Application of HC-AFW1 Hepatocarcinoma Cells for Mechanistic Studies: Regulation of Cytochrome P450 2B6 Expression by Dimethyl Sulfoxide and Early Growth Response 1

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ABSTRACT

Various exogenous compounds, for example, the drugs bupropione and propofol, but also various cytostatics, are metabolized in the liver by the enzyme cytochrome P450 (P450) CYP2B6. Transcription from the CYP2B6 gene is regulated mainly via the transcription factors constitutive androstane receptor (CAR) and pregnane-X-receptor (PXR). Most hepatic cell lines express no or only low levels of CYP2B6 because of loss of these two regulators. Dimethyl sulfoxide (DMSO) is frequently used in liver cell cultivation and is thought to affect the expression of various P450 isoforms by inducing or preserving cellular differentiation. We studied the effects of up to 1.5% of DMSO as cell culture medium supplement on P450 expression in hepatocarcinoma cells from line HC-AFW1. DMSO did not induce differentiation of the HC-AFW1 cell line, as demonstrated by unaltered levels of selected mRNA markers important for hepatocyte differentiation, and also by the lack of a DMSO effect on a broader spectrum of P450s. By contrast, CYP2B6 mRNA was strongly induced by DMSO. This process was independent of CAR or PXR activation. Interestingly, elevated transcription of CYP2B6 was accompanied by a simultaneous induction of early growth response 1 (EGR1), a transcription factor known to influence the expression of CYP2B6. Expression of wild-type EGR1 or of a truncated, dominant-negative EGR1 mutant was able to mimic or attenuate the DMSO effect, respectively. These findings demonstrate that EGR1 is involved in the regulation of CYP2B6 by DMSO in HC-AFW1 cells.

Introduction

Toxification or detoxification of many foreign substances is catalyzed by enzymes from the cytochrome P450 (P450) superfamily. Especially enzymes from P450 families 1–4 are of high importance for xenobiotic metabolism. Most of these are expressed at high levels in the liver. For a recent review of relevant human hepatic P450 enzymes, please refer to Zanger and Schwab (2013). Transcription of most P450 genes is controlled by xenosensing nuclear receptors, such as the constitutive androstane receptor (CAR) or the pregnane-X-receptor (PXR), which act as transcription factors on binding of activating ligands (Tomkins and Wallace, 2007).

The P450 isoform CYP2B6 accounts for approximately 2%–5% of total P450 in human liver and is engaged in the metabolism of various substances, for example, the drugs bupropione, ifosfamide, propofol, temazepam, or methadone (Zanger and Schwab, 2013). A number of transcription factors regulate CYP2B6 mRNA levels: CYP2B6 responds to the treatment of cells by agonists of the nuclear receptors CAR and PXR (Sueyoshi et al., 1999; Wang et al., 2003). Published studies furthermore suggest that hepatocyte nuclear factor (HNF) 4α is also involved in CYP2B6 regulation (Kamiyama et al., 2007; Benet et al., 2010). Moreover, the transcription factor early growth response (EGR) 1 plays an important role in CYP2B6 expression: multiple EGR1 binding sites have been identified within the 5′-regulatory promoter region of the CYP2B6 gene, and together with CAR and HNF4α, EGR1 synergistically activates the CYP2B6 promoter (Inoue and Negishi, 2008, 2009).

When transferred into cell culture, hepatocytes tend to lose their differentiated functions, including the activity of most drug-metabolizing P450s. Most permanent hepatoma cell lines possess only very low levels of P450s. Thus, numerous strategies have been applied that aimed to preserve P450 expression of primary cells and/or to induce differentiation processes to restore P450 expression in vitro. The methods applied comprise optimization of the extracellular matrix, three-dimensional culture approaches, microfluidic systems, as well as modulation of the composition of culture media. For a comprehensive and recent review of hepatocyte cultivation, please refer to Godoy et al. (2013). One example for a component frequently used in cell culture media aimed to support hepatocyte differentiation (or to prevent their dedifferentiation, respectively) is the polar organic solvent dimethyl sulfoxide (DMSO). Although cytotoxic at higher concentrations, DMSO at ≤2% has been described to preserve functionality and differentiation of primary rat hepatocytes, especially the maintenance of high levels of P450 enzymes and of the different HNF isoforms, important markers of hepatocyte differentiation (Rogiers et al., 1990; Lindsay et al., 1991; Padgham et al., 1992; Zurlo and Arterburn, 1996; Su and Waxman, 2004). Differentiation-inducing effects have also been reported for human Huh7 hepatoma cells (Choi et al., 2009), whereas the effect of DMSO on human primary hepatocytes appears to be rather
minor (Nishimura et al., 2003). Nonetheless, DMSO is important for the induction of differentiation of the human hepatocarcinoma cell line HepaRG, which expresses high levels of a broad spectrum of drug-metabolizing enzymes after a 2-weeks differentiation protocol driven by the presence of DMSO (Aninat et al., 2006; Antherieu et al., 2012). Protocols aimed to generate hepatocyte-like cells from induced pluripotent stem cells also use DMSO as supplement in differentiation media (Kondo et al., 2014).

The exact molecular mechanisms by which DMSO acts on cellular differentiation are not well understood yet. An early study by Villa et al. (1991) suggests hydroxyl radical scavenging by DMSO as the underlying mechanism of maintenance of hepatocyte differentiation. In addition, antiproliferative effects mediated by a downregulation of c-Myc have been implicated in the induction of differentiation by DMSO, as reviewed by Santos et al. (2003). The downregulation of pluripotency genes by DMSO may also play a role (Czyz et al., 2015). Another aspect is the maintenance of connexin-mediated cell-cell communication in the presence of DMSO (Yoshizawa et al., 1997).

In the current study, we analyzed the effects of DMSO on the expression of different P450 isoforms in HC-AFW1 cells, a novel cell line derived from a pediatric hepatocellular carcinoma (Armeanu-Ebinger et al., 2012). In contrast to many other hepatoma cell lines, HC-AFW1 cells possess considerable levels of most of the important P450s, as well as functional signal transduction through the relevant nuclear receptors, for example, CAR and PXR. Effects of DMSO in this cell line, potentially suited to induce cellular differentiation and thus improve CYP expression, have not been investigated so far; therefore, this study was conducted to analyze the impact of DMSO on P450 expression in HC-AFW1 cells. We show that DMSO does not induce a general differentiation of HC-AFW1 cells but rather selectively induces the expression of CYP2B6, an effect that was then analyzed in detail and backtracked to a transcriptional mechanism that involves the induction of EGR1.

Materials and Methods

Cell Culture and Treatment. HC-AFW1 human hepatocarcinoma cells (Armeanu-Ebinger et al., 2012) were cultured in DMEM medium supplemented with 10% fetal calf serum and penicillin/streptomycin (all purchased from Invitrogen, Karlsruhe, Germany). Cells were seeded at a density of 1.5% (v/v) DMSO for a maximum of 14 days. A medium change was performed every 48 hours. In some experiments, cells were also treated for 24 hours with 800 ng of plasmid DNA: 750 ng of the respective promoter were used: a wild-type (WT) construct and a polymorphic version of the promoter (called A3), which was cotransfected for normalization of firefly luciferase data. Cells were transfected 24 hours after seeding with 800 ng of plasmid DNA: 750 ng of the respective CYP2B6 reporter construct, or the empty pGL3 vector backbone as a control, was combined with 50 ng of plasmid pRL-CMV. When EGR1 was cotransfected, 400 ng of the luciferase reporter plasmid was cotransfected with 350 ng of the respective vector and 50 ng of pRL-CMV. Transfection was performed with Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, cells were incubated with DMSO or PB for the indicated time points before lysis with 1 mM phenobarbital (PB; Sigma, Taufkirchen, Germany; dissolved in water), 10 μM 6-(-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-3-dichlorobenzyl-oxime (CITCO; Enzo Life Sciences, Lürrach, Germany; dissolved in DMSO), or 10 μM rifampicin (RIF; Sigma; dissolved in DMSO).

Cytotoxicity Assays. The absence of treatment-related toxicity was checked for in 96-well plates by using the resazurin reduction (Alamar Blue) and neutral red uptake assays as recently described (Braeuning et al., 2012). As initial experiments, 5000 cells/well were plated on 96-well plates, and the assays were conducted in octuple determinations. In addition, resazurin reduction was monitored in parallel to all specific endpoints (e.g., mRNA expression, luciferase reporter activity) in each experiment.

Plasmid Construction, Transfections, and Luciferase Reporter Analyses.

Two variants of a pGL3-based firefly luciferase reporter vector containing approximately 2 kb of the human CYP2B6 promoter were used: a wild-type (WT) construct and a polymorphic version of the promoter (called A3), which provides enhanced activity (Zukunft et al., 2005). Deletion mutants of the A3 version of the construct were generated by restriction digest of the plasmid followed by religation. Construct 1 was obtained after digestion with MscI and EcoRV, construct 2 after digestion with MscI and SpeI, and construct 3 after digestion with PsI (see Fig. 2C for schematic representation). In addition, pCDA3.1-based expression vectors for human WT EGR1 (Inoue and Negishi, 2008) and for a truncated, dominant negative version of EGR1 were used (Chapman and Perkins, 2000; Levkovitz and Baraban, 2001).

Cells were transiently transfected with the above-mentioned reporter constructs using standard methods (Braeuning and Vetter, 2012). Renilla luciferase, encoded by the plasmid pRL-CMV (Promega, Mannheim, Germany) under the control of the constitutively active cytomegalovirus promoter, was cotransfected for normalization of firefly luciferase data. Cells were transfected 24 hours after seeding with 800 ng of plasmid DNA: 750 ng of the respective CYP2B6 reporter construct, or the empty pGL3 vector backbone as a control, was combined with 50 ng of plasmid pRL-CMV. When EGR1 was cotransfected, 400 ng of the luciferase reporter plasmid was cotransfected with 350 ng of the EGR1 expression vector and 50 ng of pRL-CMV. Transfection was performed in 24-well plates using Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, cells were incubated with DMSO or PB for the indicated time points before lysis with 1× Passive Lysis Buffer (Promega) and determination of luciferase activities, as previously described (Braeuning and Vetter, 2012).

Small interfering RNAs (siRNA) directed against EGR1 were purchased from Qiagen (Hilden, Germany) and transfected at concentrations of 10-100 nM using HiPerFect transfection reagent (Qiagen).

Gene Expression Analysis. The High Pure RNA Isolation kit (Roche, Mannheim, Germany) was used to isolate total RNA according to the manufacturer’s instructions. Reverse transcription was carried out using avian myeloblastosis virus reverse transcriptase (Promega) with oligo(dT)_18 and random (dN)_6 primers. Gene expression was analyzed using a capillary-based LightCycler system (Roche). Relative quantification of target gene expression was performed using the FastStart DNA MasterPLUS SYBR Green I kit (Roche) and the primers listed in Table 1. The BLAST algorithm and the NCBI database

### TABLE 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5'-3')</th>
<th>Reverse (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB</td>
<td>TGA TTT GGT GTC TCA GAT TCT TTT</td>
<td>AGC ACG TCA GAC CTT TCC CAT CAT</td>
</tr>
<tr>
<td>CAR</td>
<td>TGC AAG TGA TAA GAG CAA GAA GGA</td>
<td>AAG GGT GGT GTT GAG TGA TAT</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>GCC TCT TGC TTC AAG GCA TTT</td>
<td>TAC CTT GCT TGC TGC GAG GTT</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>GTC TAC CCA GCG CCA ACC CAA CCA</td>
<td>GGT GAG GAG GAG GAG GAG GAG</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>CAT CCA GCA GAT GAT GAT CAG</td>
<td>CAC GAG GAG GAG GAG GAG GAG</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>CAT CCA GCA GAT GAT GAT CAG</td>
<td>CAC GAG GAG GAG GAG GAG GAG</td>
</tr>
<tr>
<td>CYP4A11</td>
<td>TGC TCA CAG ATG TCA GTC</td>
<td>TCT CTC AAT TCC GAC GAC GAC</td>
</tr>
<tr>
<td>EGR1</td>
<td>CTG GCT AAT TCC GCA GAC GAC</td>
<td>GGT GAC GGT GGT GGT GGT GGT</td>
</tr>
<tr>
<td>HNF4α</td>
<td>CCC GCT CCA TCG CAG CAG CAG</td>
<td>TCT CTC AAT TCC GAC GAC GAC</td>
</tr>
<tr>
<td>HNF4α</td>
<td>ACC CAC CTC CAC CAC CAC</td>
<td>TCT CTC AAT TCC GAC GAC GAC</td>
</tr>
<tr>
<td>PXR</td>
<td>CAA CCG CAG TAG GAA GGA</td>
<td>CTA GAA TGG GAA GAG GAA GAG</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>CGGT GCT CAT CAC GCA GAA GGA</td>
<td>GCT GGA ATT ACC CGG CAG</td>
</tr>
</tbody>
</table>
were used to ensure specificity of the primers. Polymerase chain reaction (PCR) products were verified by melting point analyses and gel electrophoresis. Expression of 18S rRNA was used for normalization according to the method of Pfaffl (2001).

**Western Blotting.** Whole-cell lysates were prepared using standard methods as recently described (Braeuning and Schwarz, 2010); 50 µg of lysate was separated by SDS-PAGE and blotted to Polyvinyldene difluoride membranes. EGR1 protein expression was detected using an antibody directed against EGR1 (1:1000 dilution; catalog no. 4155; Cell Signaling Technology, Danvers, MA). Glyceraldehyde-3-phosphate dehydrogenase (GAP; 1:500; catalog no. MAB374; Merck Millipore, Schwalbach, Germany) was used as a loading control. For previous use of these antibodies, see Braeuning et al. (2014) and Ohnberger et al. (2015). Antibody binding was visualized by appropriate secondary antibodies (1:10,000; IRDye 800CW donkey-anti-rabbit or IRDye 680CW donkey-anti-rabbit; LI-COR Biotechnology, Bad Homburg, Germany), and images were acquired using Odyssey CLx (LI-COR) in combination with Image Studio Software Odyssey 4.0 (LI-COR).

**Statistical Analysis.** Student’s t test was used for statistical analyses in case of pairwise comparisons. For comparisons with more than two groups, analyses of variance with Bonferroni correction were conducted using SigmaPlot software. Significance was assumed at P < 0.05.

**Results**

**Selective Induction of CYP2B6 by DMSO in HC-AFW1 Cells.** DMSO is used to induce differentiation and stabilize the expression of drug-metabolizing enzymes in liver cells in vitro (see introduction section). HC-AFW1 cells were incubated with different concentrations of DMSO [0.5%, 1%, or 1.5% (v/v)] for up to 14 days to analyze potential beneficial effects of DMSO on P450 expression in this cell line. DMSO did not reduce the viability of HC-AFW1 cells in the concentrations used in this study (data not shown). Incubation with DMSO induced the expression of CYP2B6 mRNA in a concentration- and time-dependent manner, with a more than 20-fold increase in the abundance of the transcript at 2 or more days of incubation with 1.5% DMSO (Fig. 1A). This induction far exceeded the rather moderate effects on CYP2B6 mRNA (i.e., a 2- to 3-fold induction) exerted by 3 mM PB, 5 µM CITCO, or 10 µM RIF, prototypical inducers of the enzyme via activation of CAR (PB, CITCO) or PXR (RIF), respectively (Fig. 1B). The observed CYP2B6 mRNA induction was not accompanied by comparable changes in the expression of other important P450 enzymes from families 1–4, namely, CYP1A1, CYP2C, CYP2D6, CYP2E1, CYP3A4, and CYP4A11, several of which are also known to be dependent on CAR or PXR (Fig. 1, C–H).

**CYP2B6 Induction by DMSO Is Due to Transcriptional Regulation.** Analyses with a CYP2B6 promoter-driven luciferase reporter construct were conducted to determine whether the observed CYP2B6 induction was based on increased transcription of the gene. Two versions of the CYP2B6 promoter came into operation: first, a ~2 kb fragment of the CYP2B6 upstream wild-type sequence, and second, a polymorphic variant of that sequence with slightly higher activity, termed A3 (Zukunft et al., 2005). As shown in Fig. 2A, both versions of the CYP2B6 promoter, i.e., the wild-type and A3 variants, were induced in a concentration- and time-specific manner, closely reflecting the observations at the mRNA level. Transcription of the luciferase reporter gene from the empty pGL3 vector backbone was not influenced by DMSO. Again, the prototypical inducer PB exerted much less pronounced effects, while CITCO and RIF were not able to significantly influence the luciferase reporter system (Fig. 2B). Cytotoxic effects of the inducers were excluded (data not shown). Different deletion constructs of the CYP2B6 reporter plasmid were generated to attribute the induction of transcription more specifically to a certain region of the cloned promoter sequence. These constructs are schematically depicted in Fig. 2C. DMSO-induced reporter gene activities were much lower in all deletion constructs (Fig. 2D): an approximately 10-fold induction of luciferase activity was observed by 1.5% DMSO, when the CYP2B6 reporter plasmid containing the full length wild-type sequence was transfected. By contrast, the constructs no. 2 and no. 3, which contain approximately the downstream or upstream half of the ~2 kb sequence, were only able to induce the reporter by 2- to 3-fold. This indicated that more than one single transcription factor binding site must be involved in the induction of CYP2B6 transcription by DMSO, most likely with synergistic effects of the individual binding sites.

**DMSO Does Not Induce General Differentiation but EGR1 Transcription in HC-AFW1 Cells.** To unravel the mechanism by which DMSO induces CYP2B6 in HC-AFW1 cells, the expression of a number of transcriptional regulators, which have been previously implicated in the regulation of CYP2B6, was determined at the mRNA level by real-time reverse transcription (RT)-PCR: CAR, PXR, HNF4α,
and EGR1. In addition, the expression of the hepatocyte differentiation marker genes ALB (encoding albumin) and HNF1α were investigated to detect possible differentiation-inducing effects of DMSO. The results of these analyses are presented in Fig. 3: no striking effects were observed for the ALB, HNF1α, HNF4α, CAR, and PXR transcripts; the weak induction of PXR mRNA failed our criteria of statistical significance. By contrast, the expression of EGR1 mRNA was strongly increased upon treatment of HC-AFW1 cells with DMSO, showing a triplication up to a quintuplication in transcript abundance after treatment with DMSO (Fig. 3). The time and dose dependency of EGR1 induction was quite similar to that observed for CYP2B6 (Fig. 3A; compare with Figs. 1A and 2A). EGR1 mRNA induction was accompanied by a concomitant increase in cellular EGR1 protein levels (Fig. 3).

EGR1-Dependent Regulation of CYP2B6. Previous studies have demonstrated a stimulatory role of EGR1 in CYP2B6 expression (Inoue and Negishi, 2008, 2009). These studies, however, did not address the effects caused by DMSO. If EGR1 was indeed involved in the upregulation of CYP2B6 by DMSO, then overexpression of the protein should mimic the effect of DMSO, whereas silencing of EGR1 activity should alleviate DMSO-induced CYP2B6 expression. Thus, HC-AFW1 cells were first transfected with an expression vector for EGR1 together with the CYP2B6 luciferase reporter already used in Figs. 1A and 2A. Induction of CYP2B6 promoter-driven luciferase reporter activity by DMSO in HC-AFW1 cells. (A) Induction of luciferase reporter activities driven by two versions of a 2-kb fragment of the human CYP2B6 promoter after transient transfection by 24 hours of DMSO treatment. Vectors: pGL3, empty vector backbone; CYP2B6_WT, CYP2B6 reporter WT sequence; CYP2B6_A3, CYP2B6 reporter polymorphic variant with higher activity. (B) Induction of the WT promoter by the known CYP2B6 inducers PB (3 mM), Rif (10 μM), or CITCO (10 μM). (C) Schematic representation (not to scale) of the deletion constructs derived from the A3 variant of the CYP2B6 promoter luciferase reporter vector. (D) Induction of luciferase activities of the constructs depicted in (C) by 1.5% DMSO for 24 hours. Mean ± S.D. of n = 5 (empty pGL3 vector: n = 4) experiments (each performed in three biologic replicates) are presented. Statistical significance (P < 0.05) is indicated by asterisks.
the previous experiments. Cotransfection of EGR1 was able to strongly induce CYP2B6 promoter-driven luciferase reporter activity (Fig. 4A). For the WT construct, this effect was even stronger than the effect of DMSO, whereas EGR1 and DMSO were approximately equally effective on the CYP2B6 A3 promoter variant (Fig. 4A). Successful overexpression of EGR1 mRNA upon transfection of the EGR1 expression plasmid was confirmed by real-time RT-PCR (Fig. 4B). EGR1 overexpression by transfection of the EGR1 expression vector also triggered elevated CYP2B6 mRNA expression (Fig. 4C), whereas corresponding analyses of the inhibition of endogenous CYP2B6 mRNA expression by transfection of an expression vector for the zinc finger domain of EGR1, which functions as a dominant negative EGR1 (Chapman and Perkins, 2000; Levkovitz and Baraban, 2001), were impeded by very low basal levels of the transcript and limitations of the transfection procedure (data not shown). Of note, elevation of CYP2B6 mRNA was also less pronounced than the stimulation of reporter activities, a phenomenon that is most likely due to limited transfection efficiency of the cells. Silencing of EGR1 by siRNA was also performed to check whether blocking EGR1 would alleviate the effects of DMSO on CYP2B6. Using four different commercially available siRNAs against EGR1, no satisfactory reduction of the target transcript was achieved under various conditions, despite satisfactory transfection efficiency (data not shown). We therefore cotransfected the expression vector for dominant negative EGR1 with either the WT or the A3 variant of the CYP2B6 promoter reporter plasmid. As evident from Fig. 4D, a significant lessening of CYP2B6 induction by 1.5% DMSO was achieved when the dominant negative EGR1 was present.

**Discussion**

In the current study, we investigated the effects of DMSO on the expression of various enzymes from the CYP superfamily in HC-AFW1 cells. A general induction of differentiation of HC-AFW1 cells into a more hepatocyte-like phenotype was not observed in our analyses, as evidenced by unaltered levels of mRNAs for various liver-specific mRNAs encoding HNFs, nuclear receptors, or albumin. Accordingly, the levels of most P450 isoforms remained unaffected by DMSO treatment. This finding underlines that differentiation-related effects of DMSO are highly cell line–dependent, since overall P450 expression and differentiation are positively influenced by DMSO in HepaRG human hepatocarcinoma cells (Aninat et al., 2006; Antherieu et al., 2012), as well as in Huh7 (Choi et al., 2009). The molecular reasons for this different behavior of individual cell lines are still not understood.

A strong effect in HC-AFW1 cells, however, was seen for CYP2B6, which was induced by DMSO in a concentration-dependent manner. This phenomenon occurred at concentrations ≥0.5% of DMSO, which is routinely used as supplement in differentiation-inducing cell culture media. When treating cells with test chemicals dissolved in DMSO, concentrations of the solvent normally do not exceed 0.1%. Accordingly, disturbance of test results by unintended CYP2B6-inducing effects of DMSO applied in such low concentrations appears unlikely.

The fast response of the CYP2B6 gene within 24 hours after the addition of DMSO to the medium, together with the lack of modulation of hepatic differentiation markers and other CYPs, shows that this effect is a specific gene-regulatory effect and is not caused by altered cellular differentiation. To the best of our knowledge, the present article is the first report describing this particular effect on CYP2B6 expression. DMSO-induced CYP2B6 transcription might occur in other cell lines, but the specific effect of gene regulation might be masked by general effects on the differentiation of the cells, which are connected to the induction of a broader spectrum of P450s and also to increased levels of HNFs and nuclear receptors involved in CYP expression. Contrasting the situation with DMSO, where the increase in CYP2B6 is quite pronounced, the response of the CYP2B6 gene to the prototypical inducers and strong CAR or PXR agonists phenobarbital, CITCO, and rifampicin was rather moderate and transient in our analyses. Neither were the levels of other CAR or PXR target CYPs changed upon exposure of HC-AFW1 cells to DMSO, nor were the levels of CAR and PXR mRNAs significantly changed. These findings strongly suggest
that CAR and PXR, the most prominent regulators of CYP2B6 transcription (Sueyoshi et al., 1999; Wang et al., 2003), are not responsible for the phenomenon observed under DMSO treatment of the cells. Moreover, there is also no indication from the data for a specific role of HNF4α, also a known regulator of CYP2B6 expression (Inoue and Negishi, 2008, 2009), in DMSO-dependent effects in HC-AFW1 cells. Nonetheless, a certain degree of participation of HNF4α in the process cannot be ruled out, since HC-AFW1 cells express measurable levels of HNF4α and the literature has shown synergistic effects between HNF4α and EGR1 in CYP2B6 induction (Inoue and Negishi, 2008, 2009), with the latter transcription factor being pronouncedly regulated by DMSO under the conditions investigated.

A striking effect of DMSO on EGR1 mRNA levels was observed in the present study. To the best of our knowledge, such an effect has not been discovered in previous studies on the effects of DMSO on liver-derived cells (see introduction section for appropriate literature references). Several lines of evidence are in support of the hypothesis that EGR1 is responsible for the induction of CYP2B6 by DMSO: first, time and dose dependency of the increases in the two transcript levels are closely correlated; second, EGR1 has been previously shown to be pronouncedly regulated by DMSO under the conditions (own unpublished data).

In summary, the present study has revealed a novel effect of DMSO on CYP2B6 expression in human hepatocarcinoma cells. Specific induction of CYP2B6, but not of other important drug-metabolizing P450s, P450-regulating nuclear receptors or hepatocyte differentiation markers, by DMSO is mediated by induction of EGR1 mRNA.

Furthermore, the study illustrates the potential of HC-AFW1 cells to serve as a model system for the analysis of mechanisms of P450 regulation and expression in vitro, where these cells might constitute an interesting alternative to established cell models such as HepG2 or HepaRG for certain applications. In synopsis of available data, routine supplementation of culture medium with DMSO is not recommended for HC-AFW1 cells, since no general advantage with regard to differentiation is observed and since overall enzymatic activity of CYP2B6 in HC-AFW1 cells is limited even in the presence of DMSO by the genetic profile of the cells, which causes reduced function of CYP2B6 protein (own unpublished data).

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Authorship Contributions

Participated in research design: Braeuning, Schwarz.
Conducted experiments: Petzuch, Groll.

Acknowledgments

Performed data analysis: Petzuch, Braeuning.
Wrote or contributed to writing of the manuscript: Braeuning, Petzuch, Schwarz.

References


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