of numerous pesticides and because both compounds contaminate food.

The regulation of the CYP1A1 and the CYP1B1 genes is different in the liver-derived HepG2 cells. In these cells, as in the liver, CYP1B1 is not expressed even after TCDD addition. The basal level of the CYP1A1 mRNA is extremely low; however, it is highly induced by TCDD. In these cells, the pesticide α-endsulfan partially prevents TCDD induction of CYP1A1. Recent studies have shown that pesticides could induce the CYP3A gene, which codes for an enzyme that is also capable of metabolizing E2 (39). Thus, in liver-derived cells, addition of α-endsulfan to TCDD leads to a shift in the ratio of E2-metabolizing enzymes by increasing CYP3A and decreasing CYP1A1. The toxicological consequences of this shift remain unclear.

The mechanism of action of the pesticides appears to be cell specific. In MCF-7 cells, these xenobiots are likely to bind to the ER; indeed, α-endsulfan displays a similar effect as E2 on CYP1A1 gene expression, and the antiestrogen ICI 172,780 prevents this effect (Fig. 3). In contrast, α-endsulfan is unlikely to act through the ER in HepG2 cells. Indeed, E2 displays only a weak inhibitory effect on TCDD-induced CYP1A1 gene expression that has been difficult to characterize. This is probably a result of the lack of functional ER in these cells (38). ICI 172,780 was not able to antagonize the effect of α-endsulfan. One possible receptor that could mediate the effect of endosulfan is the PXR or a related receptor. These receptors can bind several steroids and xenobiots. Recent studies have shown that organochlorine pesticides can bind and activate this receptor (39–41). Furthermore, the PXR is expressed in liver cells, and rifampicin, a typical activator of PXR, mimics the effect of endosulfan (Fig. 6). Additional studies are required to firmly establish this mechanism. If confirmed, this hypothesis would imply that two xenobiotic receptors of different families, PXR and AhR, display antagonistic effects.

Most reports on the regulation of the CYP1A1 gene have focused on an induction mechanism by the AhR. Recently, the repression of this gene has also been studied, and several inhibitory mechanisms have been suggested: negative regulatory elements (42); cross-talk between transcription partners (43, 44); competition for ARNT proteins (hypoxia-inducible factor 1α and AhR repressor; Ref. 45); direct inhibition of critical transcription factors such as nuclear factor I by oxidative stress, cytokines, and inflammation (34, 46); or competitive binding to the AhR (47, 48). Competition between the ER and the AhR for nuclear factor I has also been reported (49). This antagonism is relevant for the natural hormone as well as for the synthetic ligands of the ER. Interestingly, the CYP1B1 gene, which is also induced by TCDD, is not responsive to E2 or xenoestrogens, suggesting that the repression of AhR synthesis by the ER is not a general phenomenon and depends on the promoter. Another observation suggested by this study is an additional negative regulation of CYP1A1 expression through the PXR.

In conclusion, this study shows that a combination of xenobiots can modify the CYP1A1/CYP1B1 ratio and that this could constitute one mechanism for the toxicity of such compounds. The pattern and the mechanism of regulation of these genes are distinct and display tissue specificity, which needs to be further elucidated.

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