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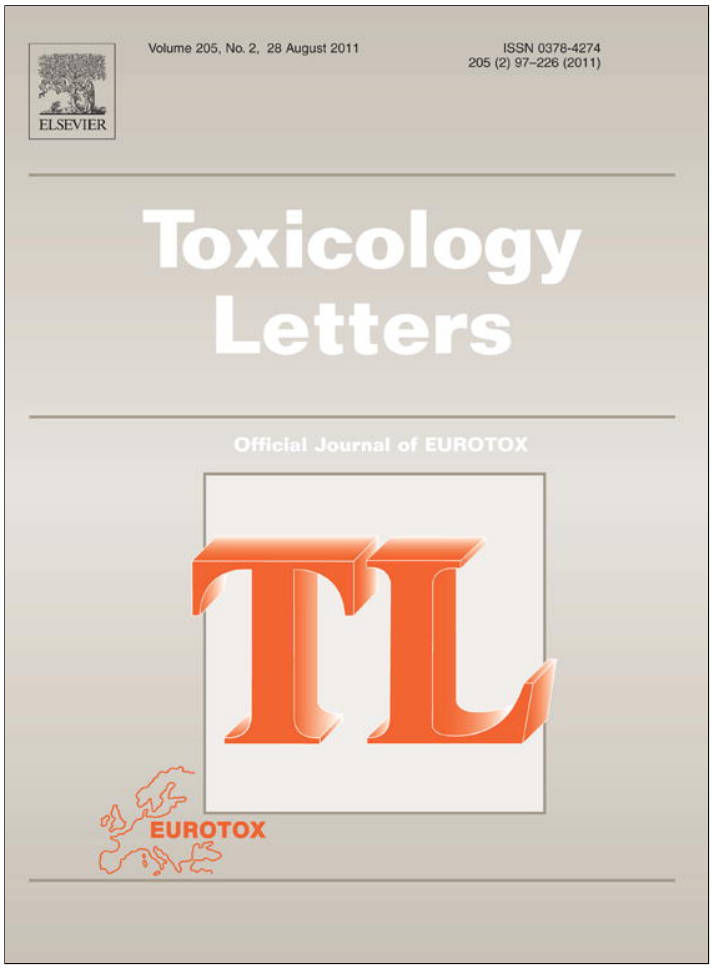

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Aflatoxins upregulate CYP3A4 mRNA expression in a process that involves the PXR transcription factor

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ABSTRACT

Pregnane X receptor (PXR) is a member of the nuclear hormone receptor (NHR) superfamily, which regulates xenobiotic and endobiotic metabolism in the liver. This transcription factor is activated by structurally diverse ligands, including drugs and environmental pollutants. PXR regulates the expression of numerous genes that function in biotransformation and the disposition of xenobiotics upon binding to an AG(G/T)TCA DNA motif in target promoter regions. We performed a screen of mycotoxins that pose a known environmental threat to human and animal health for the ability to activate PXR function in a human hepatocyte cell line, HepG2. We found that aflatoxins B1, M1, and G1 activated PXR. This activation was associated with upregulation of CYP3A4 expression and increased occupancy of PXR protein on the *CYP3A4* promoter. Using a microarray approach, we also found that aflatoxin B1 upregulated the expression of multiple genes involved in xenobiotic metabolism, including genes known to be regulated in a PXR-dependent fashion. We also observed an effect of aflatoxin B1 on the expression in other functional groups of genes, including the downregulation of genes involved in cholesterologenesis. The results of this study indicate that aflatoxin B1 is able to activate PXR, a known regulator of liver xenobiotic metabolism, in human hepatocytes, and it can upregulate the expression of PXR-dependent genes responsible for aflatoxin B1 biotransformation, including *CYP3A4*.

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1. Introduction

The NHR (nuclear hormone receptor) gene superfamily consists of genes coding for ligand-activated DNA-binding transcription factors that regulate the expression of multiple target genes (Huang et al., 2010). Several NHR proteins function as xenobiotic sensors capable of regulating xenobiotic metabolism and disposition by orchestrating the expression levels of multiple enzymes and transporters (Francis et al., 2003; Huang et al., 2010; Schulman, 2010). Many environmental toxins, including polyaromatic hydrocarbons, dioxins, phthalates, and numerous pharmacological substances, interact with NHRs (Huang et al., 2010). Such interactions could be critical for a compound's toxicity as they are in the case of endocrine disrupting environmental pollutants (le Maire et al., 2010; Ruegg et al., 2009) or affect drug metabolism and disposition, leading to drug side effects (Winterfield et al., 2003). NHR-mediated processes are also important for drug-to-drug interactions, as in the case of St. John's wort herbal medication, causing a decrease in the available doses of immunosuppressive, antiretroviral, anticancer, and contraceptive drugs, leading to a lack of therapeutic drug effects in treated patients (Borrelli and Izzo, 2009). Thus, the ability of chemical compounds to interact with a NHR could be a good indicator of a compound's potential toxicity and an important element of a compound's absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile during the drug discovery process (Sinz et al., 2008). Therefore, cell-based *in vitro* assays that detect the upregulation of the activation of NHRs trans-activating functions are employed for environmental monitoring (Murk et al., 1996) and in drug discovery research (Sinz et al., 2008).

One member of the NHR superfamily is the Pregnane X receptor (PXR), which is known to interact with the 9-cis retinoic acid receptor (RXR) to form a heterodimer that recognizes AG(G/T)TCA motifs present in the promoters of numerous genes engaged in the biotransformation and disposition of xenobiotics (Bertilsson et al., 1998; Blumberg et al., 1998). The trans-activating function of PXR is upregulated by numerous structurally diverse compounds, including pharmacological substances, such as dexamethasone, rifampicin, RU486, clotrimazole, lovastatin, and valproic acid (Cerveny et al., 2007; Lehmann et al., 1998); environmental toxicants, including organochlorine and polybrominated compounds (Cooper et al., 2008; Schulman, 2010), and endogenous substances,

Abbreviations: PXR, pregnane X receptor; NHR, nuclear hormone receptor; DEX, dexamethasone; VD3, cholecalciferol; DCA, deoxycholic acid; TBHP, tert-butylhydroperoxide; CHX, cycloheximide.

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such as bile salts and estrogens (Staudinger et al., 2001). As previously reviewed (Ihunnah et al., 2011), PXR activation leads to upregulation of phase I (i.e., CYP2B6, CYP2C9, CYP3A4, and CYP3A7) (Bertilsson et al., 1998; Drocourt et al., 2001; Lehmann et al., 1998), phase II (i.e., UGT, GST, and SULT) enzymes (Duanmu et al., 2001; Fang et al., 2007; Gardner-Stephen et al., 2004; Naspinski et al., 2008; Sonoda et al., 2002) and transporters (i.e., MDR1 and OATP2) (Cerveny et al., 2007; Frank et al., 2005). Promiscuity of PXR toward multiple structurally distinct ligands is based on particular features of the three-dimensional structure of the PXR ligand-binding domain, suggesting that PXR evolved as a molecular sensor capable of detecting numerous toxins to trigger an effective chemodefense (Jones et al., 2000; Watkins et al., 2001). Bile acid-dependent, PXR-mediated gene regulation is important for maintaining hepatic homeostasis by regulating lipid and glucose metabolism. PXR has been suggested to regulate not only xenobiotic metabolism but also other aspects of hepatic function (Echchgadda et al., 2007; Staudinger et al., 2001).

Mycotoxins are structurally diverse, low molecular weight compounds that are generated as secondary metabolites by filamentous fungi, and they constitute a serious environmental threat to human and animal health by causing mycotoxicoses (Bennett and Klich, 2003; Peraica et al., 1999). Mycotoxin exposure mostly occurs by consuming crops contaminated with filamentous fungi, and their toxic effects are both acute and chronic and are frequently organ-specific (Bennett and Klich, 2003). A well-known example of chronic and organ-specific mycotoxin-mediated toxicity is the carcinogenicity of aflatoxin B1, which is a known cause of liver cancer and is classified as a group I carcinogen (Angsubhakorn et al., 1990; Appleton, 1985; Groopman et al., 1988). The carcinogenic effect of aflatoxin B1 primarily depends on a CYP3A4-mediated biotransformation that generates a highly active genotoxic intermediate, aflatoxin-8,9 epoxide, which is capable of binding to guanine (Bechtel, 1989; Guengerich et al., 1996). Besides aflatoxin B1, there are not many examples of mycotoxin bioactivation, with the exceptions of the conversion of zearalenone to the more active metabolite alpha-zearalenol (Dong et al., 2010) and the still controversial biotransformation of ochratoxin A into its genotoxic metabolite (Lebrun et al., 2006; Wu et al., 2011). While the biotransformation of mycotoxins in different species has been the subject of numerous studies, little is known about the mycotoxins' ability to induce xenobiotic metabolic pathways, to interfere with the metabolism of other xenobiotics, and to interact with NHRs.

One possible approach for predicting a chemical compound's capability to upregulate a xenobiotics biotransformation pathway is to screen for the compound's interaction with known molecular regulators of biotransformation pathways, such as NHRs including PXR. With regard to this, PXR trans-activation assays based on genetically modified reporter cell lines have already been used for the screening of drug candidate compounds for their potential ability to induce biotransformation and to determine the risk of drug-to-drug interactions (Sinz et al., 2008). We took a similar approach, testing environmental toxins and employed a human hepatocyte cell line-based PXR trans-activation assay for screening of mycotoxins for their ability to activate PXR. As is shown in this report, we found that aflatoxin B1, and to lesser degree aflatoxins M1 and G1, activated PXR and upregulated PXR-dependent *CYP3A4* expression.

2. Materials and methods

2.1. Cell culture

The HepG2 (hepatocellular carcinoma) cell line was obtained from the ATCC (Manassas, USA) and was maintained under standard conditions in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum at 37 °C in an atmosphere of 5% CO_2. Cultures were used for experiments within 20 passages after delivery.

2.2. Plasmid construction and chemicals

The reporter plasmid $(ER6)_9$-SV40-Luc was generated by insertion of nine copies of chemically synthesized *CYP3A4* ER6 PXRE (5′-GATCAGACAGTTCATGAAGTTCATCTAGATC-3′, described previously in (Lehmann et al., 1998), and the SV40 promoter into the Acc65I/HindIII sites of the pGL4.14 vector (Promega, Madison, USA). Rifampicin, dexamethasone, SR12813, CITCO, VD3, TBHP, CHX and all mycotoxins were purchased from Sigma-Aldrich (St. Louis, USA). $ZnCl_2$ was purchased from Merck-Chemicals (Darmstadt, Germany).

2.3. Stable transfection and generation of the nhrtox-hepg2 cell line

HepG2 cells were plated into 6-well plates in DMEM medium at a density of 20×10^5 cells per well. Twenty-four hours after seeding, cells were transfected with 2 μg of $(ER6)_9$-SV40-Luc reporter plasmid using Fugene HD (Roche, Basel, Switzerland). After 14 days, selection with hygromycin B (Sigma-Aldrich) at the concentration of 100 μg/ml, individual hygromycin B-resistant colonies were isolated, expanded and screened for rifampicin-inducible expression of luciferase. A stable cell clone (*nhrtox-hepg2*) was selected for reproducible rifampicin-induced luciferase expression, expanded, and frozen in aliquots that were employed in screening experiments.

2.4. Treatment and luciferase assay

The *nhrtox-hepg2* cells were seeded into 96-well plates at a density of 15×10^4 cells per well. The next day, they were incubated with increasing concentrations of selected compounds for 24 h. Following incubation, cells were harvested, lysed and the activity of luciferase in the cell lysates was determined using an EnVision luminometer plate reader (Perkin Elmer) with a commercial luciferase substrate (BD Biosciences).

2.5. Real-time RT-PCR

Total RNA was isolated from cells using TRI Reagent® from the Molecular Research Center (Cincinnati, USA) and reverse-transcribed with the RevertAid™ H Minus M-MuLV Reverse Transcriptase (Fermentas, Vilnius, Lithuania) using an anchored oligo-dT18 primer. The level of cognate cDNA was measured by real-time RT-PCR amplification performed on a LightCycler 480 from Roche (Basel, Switzerland), using SYBR Green I Master Mix (Roche) to detect PCR products. Cycling conditions were as follows: 95 °C for 5 min followed by 45 cycles at 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 s. Intron-spanning primers were used to detect the cDNA sequence for the *CYP3A4* gene (forward primer 5′-TTCAGCAAGAAGAACAAGGACAA-3′, reverse primer 5′-GGTTGAAGAAGTCCTCCTAAGC-3′), and they were taken from the work of Cerveny et al. (2007). *HPRT1*, *HMBS*, *and RPLI3A* were selected as the most reliable reference genes in a selection procedure that was developed by Vandesompele et al. (2002). For presentation, ΔCt values were transformed into relative copy number values (the number of copies of mRNA for the *CYP3A4* gene per housekeeping gene index), as described our previous study (Ratajewski et al., 2008). HepG2 cells were plated at 50–60% confluency, treated the following day with selected compounds for 24 h, and then collected for RNA extraction.

2.6. Neutral red uptake assay

To estimate cell viability, the neutral red uptake assay (Repetto et al., 2008) was conducted. Cells were seeded into 96-well plates, and after 24 h were treated with increasing concentrations of the investigated compounds for an additional 24 h. After that time, incubation medium was removed, and cells were washed with cold PBS. After washing, cells were incubated with 50 μg/ml neutral red in HBSS for 2 h. Following incubation, the neutral red solution was removed, cells were washed with PBS, cell-bound dye was extracted using a solution containing 50% ethanol and 1% acetic acid, and a 550-nm absorbance was determined. The IC_{10} and IC_{20} (where it was possible) were calculated from linear dose-response curves.

2.7. Chromatin immunoprecipitation assay (ChIP)

HepG2 cells were treated for 24 h with the respective compounds and chromatin immunoprecipitation was performed using the EZ-ChIP kit from Millipore (Billerica, Massachusetts, USA) according to the manufacturer's instructions. The following antibodies were used: Normal Mouse IgG (part of the EZ-ChIP kit) and anti-PXR (N-16, Santa Cruz). Real-time PCR amplification was done using 1 μl of sample DNA with primers complementary to the *CYP3A4* promoter (5′-TTCTTTGCCAACTTCCAAGG-3′ and 5′-TCTGTGTTGCTCTTTGCTGG-3′; positions −235/−47 to relative to the A in the ATG translation initiation codon). Primers specific to the *GAPDH* promoter (used as negative control) were included in the EZ-ChIP. The amplification of soluble chromatin prior to immunoprecipitation was used as an input control. Quantification was performed as previously described (Walczak-Drzewiecka et al., 2008).

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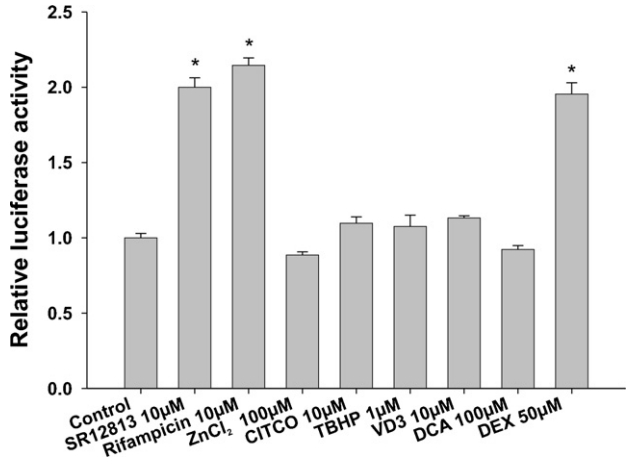

Fig. 1. Validation of the nhrtox-hepg2 reporter cells. Cells were treated for 24 h with rifampicin, SR 12813, dexamethasone (PXR activators), CITCO (CAR agonist), VD3 (VDR agonist), DCA (FXR agonist), TBHP (oxidative stress inducer) and $ZnCl_2$ (MTF1 activator), and a luciferase assay was conducted. Mean ± SEM, $n = 6$, *significantly different from control at $p < 0.01$.

2.8. Microarray analysis

Gene expression analyses were conducted using the Quick Amp Labeling Kit (Two-Color) from Agilent (Santa Clara, USA) according to the manufacturer's instructions. Total RNA (500 ng) was isolated from control HepG2 cells and HepG2 cells treated with 10 μM aflatoxin B1 for 24 h. Cy3- or Cy5-labeled cRNA was produced according to the manufacturer's protocol. For each two-color comparison, 825 ng of each Cy3- and Cy5-labeled cRNA was mixed and fragmented using the Agilent Gene Expression Hybridization Kit. Hybridizations were performed for 17 h in a 65 °C hybridization oven. Slides were washed with Gene Expression Wash Buffers 1 and 2 and then scanned with an Agilent Scanner, as indicated for 4 × 44 K format. Each analysis was performed in tetraplicate, employing a dye swap. The resultant data were processed using Gene Spring 11.5 software. Genes with a p value < 0.05 were considered differentially expressed.

2.9. Gene ontology (GO) analysis

The Gene Ontology Enrichment Analysis Software Toolkit (GEOAST) (Zheng and Wang, 2008) for functional studies of microarray data was applied. As the source of data set, we used the probe IDs from the above-mentioned Agilent microarray.

2.10. Statistics

Testing for statistical significance was done by a one-way ANOVA, followed by Tukey's test using SigmaStat 3.5 (Systat Software, Point Richmond, USA). A p value of 0.05 or lower was considered statistically significant.

3. Results

We first validated the nhrtox-hepg2 reporter cell line, containing the luciferase ORF under the control of an artificial promoter with 9 repeats of the PXR binding motif, with three known PXR activators and several control compounds. As shown in Fig. 1, incubation of nhrtox-hepg2 reporter cells with SR 12813, rifampicin and dexamethasone, but not $ZnCl_2$, CITCO, TBQ, VD3, and DCA, significantly (two-fold) increased luciferase activity. Thus, nhrtox-hepg2 cells responded to known PXR activators with increased luciferase expression. Next, we determined the cytotoxicity of tested mycotoxins in HepG2 cells (the background cell line for the development of nhrtox-hepg2) in the 24-h incubation assay. The IC_{10} and IC_{20} values obtained with the neutral red uptake assay are presented in Table 1 and were used for decisions concerning the range of concentrations of mycotoxins used in the PXR trans-activation assay. The highest tested mycotoxin concentration was equal to or lower than the respective IC_{20}. The results of PXR trans-activation assay are presented in Fig. 2A–C and show that three out of the 16 tested mycotoxins, namely aflatoxin B1, aflatoxin M1 and aflatoxin G1, resulted in significantly more than a

Table 1
The IC_{10} and IC_{20} values obtained for the indicated compounds with neutral red uptake assay. Mean ± SEM, $n = 12$.

Compound	IC_{10} [μM]	IC_{20} [μM]	Highest conc. tested
SR12813	6.5 ± 2.1	>10	10 μM
$ZnCl_2$	75.7 ± 6.4	>100	100 μM
CITCO	6.4 ± 1.4	>10	10 μM
tert-butylhydroperoxide (TBHP)	>1	>1	1 μM
Cholecalciferol (VD3)	>10	>10	10 μM
Dexamethasone (DEX)	39.0 ± 8.7	>50	50 μM
Deoxycholic acid (DCA)	>100	>100	100 μM
Rifampicin	10.0 ± 1.4	>10	10 μM
Fumonisin B1	>10	>10	10 μM
Myriocin	>10	>10	10 μM
Ochratoxin A	4.6 ± 0.9	8.9 ± 0.5	10 μM
Zearalenone	5.1 ± 1.7	9.3 ± 1.7	10 μM
Patulin	0.2 ± 0.1	0.5 ± 0.2	0.5 μM
Citrinin	>10	>10	10 μM
A-ergocryptine	7.8 ± 1.7	>10	10 μM
Ergocornine	5.3 ± 1.8	9.7 ± 1.7	10 μM
Aflatoxin B1	5.3 ± 0.3	>10	10 μM
Aflatoxin B2	>10	>10	10 μM
Aflatoxin M1	5.6 ± 0.1	>10	10 μM
Aflatoxin G1	5.3 ± 0.5	>10	10 μM
Aflatoxin G2	>10	>10	10 μM
Compound	**IC_{10} [nM]**	**IC_{20} [nM]**	**Highest conc. tested**
T-2	3.0 ± 0.4	5.1 ± 1.2	5 nM
HT-2	4.5 ± 0.1	9.5 ± 0.9	5 nM
Diacetoxyscirpenol	4.6 ± 0.2	8.7 ± 0.2	5 nM

1.5-fold increase (2.8, 2.3, and 1.7-fold, respectively) in luciferase expression in the nhrtox-hepg2 reporter cells. Thus, some of the tested mycotoxins activated PXR. We then compared the kinetics of aflatoxin B1- and rifampicin-mediated PXR activation. As seen in Fig. 2D, in both cases, the incubation of nhrtox-hepg2 cells with activator resulted in a constant time-dependent increase in luciferase activity, with aflatoxin B1 resulting in a slower rate of luciferase increase when compared to rifampicin. We next verified if the mycotoxins that activated PXR in reporter cells are also capable of increasing the expression of CYP3A4, which is known to be regulated in hepatocytes in a PXR-dependent manner. As shown in Fig. 3, incubation of HepG2 hepatocytes with 10 μM aflatoxins B1, M1 and G1 resulted in a significant increase (4.0, 2.9, 2.2-fold, respectively) in the level of CYP3A4 mRNA in HepG2 hepatocytes, as determined by the real-time RT-PCR assay. Thus, three aflatoxins, B1, M1, and G1, activated PXR and PXR-dependent CYP3A4 mRNA expression in a human hepatocyte cell line. To gain further insights into aflatoxin mediated CYP3A4 mRNA expression, its sensitivity to translation inhibitor cycloheximide was determined. Similarly to rifampicin mediated CYP3A4 expression (Gerbal-Chaloin et al., 2002) cycloheximide at concentration 1 μM inhibited aflatoxin B1 mediated CYP3A4 expression by 89% (data not shown). To further confirm that selected aflatoxins activate PXR and upregulate PXR-dependent gene expression in human hepatocytes, we decided to investigate the association of the PXR protein with the *CYP3A4* promoter region in HepG2 hepatocytes exposed to aflatoxins B1, M1, and G1 as well as rifampicin. The results of the chromatin immunoprecipitation experiments are presented in Fig. 4 and showed that in cells incubated with aflatoxins B1, M1, and G1 and with rifampicin, there is a significantly higher level (2.8, 2.7, 2.1, 2.5-fold, respectively) of PXR protein associated with the *CYP3A4* promoter sequence. Similar analysis was performed for the *GAPDH* promoter sequence (negative control) and it did not show significant differences between aflatoxin-, rifampicin-treated, and control hepatocytes. We next decided to expand our investigation of PXR-dependent gene expression in

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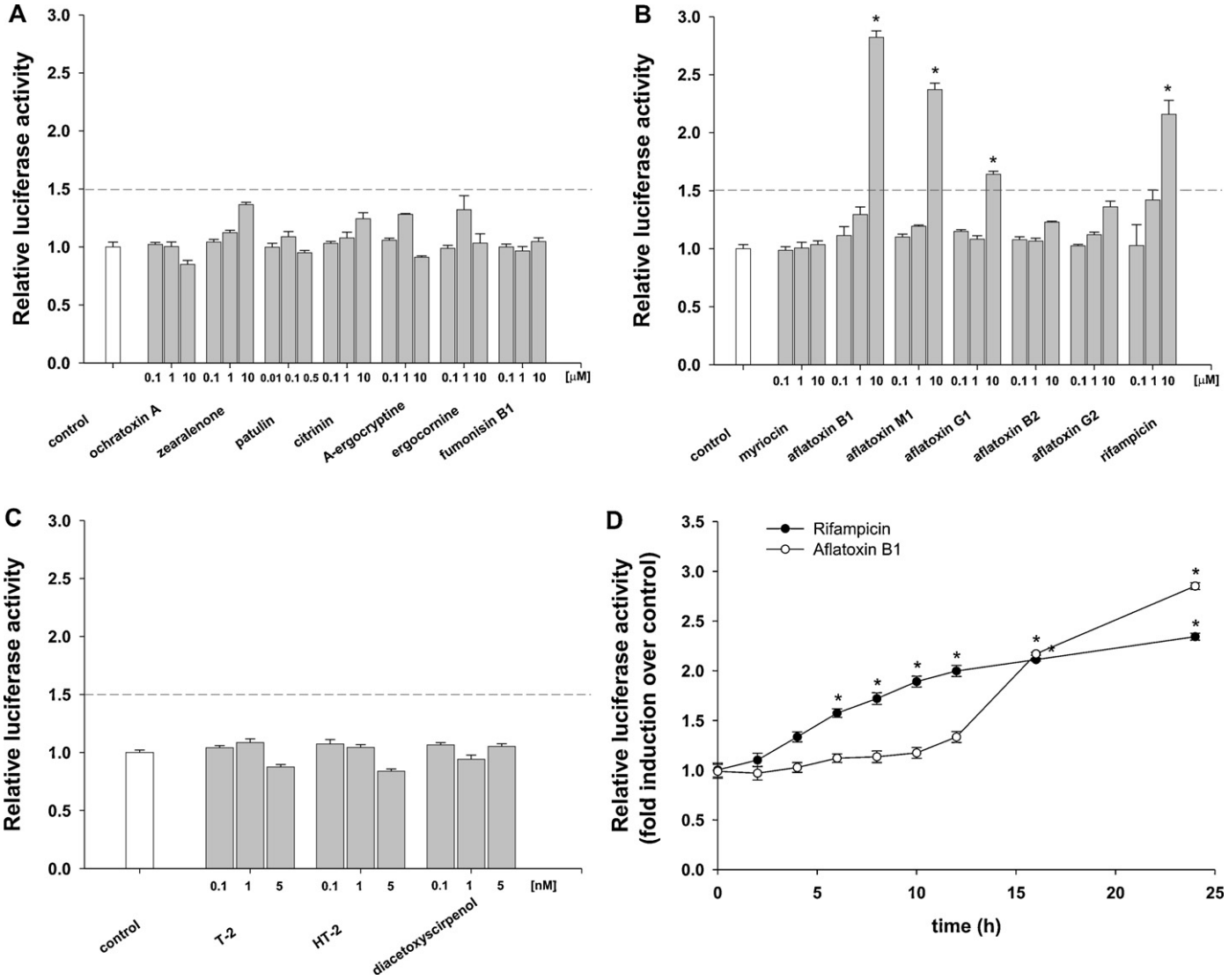

Fig. 2. Identification of aflatoxins B1, M1 and G1 as potent activators of PXR-dependent *CYP3A4* transcription in nhrtox-hepg2 cells. (A) The effect of ochratoxin A, zearalenone, patulin, citrinin, A-ergocryptine, ergocornine, and fumonisin B on the nhrtox-hepg2 assay. (B) The effect of aflatoxins on the nhrtox-hepg2 assay. (C) The effect of trichothecenes (T-2, HT-2 and diacetoxyscirpenol) on the nhrtox-hepg2 assay. (D) A time course of rifampicin (10 μM) and aflatoxin B1 (10 μM) on the nhrtox-hepg2 assay. Mean ± SEM, $n = 6$, *significantly different from control at $p < 0.01$.

HepG2 hepatocytes exposed to aflatoxin B1 using a microarray-based gene expression assay. The level of mRNA expression in HepG2 cells incubated with aflatoxin B1 was compared with that of with untreated cells. The results (Supplementary Material Table S1) showed significant ($p < 0.05$) and at least a two-fold difference in the expression level of more than 1000 genes. As seen in Table 2, aflatoxin B1-treated HepG2 cells showed a significant effect for expression of numerous genes coding for phase I and phase II enzymes important for xenobiotic metabolism and disposition, including genes such as *CYP2R1*, *CYP3A5*, *CYP3A7* and *CYP4F2* and that similarly to *CYP3A4*, are regulated by PXR (Burk et al., 2004; Ellfolk et al., 2009; Pascussi et al., 1999; Siest et al., 2008). Gene ontology analysis showed that, in addition to genes associated with xenobiotic catabolism, aflatoxin B1 treatment influenced multiple genes linked to lipid metabolism (Tables 2 and 3), zinc homeostasis (e.g., *metallothioneins* and *SLC30A1*), cadmium and copper ion binding (e.g., *LOX* and *LOXL1*), and insulin growth factor-mediated signaling (e.g., *IGFALS*, *IGFBP1*) (Supplementary Material Tables S1 and S2).

4. Discussion

Mycotoxins constitute a source of important environmental threats to human and animal health (Bennett, 1987; Bennett and Klich, 2003; Peraica et al., 1999). It is important to better understand their bioactivities, including the mechanism of their biotransformation. We have performed a screen of mycotoxins for the possible activation of the PXR nuclear hormone receptor that is an important regulator of the liver response to the presence of xenobiotics. We found that aflatoxin B1, and to lesser extent aflatoxins G1 and M1, upregulate PXR-dependent promoter activity (Fig. 1) at sub-cytotoxic concentrations (Table 1). The bioactivity of these aflatoxins was very specific and did not reflect a general cytotoxic effect of these compounds on hepatocytes, as it was observed only for three structurally similar compounds of the 16 mycotoxins tested at concentrations that showed a comparably low level of cytotoxicity. The ability of selected aflatoxins to activate PXR is consistent with the observation that all of these compounds mediated upregulation of CYP3A4 mRNA in HepG2 cells (Fig. 2). To our knowledge, this is

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Table 2
Genes of the phase I, phase II and the cholesterol metabolic process showing at least 2-fold change ($p < 0.05$) upon 10 μM aflatoxin B1 treatment in HepG2 cells determined by microarray.

Gene category	Gene symbol	GenBank Accession#	Gene description	Fold change
Phase I	*CYP2R1*	NM_024514	cytochrome P450, family 2, subfamily R, polypeptide 1	+2.2
	CYP3A4	NM_017460	cytochrome P450, family 3, subfamily A, polypeptide 4	+2.9
	CYP3A5	NM_000777	cytochrome P450, family 3, subfamily A, polypeptide 5	+2.6
	CYP3A7	NM_000765	cytochrome P450, family 3, subfamily A, polypeptide 7	+3.2
	CYP4F2	NM_001082	cytochrome P450, family 4, subfamily F, polypeptide 2	+2.3
	CYP4F8	NM_007253	cytochrome P450, family 4, subfamily F, polypeptide 8	+2.1
	CYP4F11	NM_021187	cytochrome P450, family 4, subfamily F, polypeptide 11	+2.0
	CYP21A2	NM_000500	cytochrome P450, family 21, subfamily A, polypeptide 2	+3.2
	CYP26A1	NM_057157	cytochrome P450, family 26, subfamily A, polypeptide 1	+10.2
	HSD3B1	NM_000862	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	+12.7
Phase II	*GSTA1*	NM_145740	glutathione S-transferase alpha 1	−3.2
	GSTA2	NM_000846	glutathione S-transferase alpha 2	−2.8
	GSTA3	NM_000847	glutathione S-transferase alpha 3	−2.7
	GSTT2	NM_000854	glutathione S-transferase theta 2	+2.9
	GPX1	NM_201397	glutathione peroxidase 1	+2.9
	UGT2B7	NM_001074	UDP glucuronosyltransferase 2 family, polypeptide B7	+2.8
	UGT2B10	NM_001075	UDP glucuronosyltransferase 2 family, polypeptide B10	+2.3
	UGT2B17	NM_001077	UDP glucuronosyltransferase 2 family, polypeptide B17	+3.4
	UGT2B28	NM_053039	UDP glucuronosyltransferase 2 family, polypeptide B28	+2.9
Cholesterol metabolic process	*SOAT1*	NM_003101.4	sterol O-acyltransferase 1	−2.1
	HMGCS1	NM_002130	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)	−2.7
	AKR1D1	NM_005989	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	−3.1
	APOL1	NM_145343	apolipoprotein L, 1	−2.0
	LSS	NM_001001438	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	−2.4
	MVK	NM_000431	mevalonate kinase	−2.2
	SREBF1	NM_001005291	sterol regulatory element binding transcription factor 1	−2.2
	HMGCR	NM_000859	3-hydroxy-3-methylglutaryl-CoA reductase	−2.5
	RXRA	NM_002957	retinoid X receptor, alpha	−2.4
	SQLE	NM_003129	squalene epoxidase	−2.5
	TNFSF4	NM_003326	tumor necrosis factor (ligand) superfamily, member 4	+3.6
	PCSK9	NM_174936	proprotein convertase subtilisin/kexin type 9	−4.0
	FDXR	NM_004110	ferredoxin reductase	+4.2

the first report of aflatoxin B1-mediated upregulation of CYP3A4 expression in human cells, and might be of particular interest because aflatoxin B1 is one of the most intensely studied mycotoxins. It presents an example of a well-established mechanism of hepatocarcinogenicity that depends predominantly on CYP3A4-mediated oxidation of aflatoxin B1 in human liver to the highly reactive compound, aflatoxin-8,9 epoxide (half-life in water lower than 1 s), which forms DNA and protein adducts (Appleton, 1985; Akao et al., 1992; Groopman et al., 1993; Bren et al., 2007). Thus, the CYP3A4-mediated biotransformation of aflatoxin B1 that occurs in liver is critical for its adverse health effects, and our observations provide important novel information for better understanding of this process.

Xenobiotic metabolic enzymes, such as CYP3A4, are frequently induced by their own substrate, and the molecular mechanisms behind this positive regulatory feedback involves the interaction of xenobiotics with NHRs, such as PXR, that recognize variety of chemical structures to trigger changes in gene expression (Carnahan and Redinbo, 2005). The *CYP3A4* promoter contains several PXR binding motifs, and the binding of PXR to these DNA sequences is critical for the upregulation of *CYP3A4* by several xenobiotics, including dexamethasone, rifampicin, RU486, clotrimazole, and lovastatin (Lehmann et al., 1998). A similar mechanism could be proposed for aflatoxin B1-mediated upregulation of CYP3A4 expression (Fig. 3), which at a similar concentration upregulates PXR-dependent promoter activity (Fig. 2). This hypothesis is further supported by the results of the chromatin immunoprecipitation experiment, which demonstrates that an aflatoxin B1-mediated increase in PXR promoter occupancy of *CYP3A4* but not the control *GAPDH* promoter suggesting a direct link between the ability of aflatoxin B1 to upregulate the PXR trans-activation function and CYP3A4 expression. One possible explanation for these observations is the hypothesis

Table 3
Example of the GO terms linked to lipid metabolism, identified using the Gene Ontology Enrichment Analysis Software Toolkit (GEOAST). GOID - accession number in gene ontology, *p*-value is from hypergeometric test by default for the over-representation of aflatoxin B1 target genes in the GO category.

GOID	Term	Number of genes	*p*-value
GO:0008152	metabolic process	402	6.88×10^{-5}
GO:0044238	primary metabolic process	359	1.32×10^{-3}
GO:0006629	lipid metabolic process	92	6.75×10^{-16}
GO:0008610	lipid biosynthetic process	39	5.69×10^{-7}
GO:0006694	steroid biosynthetic process	15	1.64×10^{-4}
GO:0016126	sterol biosynthetic process	9	6.64×10^{-4}
GO:0008202	steroid metabolic process	30	2.04×10^{-7}
GO:0008207	C21-steroid hormone metabolic process	5	3.11×10^{-2}
GO:0016125	sterol metabolic process	18	2.31×10^{-5}
GO:0008203	cholesterol metabolic process	13	4.25×10^{-6}

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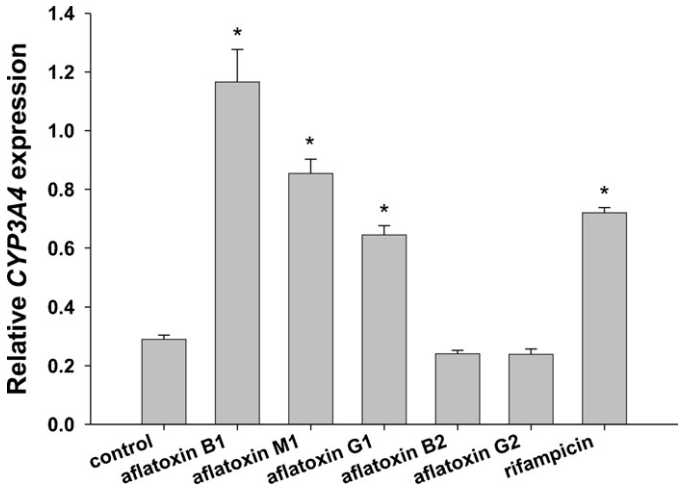

Fig. 3. Aflatoxins B1, M1 and G1 (10 μM) induce increased CYP3A4 mRNA levels in HepG2 cells. Cells were treated with experimental compounds for 24 h. Rifampicin (10 μM) was used as a positive control. The amount of CYP3A4 mRNA was measured by real-time RT-PCR and is expressed as the number of mRNA copies per the housekeeping gene index (HPRT1, HMBS and RPLI3A). Mean ± SEM, $n = 4$, *significantly different from control at $p < 0.01$.

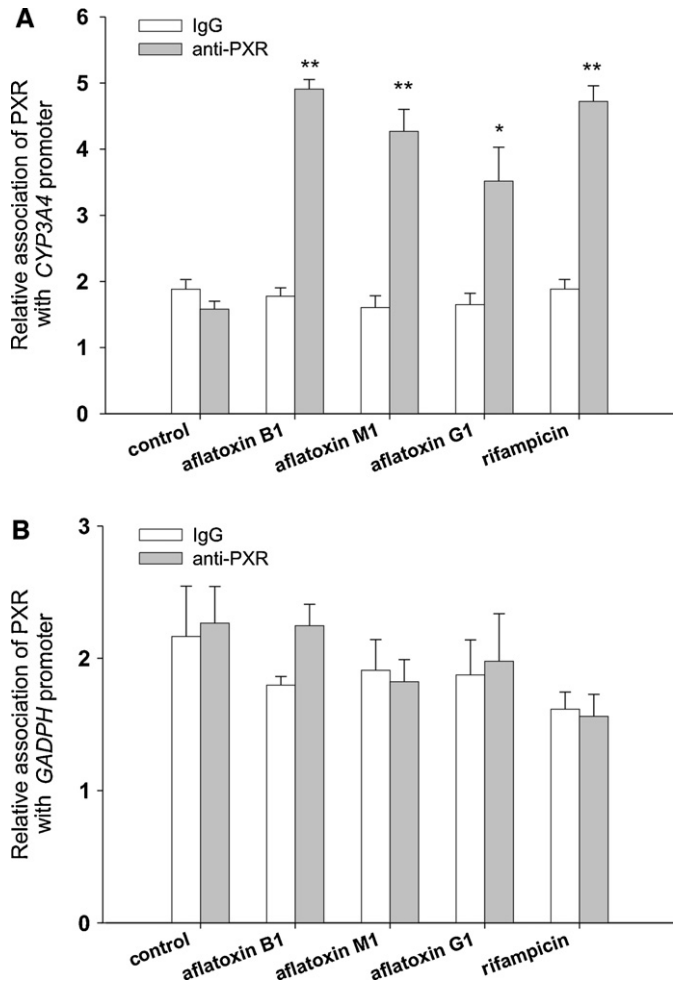

Fig. 4. PXR binding to the *CYP3A4* (A) and *GADPH* (B) promoters in HepG2 cells treated with aflatoxin B1, M1, G1 or rifampicin as demonstrated by chromatin immunoprecipitation assay. The amount of immunoprecipitated gene promoter sequence occupancy was determined by real-time PCR, expressed in arbitrary relative values, and standardized against the amount of input material (Mean ± SEM, $n = 6$). *Statistically significant at $p < 0.05$, **Statistically significant at $p < 0.01$.

that aflatoxin B1 is a ligand for PXR. However, we do not have data demonstrating such a direct interaction. Additionally, aflatoxin B1 activated PXR with slower kinetics than those observed with the model PXR ligand rifampicin (Fig. 2D), which might suggest that a metabolite of aflatoxin, rather than aflatoxin itself, is responsible for the observed effects. An alternative explanation is that aflatoxin B1 or its metabolite indirectly upregulates PXR inhibiting signaling pathways known to negatively regulate this nuclear receptor by phosphorylating the PXR protein and downregulating its activity and the possibilities include the cAMP-PKA, Cdk2, PI3′-Akt, and p70S6K pathways (Ding and Staudinger, 2005; Kodama et al., 2004; Lin et al., 2008; Pondugula et al., 2009).

The ability of aflatoxin B1 to upregulate PXR activity is consistent with the results of the whole genome expression analysis of HepG2 cells exposed to this mycotoxin (Tables 2 and 3, and Supplementary Material Table S1), which shows changes in the expression of multiple genes, including several genes critical for xenobiotic metabolism in liver. Thus, aflatoxin B1-mediated upregulation of several genes coding for phase I and II (Table 2) enzymes, including *CYP2R1*, *CYP3A4*, *CYP3A5*, *CYP3A7*, *CYP4F2*, *GSTT1*, and *GPX1*, which are known to be regulated by PXR (Bertilsson et al., 1998; Drocourt et al., 2001; Kluth et al., 2007; Lehmann et al., 1998; Naspinski et al., 2008). The expression levels of numerous other genes potentially important for aflatoxin B1-mediated liver damage were also affected. Ontology analysis revealed overrepresentation of GO terms linked to lipid metabolism (Table 3). One functional category of genes regulated by aflatoxin B1 in HepG2 hepatocytes that is of special interest was genes involved in cholesterol synthesis (Table 2). Aflatoxin B1 downregulated multiple genes involved in cholesterologenesis, including *SREBF1*, which encodes a transcription factor that regulates genes involved in this process (Horton et al., 2002) and genes encoding enzymatic proteins directly involved in cholesterol biosynthesis, such as *HMGCS1*, *AKR1D1*, *LSS*, and *HMGCR*. These changes in gene expression are consistent with *in vivo* observations showing that hypocholesterolemia is a common clinical feature observed in acute aflatoxicosis in different species (Bassir and Alozie, 1979; Shukla and Pachauri, 1995; Amaya-Farfan, 1999; Dereszynski et al., 2008; Gowda et al., 2008). These observations are especially intriguing in the context of the role of PXR in regulating cholesterol biosynthesis, transport and catabolism (Rezen et al., 2011). It is possible that aflatoxin B1-mediated PXR activation might also play a role in the observed downregulation of certain cholesterologenesis-linked genes. For example, *SREBF1* is positively regulated by the transcription factor HNF4, and inhibitory crosstalk between PXR and HNF4 that depends on their competition for the co-activator protein PGC-1alpha was previously demonstrated (Bhalla et al., 2004).

In conclusion, we demonstrated that aflatoxin B1 is able to activate the transcription factor that is a master regulator of liver xenobiotic metabolism in the human hepatocytes, PXR, and that it upregulates multiple PXR-dependent genes, including *CYP3A4*, which is responsible for aflatoxin B1 biotransformation into a hepatocarcinogenic metabolite.

Conflict of interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.toxlet.2011.05.1034.

References

Akao, M., Kuroda, K., Gonoi, T., Kishikawa, S., 1992. Isolation of a metastasizing cancer cell line from an aflatoxin B1-induced rat liver tumor. Chem. Pharm. Bull. (Tokyo) 40, 1299–1302.

Amaya-Farfan, J., 1999. Aflatoxin B1-induced hepatic steatosis: role of carbonyl compounds and active diols on steatogenesis. Lancet 353, 747–748.

Angsubhakorn, S., Get-Ngern, P., Miyamoto, M., Bhamarapravati, N., 1990. A single dose-response effect of aflatoxin B1 on rapid liver cancer induction in two strains of rats. Int. J. Cancer 46, 664–668.

Appleton, B.S., 1985. Aflatoxin exposure and human liver-cancer risk. Food Chem. Toxicol. 23, 129–130.

Bassir, O., Alozie, T.C., 1979. Short-term effects of aflatoxin B1 on serum lipids in subhuman primates. Lab. Anim. 13, 67–68.

Bechtel, D.H., 1989. Molecular dosimetry of hepatic aflatoxin B1-DNA adducts: linear correlation with hepatic cancer risk. Regul. Toxicol. Pharmacol. 10, 74–81.

Bennett, J.W., 1987. Mycotoxins, mycotoxicoses, mycotoxicology and Mycopathologia. Mycopathologia 100, 3–5.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497–516.

Bertilsson, G., Heidrich, J., Svensson, K., Asman, M., Jendeberg, L., Sydow-Backman, M., Ohlsson, R., Postlind, H., Blomquist, P., Berkenstam, A., 1998. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. Proc. Natl. Acad. Sci. U.S.A. 95, 12208–12213.

Bhalla, S., Ozalp, C., Fang, S., Xiang, L., Kemper, J.K., 2004. Ligand-activated pregnane X receptor interferes with HNF-4 signaling by targeting a common coactivator PGC-1alpha. Functional implications in hepatic cholesterol and glucose metabolism. J. Biol. Chem. 279, 45139–45147.

Blumberg, B., Sabbagh Jr., W., Juguilon, H., Bolado Jr., J., van Meter, C.M., Ong, E.S., Evans, R.M., 1998. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. Genes Dev. 12, 3195–3205.

Borrelli, F., Izzo, A.A., 2009. Herb-drug interactions with St John's wort (Hypericum perforatum): an update on clinical observations. AAPS J. 11, 710–727.

Bren, U., Guengerich, F.P., Mavri, J., 2007. Guanine alkylation by the potent carcinogen aflatoxin B1: quantum chemical calculations. Chem. Res. Toxicol. 20, 1134–1140.

Burk, O., Koch, I., Raucy, J., Hustert, E., Eichelbaum, M., Brockmoller, J., Zanger, U.M., Wojnowski, L., 2004. The induction of cytochrome P450 3A5 (CYP3A5) in the human liver and intestine is mediated by the xenobiotic sensors pregnane X receptor (PXR) and constitutively activated receptor (CAR). J. Biol. Chem. 279, 38379–38385.

Carnahan, V.E., Redinbo, M.R., 2005. Structure and function of the human nuclear xenobiotic receptor PXR. Curr. Drug Metab. 6, 357–367.

Cerveny, L., Svecova, L., Anzenbacherova, E., Vrzal, R., Staud, F., Dvorak, Z., Ulrichova, J., Anzenbacher, P., Pavek, P., 2007. Valproic acid induces CYP3A4 and MDR1 gene expression by activation of constitutive androstane receptor and pregnane X receptor pathways. Drug Metab. Dispos. 35, 1032–1041.

Cooper, B.W., Cho, T.M., Thompson, P.M., Wallace, A.D., 2008. Phthalate induction of CYP3A4 is dependent on glucocorticoid regulation of PXR expression. Toxicol. Sci. 103, 268–277.

Dereszynski, D.M., Center, S.A., Randolph, J.F., Brooks, M.B., Hadden, A.G., Palyada, K.S., McDonough, S.P., Messick, J., Stokol, T., Bischoff, K.L., Gluckman, S., Sanders, S.Y., 2008. Clinical and clinicopathologic features of dogs that consumed food-borne hepatotoxic aflatoxins: 72 cases (2005-2006). J. Am. Vet. Med. Assoc. 232, 1329–1337.

Ding, X., Staudinger, J.L., 2005. Induction of drug metabolism by forskolin: the role of the pregnane X receptor and the protein kinase a signal transduction pathway. J. Pharmacol. Exp. Ther. 312, 849–856.

Dong, M., Tulayakul, P., Li, J.Y., Dong, K.S., Manabe, N., Kumagai, S., 2010. Metabolic conversion of zearalenone to alpha-zearalenol by goat tissues. J. Vet. Med. Sci. 72, 307–312.

Drocourt, L., Pascussi, J.M., Assenat, E., Fabre, J.M., Maurel, P., Vilarem, M.J., 2001. Calcium channel modulators of the dihydropyridine family are human pregnane X receptor activators and inducers of CYP3A, CYP2B, and CYP2C in human hepatocytes. Drug Metab. Dispos. 29, 1325–1331.

Duanmu, Z., Kocarek, T.A., Runge-Morris, M., 2001. Transcriptional regulation of rat hepatic aryl sulfotransferase (SULT1A1) gene expression by glucocorticoids. Drug Metab. Dispos. 29, 1130–1135.

Echchgadda, I., Song, C.S., Oh, T., Ahmed, M., De La Cruz, I.J., Chatterjee, B., 2007. The xenobiotic-sensing nuclear receptors pregnane X receptor, constitutive androstane receptor, and orphan nuclear receptor hepatocyte nuclear factor 4alpha in the regulation of human steroid-/bile acid-sulfotransferase. Mol. Endocrinol. 21, 2099–2111.

Ellfolk, M., Norlin, M., Gyllensten, K., Wikvall, K., 2009. Regulation of human vitamin D(3) 25-hydroxylases in dermal fibroblasts and prostate cancer LNCaP cells. Mol. Pharmacol. 75, 1392–1399.

Fang, H.L., Strom, S.C., Ellis, E., Duanmu, Z., Fu, J., Duniec-Dmuchowski, Z., Falany, C.N., Falany, J.L., Kocarek, T.A., Runge-Morris, M., 2007. Positive and negative regulation of human hepatic hydroxysteroid sulfotransferase (SULT2A1) gene transcription by rifampicin: roles of hepatocyte nuclear factor 4alpha and pregnane X receptor. J. Pharmacol. Exp. Ther. 323, 586–598.

Francis, G.A., Fayard, E., Picard, F., Auwerx, J., 2003. Nuclear receptors and the control of metabolism. Annu. Rev. Physiol. 65, 261–311.

Frank, C., Makkonen, H., Dunlop, T.W., Matilainen, M., Vaisanen, S., Carlberg, C., 2005. Identification of pregnane X receptor binding sites in the regulatory regions of genes involved in bile acid homeostasis. J. Mol. Biol. 346, 505–519.

Gardner-Stephen, D., Heydel, J.M., Goyal, A., Lu, Y., Xie, W., Lindblom, T., Mackenzie, P., Radominska-Pandya, A., 2004. Human PXR variants and their differential effects on the regulation of human UDP-glucuronosyltransferase gene expression. Drug Metab. Dispos. 32, 340–347.

Gerbal-Chaloin, S., Daujat, M., Pascussi, J.M., Pichard-Garcia, L., Vilarem, M.J., Maurel, P., 2002. Transcriptional regulation of CYP2C9 gene. Role of glucocorticoid receptor and constitutive androstane receptor. J. Biol. Chem. 277 (1), 209–217.

Gowda, N.K., Ledoux, D.R., Rottinghaus, G.E., Bermudez, A.J., Chen, Y.C., 2008. Efficacy of turmeric (Curcuma longa), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. Poult. Sci. 87, 1125–1130.

Groopman, J.D., Cain, L.G., Kensler, T.W., 1988. Aflatoxin exposure in human populations: measurements and relationship to cancer. Crit. Rev. Toxicol. 19, 113–145.

Groopman, J.D., Wild, C.P., Hasler, J., Junshi, C., Wogan, G.N., Kensler, T.W., 1993. Molecular epidemiology of aflatoxin exposures: validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. Environ. Health Perspect. 99, 107–113.

Guengerich, F.P., Johnson, W.W., Ueng, Y.F., Yamazaki, H., Shimada, T., 1996. Involvement of cytochrome P450, glutathione S-transferase, and epoxide hydrolase in the metabolism of aflatoxin B1 and relevance to risk of human liver cancer. Environ. Health Perspect. 104 (Suppl. 3), 557–562.

Horton, J.D., Goldstein, J.L., Brown, M.S., 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. 109, 1125–1131.

Huang, P., Chandra, V., Rastinejad, F., 2010. Structural overview of the nuclear receptor superfamily: insights into physiology and therapeutics. Annu. Rev. Physiol. 72, 247–272.

Ihunnah, C.A., Jiang, M., Xie, W., 2011. Nuclear receptor PXR, transcriptional circuits and metabolic relevance. Biochim. Biophys. Acta, in press.

Jones, S.A., Moore, L.B., Shenk, J.L., Wisely, G.B., Hamilton, G.A., McKee, D.D., Tomkinson, N.C., LeCluyse, E.L., Lambert, M.H., Willson, T.M., Kliewer, S.A., Moore, J.T., 2000. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. Mol. Endocrinol. 14, 27–39.

Kluth, D., Banning, A., Paur, I., Blomhoff, R., Brigelius-Flohe, R., 2007. Modulation of pregnane X receptor- and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds. Free Radic. Biol. Med. 42, 315–325.

Kodama, S., Koike, C., Negishi, M., Yamamoto, Y., 2004. Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. Mol. Cell. Biol. 24, 7931–7940.

le Maire, A., Bourguet, W., Balaguer, P., 2010. A structural view of nuclear hormone receptor: endocrine disruptor interactions. Cell. Mol. Life Sci. 67, 1219–1237.

Lebrun, S., Golka, K., Schulze, H., Follmann, W., 2006. Glutathione S-transferase polymorphisms and ochratoxin A toxicity in primary human urothelial cells. Toxicology 224, 81–90.

Lehmann, J.M., McKee, D.D., Watson, M.A., Willson, T.M., Moore, J.T., Kliewer, S.A., 1998. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. J. Clin. Invest. 102, 1016–1023.

Lin, W., Wu, J., Dong, H., Bouck, D., Zeng, F.Y., Chen, T., 2008. Cyclin-dependent kinase 2 negatively regulates human pregnane X receptor-mediated CYP3A4 gene expression in HepG2 liver carcinoma cells. J. Biol. Chem. 283, 30650–30657.

Murk, A.J., Legler, J., Denison, M.S., Giesy, J.P., van de Guchte, C., Brouwer, A., 1996. Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water. Fundam. Appl. Toxicol. 33, 149–160.

Naspinski, C., Gu, X., Zhou, G.D., Mertens-Talcott, S.U., Donnelly, K.C., Tian, Y., 2008. Pregnane X receptor protects HepG2 cells from BaP-induced DNA damage. Toxicol. Sci. 104, 67–73.

Pascussi, J.M., Jounaidi, Y., Drocourt, L., Domergue, J., Balabaud, C., Maurel, P., Vilarem, M.J., 1999. Evidence for the presence of a functional pregnane X receptor response element in the CYP3A7 promoter gene. Biochem. Biophys. Res. Commun. 260, 377–381.

Peraica, M., Radic, B., Lucic, A., Pavlovic, M., 1999. Toxic effects of mycotoxins in humans. Bull. World Health Organ. 77, 754–766.

Pondugula, S.R., Brimer-Cline, C., Wu, J., Schuetz, E.G., Tyagi, R.K., Chen, T., 2009. A phosphomimetic mutation at threonine-57 abolishes transactivation activity and alters nuclear localization pattern of human pregnane x receptor. Drug Metab. Dispos. 37, 719–730.

Ratajewski, M., Van de Ven, W.J., Bartosz, G., Pulaski, L., 2008. The human pseudoxanthoma elasticum gene ABCC6 is transcriptionally regulated by PLAG family transcription factors. Hum. Genet. 124, 451–463.

Repetto, G., del Peso, A., Zurita, J.L., 2008. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat. Protoc. 3, 1125–1131.

Rezen, T., Rozman, D., Pascussi, J.M., Monostory, K., 2011. Interplay between cholesterol and drug metabolism. Biochim. Biophys. Acta 1814, 146–160.

Ruegg, J., Penttinen-Damdimopoulou, P., Makela, S., Pongratz, I., Gustafsson, J.A., 2009. Receptors mediating toxicity and their involvement in endocrine disruption. EXS 99, 289–323.

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Schulman, I.G., 2010. Nuclear receptors as drug targets for metabolic disease. Adv. Drug Deliv. Rev. 62, 1307–1315.
Shukla, S.K., Pachauri, S.P., 1995. Blood biochemical profiles in induced aflatoxicosis of cockerels. Br. Poult. Sci. 36, 155–160.
Siest, G., Jeannesson, E., Marteau, J.B., Samara, A., Marie, B., Pfister, M., Visvikis-Siest, S., 2008. Transcription factor and drug-metabolizing enzyme gene expression in lymphocytes from healthy human subjects. Drug Metab. Dispos. 36, 182–189.
Sinz, M., Wallace, G., Sahi, J., 2008. Current industrial practices in assessing CYP450 enzyme induction: preclinical and clinical. AAPS J. 10, 391–400.
Sonoda, J., Xie, W., Rosenfeld, J.M., Barwick, J.L., Guzelian, P.S., Evans, R.M., 2002. Regulation of a xenobiotic sulfonation cascade by nuclear pregnane X receptor (PXR). Proc. Natl. Acad. Sci. U.S.A. 99, 13801–13806.
Staudinger, J.L., Goodwin, B., Jones, S.A., Hawkins-Brown, D., MacKenzie, K.I., LaTour, A., Liu, Y., Klaassen, C.D., Brown, K.K., Reinhard, J., Willson, T.M., Koller, B.H., Kliewer, S.A., 2001. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc. Natl. Acad. Sci. U.S.A. 98, 3369–3374.
Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3, RESEARCH0034.
Walczak-Drzewiecka, A., Ratajewski, M., Wagner, W., Dastych, J., 2008. HIF-1alpha is up-regulated in activated mast cells by a process that involves calcineurin and NFAT. J. Immunol. 181, 1665–1672.
Watkins, R.E., Wisely, G.B., Moore, L.B., Collins, J.L., Lambert, M.H., Williams, S.P., Willson, T.M., Kliewer, S.A., Redinbo, M.R., 2001. The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. Science 292, 2329–2333.
Winterfield, L., Cather, J., Menter, A., 2003. Changing paradigms in dermatology: nuclear hormone receptors. Clin. Dermatol. 21, 447–454.
Wu, Q., Dohnal, V., Huang, L., Kuca, K., Wang, X., Chen, G., Yuan, Z., 2011. Metabolic pathways of ochratoxin a. Curr. Drug Metab. 12, 1–10.
Zheng, Q., Wang, X.J., 2008. GOEAST: a web-based software toolkit for Gene Ontology enrichment analysis. Nucleic Acids Res. 36, W358–W363.