CYTOCHROME P450 GENE INDUCTION IN RATS EX VIVO ASSESSED BY QUANTITATIVE REAL-TIME REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (TAQMAN)

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ABSTRACT:

Drug-induced changes in expression of cytochrome P450 (P450) genes are a significant issue in the preclinical development of pharmaceuticals. For example, preclinically, P450 induction can affect safety studies by reducing the systemic exposure of a compound undergoing toxicological evaluation, thus limiting the exposure that can be safely investigated in patients. Therefore, the induction potential of candidate drugs has been studied as part of the drug development process, typically using protein and/or catalytic end points. However, measuring changes in the levels of mRNA using TaqMan technology offers the opportunity to investigate this issue with the advantages of better dynamic range and specific enzyme identification. Here, we describe the TaqMan application to study ex vivo the P450 gene induction in the rat. Initially, livers from rats dosed with the prototypic P450 inducers β-naphthoflavone (BNF), phenobarbital (PB), dexamethasone (DEX), and clofibric acid (CLO) were analyzed for mRNA levels of CYP1A1, 1A2, 2B1, 2B2, 2E1, 3A2, 3A23, and 4A1 and compared with control animals. The maximum fold induction of mRNA varied: 2500-fold for CYP1A1 with BNF, 680-fold for CYP2B1 with PB, 59-fold for CYP3A23 with DEX, and 16-fold for CYP4A1 with CLO.

The cytochromes P450 (P450s) are a ubiquitous superfamily of heme-containing monoxygenase enzymes that play a fundamental role in the metabolism of a variety of chemically diverse compounds, including endogenous chemicals and pharmaceutical agents (Nelson et al., 1996; Nelson, 1999; Parkinson, 2001; P450 nomenclature on http://dnelson.utmem.edu/CytochromeP450.html). Therefore, these enzymes are involved in many pharmacokinetic drug-drug interactions (DDIs), where multiple drug therapy results in the interference of one drug with the metabolism of another (Guengerich, 1997). Drug interactions mediated by P450 induction is a well established mechanism of pharmacokinetic DDIs and can result either in a marked increase in production of a toxic metabolite (Lin and Lu, 1998) or can reduce the efficacy of other coadministered medications because of an increase in clearance (Park et al., 1996). A more comprehensive listing of drugs that are inducers of P450 enzymes can be found in Dave Flockhart’s P450 drug interaction tables (http://medicine.iupui.edu/flockhart/) and is reported by Michalets (1998).

Estimating the potential of a candidate drug to be an inducer of P450 expression is an important consideration for the early stages of discovery and development of new chemical entities (NCEs). In particular, toxicokinetic findings associated with a candidate drug inducing its own metabolism (autoinduction) may include subproportional increases in exposure (Cmax, AUC), with increasing doses and reduced exposure after repeat administration (Worboys and Carlile, 2001). However, if the enzyme(s) induced are not responsible for the clearance of the candidate drug, these parameters will not be affected but may result in altered kinetics of other drugs. In both cases, these treatment-related effects may be associated with liver weight increases and histological findings of hepatomegaly through induction of cellular hypertrophy and hyperplasia of the liver (Staudinger et al., 2001). It has also been reported that P450 induction in the rat may also be associated with thyroid toxicity (Hood et al., 1999).

Currently, quantification of P450 induction in preclinical species can be tested as part of toxicological/safety evaluation before the candidate drug is selected for clinical development. This is achieved primarily by measuring changes in their enzyme activity by catalytic end points or by quantification of gross changes in P450 protein levels using specific antibodies suitable for Western blot analysis. However, because the major mechanism of P450 induction is via increased rates

ABBREVIATIONS: P450, cytochrome P450; DDI, drug-drug interaction; NCE, new chemical entity; qRT-PCR, quantitative real-time reverse transcriptase-polymerase chain reaction; DEPC, diethyl pyrocarbonate; RT, reverse transcriptase; BNF, β-naphthoflavone; PB, phenobarbital; DEX, dexamethasone; CLO, clofibric acid; Tm, melting temperature; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PXR, pregnane X receptor; CAR, constitutively activated/androstane receptor; EROD, 7-ethoxyresorufin O-dealkylation; test 6β-oh, testosterone 6β-hydroxylation; TDI, time-dependent inhibitor.
of transcription, this testing can be addressed by studying changes in specific P450 gene expression by measuring messenger RNA (mRNA) levels using quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) (Gibson et al., 1996; Godfrey and Kelly, 2005).

Here, we report the sensitivity, specificity, wide dynamic range, reproducibility, accuracy, and application of an automated qRT-PCR protocol (TaqMan) for the measurement of mRNA levels of P450 genes in total RNA extracted from rat liver. To evaluate this method, we assessed the inducibility of CYP1A1, CYP2B, CYP3A, and CYP4A after administration of the prototypical P450 inducers β-naphthoflavone, phenobarbital, dexamethasone, and clofibric acid, respectively, to rodents. CYP2E1 mRNA levels were also analyzed, although it is recognized that this enzyme is regulated by mRNA stabilization, increased mRNA translatability, and decreased protein degradation. Once evaluated, we assessed the potential of a significant number of putative drug candidates (n = 71) to cause induction of the major rat P450s (CYP1A, 2B, 2E, 3A, and 4A), and these data along with the positive controls are reported here.

Materials and Methods

Materials. β-Naphthoflavone, sodium phenobarbital, dexamethasone, and clofibric acid were obtained from Sigma-Aldrich Co. Ltd. (Poole, UK). The RNeasy 96 total RNA Isolation Kit, RLT lysis buffer, Hotstart Taq Mastermix, and MinElute PCR purification kit were obtained from Qiagen Ltd. (Crawley, UK). DNase I (RNase-free, 2 units/μl) and DEPC-treated water were obtained from Ambion (Camb, UK). Ribogreen RNA Quantitation Kit was purchased from Molecular Probes Inc. (Eugene, OR). Oligo(dT)12–18 primer (0.5 μg/μl) kit, 10 mM 2′-deoxynucleoside 5′-triphosphate mix, the Superscript II Reverse Transcriptase (RT) (200 μl/kit) containing 0.1 M dithiothreitol and 5× first-strand buffer, and 4% E-Gel were purchased from Invitrogen Ltd. (Paisley, UK). Oligonucleotide fluorogenic probes, TaqMan Universal PCR Mastermix, and 96-well optical reaction plates with optical adhesive films were purchased from Applied Biosystems (Warrington, UK). Oligonucleotide primers were purchased from Sigma-Genosys Ltd. (Camb, UK), with rat genomic DNA (0.2 μg/μl) obtained from Bioline (London, UK). DNA polymerase promoter primers T7 and SP6 were purchased from Promega (Southampton, UK). All other reagents used in this study were of molecular biology or analytical grade and purchased from commercial sources.

Animals. Male Sprague-Dawley rats (200–250 g) were obtained from Charles River (Margate, Kent, UK), and this strain of rat was chosen because of the considerable knowledge within GloxSmithKline of the strain’s general pathology and response to a wide variety of drugs. Rats were housed at 19–23°C with a 12-h light/dark cycle and had free access to food and water. Animals were allowed to acclimatize to their environment for at least 5 days before drug administration.

Administration of P450 Inducers. To evaluate the TaqMan protocol outlined in this report, rats were administered prototypical P450 inducers by daily intraperitoneal injections of β-naphthoflavone (BNF; CYP1A1 inducer at 80 mg/kg in corn oil), phenobarbital (PB; CYP2B1 inducer at 80 mg/kg in saline), dexamethasone (DEX; CYP3A inducer at 150 mg/kg in corn oil), or clofibric acid (CLO; CYP4A inducer at 200 mg/kg in corn oil) for 3 consecutive days. Three rats were used in each group, with control animals being administered with vehicle only (a separate group for corn oil and saline controls). Animals were killed 24 h after their last dose, and the livers were immediately excised. Liver samples were placed in foil bags and snap-frozen before drug administration.

Drug Candidate Studies. Once evaluated, the TaqMan protocol was used to investigate the induction potential of a number of putative drug candidates (n = 71). Livers were typically collected from 4- to 7-day rat toxicology studies, as described above, after administration of a vehicle control and three drug concentrations (n = 3 male and/or female Sprague-Dawley rats/dose group).

Extraction of Total RNA from Liver. Liver homogenates (7% (w/v)) were prepared in RLT lysis buffer using a polytron homogenizer (20 × 1000 rpm, <30-s bursts at room temperature) from a representative portion of liver (approximately 1 g of tissue). Although a fraction of this quantity was required for the TaqMan protocol, it ensured a homogeneous sample of liver was analyzed. The homogenates were further diluted with RLT lysis buffer to give a final homogeneate preparation of 0.28% (w/v). An equal volume of ethanol was then added to the 0.28% (w/v) homogenate using a Zymark rapidplate robot (Zymark Corp., Hopkinton, MA). Total RNA was extracted from quadruplicate samples of the liver homogenate (600 μl of homogenate/ethanol mix per extraction column) using a RNeasy 96 kit on a Qiagen 3000 robot with vacuum, according to the manufacturer’s protocol. The RNA was eluted from each column with two aliquots of 140 μl of RNase-free water, each aliquot being spun at ca. 5600g for 4 min at room temperature.

DNaSe Treatment. Total RNA aliquots (approximately 1 μg) were treated with a DNase bulk mix, which consisted of Rnase-free DNase I (0.5 units), 10× DNase I buffer (10 μl), and DEPC-treated water (0.75 μl). The DNase-treated plate was incubated in a thermocycler at 37°C for 10 min, followed by 75°C for 5 min. This eliminated any contaminating genomic DNA and allowed gene(s) to be quantified using a rat genomic DNA standard curve (described later). Any remaining total RNA left untreated was stored at ca. −80°C.

Quantitation of Total RNA. The quantity of total DNA (DNase-treated) was determined by measurement of a fluorescent RNA-binding probe, Ribogreen, according to the manufacturer’s RNA quantitation kit instructions (Molecular Probes) (Jones et al., 1998). The Ribogreen assay was prepared on a Qiaegen 3000 robot using a ribosomal RNA standard curve (final concentration ranging from 15.62 to 1000 ng/ml). One hundred microliters of the fluorescent RNA-binding probe (diluted 1 in 200) was added to both the RNA isolates and RNA standards (5 μl of RNA or RNA standard in 95 μl of TE buffer). The fluorescence in each well was measured using a Polara plate reader (BMG Labtechnologies Ltd., Offenburg, Germany) set at 480-nm excitation and 520-nm emission. The concentration of the total RNA isolates was interpolated from the standard curve.

No-Amplification Controls. A no-amplification control plate was set up to evaluate the efficiency of the DNase process and to test for the degree of any contaminating genomic DNA in the RNA sample. Aliquots of DNase-treated total RNA (5 μl) were not subjected to cDNA synthesis (were not reverse-transcribed) but added directly to a TaqMan reaction mix and quantified by real-time qRT-PCR (TaqMan) as described later.

cDNA Synthesis. First-strand cDNA synthesis was performed on the remaining DNase-treated total RNA, which was reverse-transcribed using Oligo(dT)12–18 primer (0.5 μg), heated to 70°C for 10 min with immediate cooling on ice and Superscript II bulk mix (200 units of Superscript II RNase H− reverse transcriptase, 2′ deoxynucleoside-5′-triphosphate mix, dithiothreitol, and 5× first-strand buffer), and heated to 42°C for 50 min followed by 70°C for 15 min, according to the manufacturer’s instructions. In addition, duplicate no-template control samples were run in identical conditions as those described above, except the RNA sample was substituted for DEPC-treated water. All resulting cDNA was subjected to PCR amplification using TaqMan technology.

Principles of TaqMan Technology (Theory). Relative differences in mRNA expression (i.e., target gene expression) were assessed based on different PCR cycling threshold (Ct) values, which were determined by the PE Biosystems ABI 7900 sequencer software (Sequence Detection System version 2.0; Perkin Elmer-Applied Biosystems, Foster City, CA). A difference of one Ct value is equivalent to 2-fold difference in gene expression (an exponential relationship). Quantitation of the initial number of copies of mRNA of the target gene detected was calculated from the experimental Ct value by interpolation from the standard curve generated using known amounts of rat genomic DNA (gDNA).

Primers and Fluorogenic Probe Design. The primers and probes were designed to a definitive gene sequence in a single exon to allow the amplification of a gDNA standard curve (contrary to Gibson et al., 1996) and as close as possible to the 3′-coding region of the target gene sequence obtained from GenBank (http://www.ncbi.nlm.nih.gov/GenBank). Regions that satisfied this criterion were then put into Primer Express, and primer and probe sequences were designed according to the parameters incorporated in the Primer Express software (versions 1 and 2; PE Applied Biosystems). Optimal primers and probes were 20 to 80% GC-rich between 9 to 40 bases in length, primer Tm values were 58–60°C (<2°C difference between forward and reverse primer), with probes ideally having a Tm 10°C higher than the primer Tm. The probe...
selected was close to the 3'-end of the forward primer had more bases of Cs than Gs and with <4 contiguous Gs in the strand [as recommended by the manufacturer and described by Livak et al. (1995)]. Probes with a G at the 5'-end were avoided as this has been shown by the manufacturer to exert a quenching effect on the reporter fluorochrome. Finally, the amplicons [typically 70–100 base pairs (bp) long] were homology-searched to ensure that they were specific for the target mRNA transcript using an National Center for Biotechnology Information BLAST search. The final TaqMan probe/primer sequences designed to be specific for rat CYP1A1, 1A2, 1B1, 2B1, 2B2, 2E1, 3A2, 3A23, 4A1, and the housekeeping gene glyceraldehyde-3-phosphate (GAPDH) are summarized with their sequences and corresponding accession number of GenBank.

**Determination of the specificity of the primer set**

To confirm the specificity of the primer set, the PCR products were analyzed by agarose gel electrophoresis and the gene product was identified by sequencing using a Hotstart Taq Polymerase Kit (Qiagen) and sequencing reagents. After sealing the plate with an optical adhesive cover, the thermocycling conditions were initiated with an enzyme activation step of 50°C for 2 min with an enzyme activation step of 95°C for 10 min, followed by 40 PCR cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

Purification of the PCR product (10 μl) was performed using a MinElute PCR purification kit (Qiagen) according to the manufacturer’s protocol. The DNA was eluted with 10 μl of PCR-grade water by centrifugation at ca. 5600 g for 1 min at room temperature.

Once purified, the PCR product was loaded into wells of 4% E-Gel (5 μl of PCR product in 15 μl of PCR-grade water per well) and run at 75 V for approximately 30 min in parallel with a 10-bp DNA ladder (2 μl of 10 bp of DNA in 18 μl of PCR-grade water). The molecular weight of the amplified product (seen as a single band with a transilluminator) was identified using the DNA ladder.

TABLE 1

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<td>*RP</td>
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FP, forward primer; RP, reverse primer.
* Sequence designed to the reverse compliment.
* GenBank.

**PPARα and PPARγ**

The expression of PPARα and PPARγ doi...
standard curve calculation was based on the assumption that the PCR reaction was exhibiting 100% amplification efficiency (i.e., the PCR products will double with each cycle during exponential amplification) (Leutenegger et al., 2001). The slope of the gDNA standard curve plot was used to estimate PCR efficiency (E) according to the equation $E = \frac{1}{\text{slope}}$ (calculation for real-time PCR efficiency is detailed on http://www.gene-quantification.info/; described by Rasmussen, 2001). An ideal PCR reaction would exhibit a standard curve slope of $\sim 3.32$, with a PCR efficiency of $E = 2$.

Quantitation of Gene Expression. The specific gene expression was quantitatively detected for the following genes: CYP1A1, 1A2, 2B1, 2B2, 2E1, 3A2, 3A23, 4A1, and GAPDH. Data have been reported as fold induction of mRNA by 2500- and 23-fold, respectively, with all other P450 genes determined being relatively unaffected after 3 days of treatment. The difference in magnitude of fold induction between CYP1A1 and CYP1A2 is largely due to their relative constitutive expressions, with CYP1A1 being extremely low compared with a high expression of CYP1A2 but also because CYP1A1 was the major inducible CYP1A enzyme.

Induction Potential of Putative Drug Candidates/NCEs. The ability of prototypical inducers to induce P450 gene expression was examined in ex vivo rat liver and was the basis for validating the TaqMan technique. Data for the fold induction of control after treatment with the classic inducers BNF, PB, DEX, and CLO are shown in Table 3.

BNF (80 mg/kg/day) specifically induced CYP1A1 and CYP1A2 mRNA by 2500- and 23-fold, respectively, with all other P450 genes determined being relatively unaffected after 3 days of treatment. The difference in magnitude of fold induction between CYP1A1 and CYP1A2 is largely due to their relative constitutive expressions, with CYP1A1 being extremely low compared with a high expression of CYP1A2 but also because CYP1A1 was the major inducible CYP1A enzyme.

As expected from the literature (Huss and Kasper, 1998), the two rat CYP3A genes were differentially responsive to induction by the glucocorticoid CYP3A inducer DEX (150 mg/kg/day). Specifically, there was a marked increase in the CYP3A23 mRNA (the major glucocorticoid-responsive rat CYP3A gene), which corresponded to a 59-fold induction of control. Whereas CYP3A23 was classified as the “male-specific” gene and represents the predominant CYP3A form in control liver, it was less responsive to DEX (7-fold induction of control). CYP2B mRNA expression was also induced by DEX, with CYP2B2 being induced to a greater degree by DEX than PB (mean -fold induction of control was 49 and 50 for CYP2B1 and CYP2B2, respectively). This up-regulation of CYP2B is to be expected given the cross-talk that is reported between the intracellular “orphan” nuclear receptors PXR and the constitutively activated/androstane receptor CAR, which mediates the induction of CYP2B by xenobiotics (Waxman, 1999; Honkakoski et al., 2003).

Prototypical P450 Inducers. The ability of prototypical inducers to induce P450 gene expression was examined in ex vivo rat liver and was the basis for validating the TaqMan technique. Data for the fold induction of control after treatment with the classic inducers BNF, PB, DEX, and CLO are shown in Table 3.

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Administration of the peroxisome proliferator clofibrate acid (200 mg/kg) up-regulated mRNA levels of CYP4A1 by 16-fold compared with the vehicle-treated control group. There was also evidence of clofibrate acid having an effect on other P450 enzymes. In particular, CYP2B2 increases were comparable with those seen with PB and are in agreement with other reported studies (22-fold induction of control) (Bars et al., 1993; Pan et al., 2002).

Induction Potential of Putative Drug Candidates/NCEs. After completion of the TaqMan assay evaluation, the methodology was...
Corresponding catalytic induction data for the same NCE, representing 2- to 4-fold induction as triangles, and >4-fold induction as a square. NCEs with no comparable catalytic data are shown as clear circles. The maximum response by the prototypical CYP1A inducer BNF (80 mg/kg/day) is shown by a star. The compound with known TDI properties is indicated by an arrow.

studied catalytically, any potential inductive effect of these drug candidates may be masked.

Discussion

The examination of hepatic mRNA levels of specific P450 gene expressions using TaqMan technology has a number of advantages over conventional methods for assessing the potential of a drug to cause P450 induction. The key advantages of this method that makes it both precise and reproducible (Gibson et al., 1996; Bustin, 2000; Ginzinger, 2002) are features, such as it is a completely homogenous assay with a specific target gene being detected. It is further enhanced by the detection system being fully automated with a 96-well sample format, which ultimately leads to an accelerated throughput. The TaqMan method is also exquisitely sensitive, being able to amplify small amounts of mRNA in contrast to commonly used methodologies, which typically require relatively large amounts of total RNA and are unsuitable for high throughput and usually only semiquantitative in nature.

The TaqMan technology also has the advantage of quantifying changes in gene expression over a wide dynamic range, due in part to its ability to measure low levels of gene expression. For example, CYP1A1 is constitutively expressed at extremely low levels in the rat liver and is poorly detected (if at all) using conventional Western blots. In addition, CYP3A levels in the female rat quantified by Western blots. In addition, CYP3A levels in the female rat quantified by
catalytic activity are typically low. However, using qRT-PCR in this study, the basal expression of CYP1A1, CYP3A2, and CYP3A23 mRNA was readily measured.

To investigate the applicability of qRT-PCR to measure P450 induction, the effects of prototypical inducers on the major rat P450 genes were measured to evaluate the TaqMan protocol. The study clearly demonstrated that there is a marked increase in the mRNA levels of the induced P450 enzyme after administration of the corresponding prototypical inducer. The maximum fold induction for individual P450 enzymes varied widely, ranging from approximately 7-fold for CYP3A2 to 2500-fold for CYP1A1. Note that the large differences in the potential magnitude of induction across the P450 genes result in cases where increases in expression may be significant for one gene (e.g., 20-fold for 3A23 or 16-fold for CYP4A) but meaningless for another (e.g., 20-fold is minor for 1A1). These results were similar to those reported by Goodsaaid et al. (2003). There was also evidence of induction of both CYP2B by DEX and CYP3A by PB. This interplay or cross-talk between the “orphan” nuclear hormone receptors, suggesting that they can activate each other’s target genes, is now well documented (Handschin and Meyer, 2003; Honkakoski et al., 2003).

During the evaluation of the TaqMan protocol, another important consideration was whether to normalize to a “housekeeping” gene. Historically, quantitative gene expression assays were often referenced to an internal control, such as the ubiquitously expressed housekeeping gene GAPDH, because it is present in all nucleated cell types as a key enzyme in glycolysis. However, recent publications have shown that housekeeping genes such as GAPDH are not ideal as an internal reference, in view of the fact that their expression levels can be up- or down-regulated in response to experimental conditions and increased drug exposure (Schmitgen and Zakraskjek, 2000). In this study, to account for the differences in RNA loading between the samples, the data were normalized to accurately quantitated total RNA and not the housekeeping gene. However, GAPDH mRNA levels were measured in all samples to provide a marker of mRNA integrity. Thereafter, ex vivo measurements of P450s in livers from rats were taken to estimate the inductive potential of putative drug candidates undergoing toxicological evaluation. To evaluate the potency of the NCEs ability to up-regulate P450 gene expression, the prototypical inducer response was considered the maximal potency indicator. It was also assumed that a change in mRNA levels would be indicative of an eventual change in P450 enzyme levels. Generally, there was good agreement between the up-regulation of P450 mRNA levels as determined by TaqMan and previously generated P450 enzyme activities, although not in a directly proportional manner. However, some NCEs seemed to have the potential to induce P450 gene expression but did in fact have little inductive effect when measured by conventional catalytic methods. A number of these candidate drugs were later established to cause a time-dependent loss in P450 enzyme activity. The few remaining NCEs that demonstrated increases in mRNA levels without a functional change and that could not be explained by the effects of time-dependent inhibition were often identified as inducing other P450 enzymes by both TaqMan and catalytic analysis. Thus, in the absence of any supporting TaqMan data, we may underpredict the inductive drive these compounds have on the liver. Although inhibition and induction may be considered to have canceled one another out many other genes and proteins are induced by, for example, PXR ligands that would not be subject to ameliorating inhibitory effects by the compound.

Having established the TaqMan methodology to examine the ability of a drug to modulate P450 gene expression using rat ex vivo livers, it can be adapted to measure mRNA changes in other tissue (Medhurst et al., 2000), species (Pan et al., 2000), in vitro systems, and other gene targets. Many studies have been published using cultured hepatocytes as a model system to evaluate the induction of P450 enzymes of rat (Buczynski et al., 2001), mouse (Pan et al., 2000), and human (Bowen et al., 2000; Rodriguez-Antona et al., 2000; Perez et al., 2003), with more limited articles using cultured precision-cut rat liver slices (Pan et al., 2002; Meredith et al., 2003). Although attention is primarily placed on P450-mediated drug interactions, the importance of other mechanisms as a cause of DDIs is slowly being recognized. In particular, nonoxidative pathways such as the response of UDP-glucuronosyltransferases to inducers (Soars et al., 2004) and also the efflux transporter proteins, e.g., P-glycoprotein (Lin and Yamazaki, 2003) and the multidrug resistance-associated protein 2 Mrp2 (Johnson and Klaassen, 2003) are being recognized.

We have proven that TaqMan is a powerful tool in measuring changes in the expression of P450 genes in rats ex vivo and is a technique that can be used to study the potential of a drug to induce drug-metabolizing enzymes as part of their safety evaluation (Worboys and Carlile, 2001). Thus, evaluating drug-mediated induction at an early stage of development can aid the selection of a drug candidate over structurally similar backups and facilitate in interpreting the toxicokinetics and toxicology as well as indicating a potential risk of clinical DDIs.

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References

FIG. 2. Fold induction of CYP3A2/3A23 mRNA as determined by TaqMan for 71 NCEs and CYP3A catalytic activity. All data points represent fold induction of CYP3A2/3A23 mRNA as determined by TaqMan. Comparable catalytic CYP3A data (6β-hydroxy testosterone) for the same NCE, representing <2-fold induction, are shown as solid circles, 2- to 4-fold induction as triangles, and >4-fold induction as a square. NCEs with no comparable catalytic data are shown as clear circles. The maximum response by the prototypical CYP3A inducer DEX (150 mg/kg/day) is shown by a star. Compounds with known TDI properties are indicated by arrows.


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