Species and strain differences in drug metabolism in liver and intestine
Martignoni, Marcella

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Chapter 3

An *in vivo* and *in vitro* comparison of CYP induction in rat liver and intestine using slices and quantitative RT-PCR

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Abstract

Xenobiotics, including drugs, can influence cytochrome P450 (CYP) activity by upregulating the transcription of CYP genes. To minimize potential drug interactions, it is important to ascertain whether a compound will be an inducer of CYP enzymes, early in the development of new therapeutic agents. The present in vivo and in vitro studies demonstrate the use of liver and intestinal slices as an in vitro model to predict potential CYP induction in vivo.

Rat liver slices and intestinal slices were incubated, for 24h and 6h respectively, with β-naphthoflavone (βNF), phenobarbital (PB) or dexamethasone (DEX). In rat liver slices also the enzyme activity was investigated after 48h incubation. In addition, in an in vivo study, rats were treated with the same compounds for three days. In vivo and in vitro CYP mRNA levels were measured by using real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). In both rat liver and intestinal slices, βNF significantly induced CYP1A1, CYP1A2 and CYP2B1 mRNA levels. PB significantly induced CYP2B1. In liver slices a minor induction of CYP1A1 and CYP3A1 by PB was observed, whereas DEX significantly induced CYP3A1, CYP2B1 and CYP1A2 mRNA levels. The induction profiles (qualitative and quantitative), observed in vivo and in vitro are quite similar. All together, these data demonstrate that liver and intestinal slices are a useful and predictive tool to study CYP induction.

Introduction

Cytochrome P450 enzymes form a gene superfamily that are involved in the metabolism of a variety of chemical diverse substances, ranging from endogenous compounds to xenobiotics, including drugs, carcinogens, and environmental pollutants. Although the regulation of CYP enzymes is still not completely understood, it is well known that several CYP genes can be induced by drugs. Induction of CYPs by a drug is an undesirable drug interaction, as it can lead to enhanced metabolism of a co-administered drug that is a substrate of the induced CYP isoform. The interaction is less likely to result in a safety issue, but may impact efficacy of one or more medications [1]. In contrast to the clinic, CYP induction is a rather common finding in preclinical safety studies [2]. This is partly due to the use of doses up to orders of magnitude greater than the clinical dose. In addition, in preclinical studies several non-genotoxic CYP inducers have been shown to induce liver tumours in rodents [3]. Despite that it is well known that species differences in CYP induction exist, making the extrapolation from animals to humans unreliable, it is not uncommon that regulatory agencies request information on CYP induction potential of new molecular entities obtained from laboratory animals studies. However, these studies require large numbers of animals, a significant amount of test compound and are time and resource demanding. Therefore, it is desirable to have in vitro models available to address potential CYP induction of drug candidates. Various in vitro models for assessing enzyme induction in the liver have been described and include precision-cut liver slices [4,5], primary hepatocytes [6,7], and reporter gene constructs [8,9]. In primary hepatocytes or precision-cut liver slices, CYP induction can be evaluated by measurements of changes in CYP mediated enzyme activities, Western
CYP induction in rat liver and intestine slices

 blot analyses for gross changes in CYP protein levels and by determination of CYP mRNA levels. Historically, the liver slice model has not been widely used for CYP induction assessment as compared to primary hepatocytes. However, the successful use of liver slices for CYP induction, determined at the level of enzyme activity, apoprotein and/or mRNA expression, is well documented [10,11].

In the recent years, the intestine has been more and more investigated because of its significantly contribution to the biotransformation of xenobiotics [12], and consequently, its implications on clinical interactions [13]. As information on CYP induction in the intestine is very limited, we felt that it would be useful to investigate the potential of well-known CYP inducers to upregulate CYP genes levels in the intestine.

Analogous to precision-cut liver slices, an in vitro model to study the intestine has been developed recently [14]. Until now, slices from intestinal tissue have only been used rarely and never for studying enzyme induction. Examples with intestinal slices regard only the metabolism of drugs in human intestinal slices [15-17] and the metabolic activation in rat colon slices [18].

An attractive approach for maximizing the amount of induction data that can be obtained from liver and intestine slices is the determination of CYP mRNA levels by using real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) methodology (Taqman®). This methodology requires small amounts of total RNA, is suitable for high-throughput and is capable of monitoring signal amplification during the course of each PCR cycle, allowing quantitative measurement.

The objective of this study was to evaluate the effect of some well described CYP inducers on some selected CYP isoforms in cultured liver and intestine slices using the RT-PCR methodology in combination with enzyme activity measurements. To assess predictability of the in vitro approach, results were compared with induction data obtained from in vivo experiments.

Materials and Methods

Chemicals. The following compounds were obtained from the sources indicated: sodium phenobarbital (PB), dexamethasone (DEX), β-naphthoflavone (β-NF), D-glucose, insulin, hydrocortisone-21 hemisuccinate, gentamicin sulphate, 7-ethoxyresorufin, resorufin, NADPH, low melting agarose (type VII-A), corticosterone and DMSO were from Sigma-Aldrich (St. Louis, MO, USA); testosterone was from Fluka (Buchs, Switzerland); testosterone metabolites were from Ultrafine Chemicals (Manchester, UK). L-Methionine, fungizone, 5 x first strand buffer, RnaseOUT, Superscript, dATP, dGTP, dCTP, dTTP, random primers, DTT, BSA and Williams’ Medium E were obtained from Gibco (Paisley, Scotland, UK). Fetal calf serum was from Biological Industries Co (Kibbutz Beit Haemek, Israel). RNA 6000 Nano Assay was from Agilent Technologies (Palo Alto, CA, USA). RiboGreen® RNA Quantitation kit was from Molecular Probes (Eugene, OR, USA). Qiagen Rneasy® mini and micro kit were from Qiagen Ltd (Crawley, UK). RNAlater™ was from Ambion (Austin, TX, USA). TaqMan® Universal PCR Master Mix Reagents, SYBR® Green PCR Master Mix Reagents and TaqMan® probes were obtained from Applied Biosystems (Foster City, CA, USA). The oligonucleotide primers were synthesized by Nerviano Medical Sciences Labs (Nerviano, Italy), except for 18S rRNA that was obtained form Applied Biosystems (Foster City, CA, USA).

Animals. Male Wistar rats from Charles River (Como, Italy) were maintained under a 12-h light/dark cycle in standard cages and bedding with free access to standard commercial food pellets and tap water. Groups of three male rats (200-220 g) were treated both i.p. and p.o. with β-NF (80 mg kg⁻¹ day⁻¹, corn oil), PB (80 mg kg⁻¹ day⁻¹, saline) and DEX (100 mg kg⁻¹ day⁻¹, corn oil) for three days. Control animals received only the vehicle (corn oil or saline). Animals were
CYP induction in rat liver and intestine slices

sacrificed 24 h after the last treatment. Samples (about 30 mg) of the livers from rats treated i.p., and duodenum, ileum and colon from rats treated p.o. were stored in RNealater™ and kept at 4°C before extraction of RNA.

Liver and intestine slice preparation. After i.p. anaesthesia with sodium tiopental 100 mg/kg (rat), the livers and the first 25-30 cm intestine (thus mainly duodenum of the small intestine) from rats were excised and stored in ice-cold Williams’ Medium E until use (max. 0.5 h). Liver slices (diameter 8 mm) were prepared in ice-cold Williams’ Medium E that was oxygenated with 95% O2 / 5% CO2 and supplemented with extra glucose (25 mM) and gentamicin (50 µg/ml), using a Krumdieck tissue slicer [19]. The slices obtained were subsequently stored in ice-cold Williams’ Medium E until use (within 0.5 h after the preparation).

Agarose filled and embedded slices were prepared as described elsewhere [14]. Shortly, the excised 25 cm of the small intestine was first cut in two parts that were subsequently ligated on one side. These parts were then filled with 3% (w/v) low melting agarose solution in 0.9% (w/v) NaCl at 37°C and allowed to gel in ice-cold Williams’ Medium E. The agarose-filled intestine was cut in 1 cm parts and these were embedded in the agarose solution at 37°C using the Tissue Embedding Unit from Alabama R&D (Munford, AL, USA) and allowed to gel so that agarose gel cylinders of a diameter of 16 mm were formed. These cylinders were used to prepare precision-cut intestinal slices, with a diameter of 16 mm and a thickness of 0.25 mm, using a Krumdieck tissue slicer as described above for liver slices. When the slices were transferred to the incubation plates, the agarose surrounding the slices was separated from the slice, so that only the ring of intestinal tissue (diameter about 3-5 mm) was used.

Incubations. Liver and intestinal slices were individually incubated in 6 well culture plates (Falcon, France) in respectively 2 or 3.2 ml Williams’ Medium E under 95% O2 / 5% CO2 atmosphere (one slice per well) at 37°C while shaking horizontally at 90-100 times min-1. For each experiment, the slices were incubated in triplicate. The culture medium consisted of Williams’ Medium E containing glucose (25 mM), gentamicin (50 µg/ml), insulin (1 µM), hydrocortisone (10 µM), fungizone (2.5 µg/ml), methionine (0.5 mM) and fetal calf serum (5%).

After 1 hour, treatment was started by replacing the culture medium with medium containing the required concentrations of the test compounds (βNF 50 µM, PB 1 mM and DEX 100 µM) [20,21]. The test compounds were dissolved in DMSO and added to the culture medium so that the final DMSO concentration was 0.1% (v/v) in all wells including the control incubations. After culture for 48 hours, 7-ethoxyresorufin O-deethylase and testosterone hydroxylase activities were investigated using three homogenates of three rat liver slices for each substrate, employing substrate concentrations of 5 µM and 250 µM, respectively. After culture for 6 hours (intestinal slices) or 24 hours (liver slices), three slices from each treatment were transferred to RNealater™ and stored at 4°C until RNA extraction.

Testosterone metabolism by rat liver slices. Liver slices were homogenized in 3 ml of 0.1 M phosphate buffer using a Potter homogeniser. An aliquot of the homogenate (400 µl) was incubated with 250 µM testosterone and NADPH (1 mM) for 30 minutes. Testosterone was administered to the incubation medium as a 200-times concentrated solution in methanol. Reactions were terminated, analyzed and quantified as described earlier [19].

Cytochrome P4501A1/1A2 ethoxyresorufin-O-deethylase (EROD) assay. Liver slices were homogenized as described above for testosterone metabolism. An aliquot of the homogenate (160 µl) was incubated with 7-ethoxyresorufin (5 µM) and NADPH (1 mM) for 10 minutes. 7-ethoxyresorufin was administered to the incubation medium as a 200-times concentrated solution in DMSO. Reactions were terminated by addition of equal volume of acetonitrile and the fluorescence was immediately read (λex = 530, λem = 590 nm). The quantification of resorufin present was performed by comparing with a resorufin standard curve.

RNA preparation from liver and intestinal slices and tissue. Total RNA was extracted from cultured liver and intestinal slices and from rat liver and intestinal samples using Qiagen Rneasy® mini or micro (for intestinal slices) kit. The quality of the isolated RNA was assessed using the RNA 6000 Nano Assay and the Agilent 2100 bioanalyzer (Palo Alto, CA, USA). The bioanalyzer uses gel electrophoresis in the confines of a microfabricated chip and highly sensitive laser induced fluorescence detection using an intercalating dye, which is added to the polymer. RNA concentration was determined using a RiboGreen™ RNA Quantitation kit.
Reverse transcription. The reaction mixture (final volume 40 µl) was prepared with final concentrations as follows: 1 x first strand buffer, 64 units RnaseOUT, 200 units Superscript, 0.6 mM of dNTP (dATP, dGTP, dCTP and dTTP), 0.75 µg random primers, 10 mM DTT and 16 ng BSA. To this mixture 1 µg of total RNA extracted was added. The reverse transcription reaction was performed for 10 min at 25°C, 60 min at 42°C and 30 min at 37°C.

Design of primers and probes. The cDNA sequences of rat CYP1A1, CYP1A2, CYP2B1, CYP3A1 and β-actin were obtained from GenBank accession no. X00469, accession no. NM012541, accession no. J00719, accession no. L24207 and accession no. NM031144. PCR primers and probe sequences were designed using PrimerExpress software (Applied Biosystems) and shown in Table 1. Nucleotide primers and probe sequences were checked against the NCBI BLAST database to ensure specificity for the selected gene.

Real-time quantitative RT-PCR. Real-time quantitative RT-PCR was performed, employing either a TaqMan 7900 sequence detector system (Applied Biosystems) or an iCycler iQ™ Real-time PCR detector system (Bio-Rad). The PCR reaction was performed in either a 384 well plate or a 96 well plate. The reaction mixture (13.5 µl) was added in each well to give the following concentration: 1 x master mix reagents, 200-300 nM of each primer and 200 nM probe for each CYP mRNA assay, except for CYP3A1 for which SYBRGreen was used. cDNA (1.5 µl) was added to each well and the final volume was 15 µl. The thermal cycle condition was 50°C for 2 min, 95°C for 10 min to activate AmpliTaq Gold DNA polymerase, denaturation at 95°C for 15 sec and anneal/extension at 59°C for 1 min (40 cycles). Quantitative PCR for 18S rRNA or β-actin was also performed to normalize for RNA loading. Due to the interference of the probe labels, these reactions were performed separately. We found similar results for 18S rRNA and β-actin, suggesting that both of them can be used.

Statistical analysis. Differences among group mean values were assessed using two-tailed, two sample t test, and assuming equal variance. A difference of $P < 0.05$ was considered statistically significant.

Results

Quantitative RT-PCR. Taqman primer and probe sets were designed for rat CYP1A1, CYP1A2, CYP2B1 and CYP3A1. Total RNA was extracted from liver and intestinal samples and slices (control and CYP inducer treated). cDNA was prepared by reverse transcription and the levels of CYP mRNAs were determined by real-time quantitative RT-PCR methodology, using primers and probes listed in Table 1. The levels of all CYP mRNA were normalized with the expression of 18S or β-actin, which was amplified in a separate reaction.
Table 1. Taqman® primer and probe sequences of rat CYP mRNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers/Probes</th>
<th>5' —&gt; 3' Sequence</th>
<th>Amplicon Size (bp)</th>
</tr>
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<tr>
<td>CYP1A1</td>
<td>Forward</td>
<td>GAAGAAGCTAATCAAAGAGCACTACAGG</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>CAATGCTCAATGAGGCTGTCG</td>
<td></td>
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<tr>
<td></td>
<td>Probe</td>
<td>CATTGAGAAGGGCCACATCCGGG</td>
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<tr>
<td>CYP1A2</td>
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<td>TCCACATTCCCAAGGAGTGCT</td>
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<tr>
<td></td>
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<td>TAAGAAACCGCTCTGGGCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>AGTGGCAGGTCACCATGATGAGAAGCA</td>
<td></td>
</tr>
<tr>
<td>CYP2B1</td>
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<td>TGAAAACCTCTGAATCTCGTGGATA</td>
<td></td>
</tr>
<tr>
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<td>Probe</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>TCGGGTTGTTGAGGGAATCA</td>
<td></td>
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</table>

**Induction studies in rats.** *In vivo.* Treatment of rats with βNF resulted in a marked induction of hepatic CYP1A1 (~1200-fold, Fig. 1A) and in less prominent induction in duodenum (~10-fold, Fig. 1A). In ileum a 3-fold induction was observed, while in colon CYP1A1 was detected only after βNF treatment. βNF strongly induced CYP1A2 in intestine (~135-fold, Fig. 1B) and although less prominent, also induced hepatic CYP1A2 (~20-fold, Fig. 1B). βNF also induced CYP1A2 in ileum but the level of induction can not be evaluated because of the lack of detection in untreated animals. Hepatic and intestinal CYP2B1 and CYP3A1 were not affected by βNF treatment. PB significantly induced hepatic CYP2B1 (~3700-fold, Fig. 1C) and to a lesser extent also CYP1A1 and CYP3A1 in the liver (13-fold and 11-fold, Fig. 1A and 1D). Although less prominent, PB induced CYP2B1 in duodenum and ileum (21-fold and 8-fold respectively, Fig. 1C). Levels of CYP3A1 in the liver were significantly increased after treatment with DEX (35-fold, Fig. 1D) and a less pronounced, but significant induction was also observed towards hepatic CYP2B1 by DEX (15-fold, Fig. 1C). DEX also induced CYP3A1 in duodenum and ileum, but the level of induction could not be evaluated because of the lack of detection in untreated animals.
CYP induction in rat liver and intestine slices

Figure 1. Effect of classic inducers on expression of CYP mRNAs in rat liver and intestine assayed by real-time PCR. After treatment with βNF, PB and Dex for three days, the levels of CYP1A1 (A), CYP1A2 (B), CYP2B1 (C) and CYP3A1 (D) were investigated. Total RNA (3.5 ng) was loaded for one-step real-time RT-PCR assay and the levels of CYP mRNAs were normalized for RNA loading by taking the ratio of 18S and expressed as fold induction over untreated animals. Results are the mean ± SEM for groups of three rats. Values significantly different from control: *p < 0.05.

Induction by βNF in ileum, but not quantifiable because of lack of signal from untreated animals. See results for comments

Induction by DEX in duodenum and ileum, but not quantifiable because of lack of signal from untreated animals. See results for comments
In vitro. Precision cut rat liver and intestinal slices were cultured in the presence of either 50 µM βNF, 1 mM PB or 100 µM DEX. Their viability was judged by the quality of the RNA. Total RNA was extracted from the preparations and analysed for integrity by gel separation of the 18S and 28S bands using an Agilent 2100 Bioanalyser. As shown in Figure 2, total RNA in slices incubated for 6 and 24 hours showed a typical, intact RNA pattern, comparable to tissue that was not incubated.

In liver slices, after 24 hours of βNF exposure, mRNA levels of CYP1A1, CYP1A2 and CYP2B1 were ~500-, 5- and ~ 50-fold increased (Fig. 3A, 3B and 3C). PB induced CYP2B1 (~600-fold, Fig. 3C) and slightly CYP1A1 and CYP3A1 (3-fold, Fig. 3A and 3D), but did not significantly increase CYP1A2 mRNA level. A marked induction of CYP2B1 (~600-fold) and CYP3A1 (~70-fold) was observed after treatment with DEX (Fig. 3C and 3D). CYP1A2 was also slightly induced by DEX (2-fold, Fig. 3B). In rat intestinal slices, βNF strongly induced CYP1A1 (~ 300-fold, Fig. 3A) and to a less extent also CYP2B1 (~2-fold, Fig. 3C) after 6 hours treatment. βNF also induced CYP1A2 in intestinal slices, but the level of induction could not be evaluated because of the lack of signal in untreated slices. PB significantly induced CYP2B1 (~ 4-fold, Fig. 3C). DEX did not induce any CYP mRNA.

Figure 2. Total RNA integrity assessment form slices incubated for 0, 6 and 24h. The fluorescence peaks at 40-41 and 47-48 sec gel electrophoresis run time represent the 18S and 28S bands, respectively.
Figure 3. Effect of classic inducers on expression of CYP mRNAs in rat liver and duodenum slices, assayed by real-time PCR. After treatment slices with βNF (50µM), PB (1mM) and Dex (100µM) for either 6 hours (duodenum slices) or 24 hours (liver slices), the levels of CYP1A1 (A), CYP1A2 (B), CYP2B1 (C) and CYP3A1 (D) were investigated. Total RNA (3.5 ng) was loaded for one-step real time-RT-PCR assay and the levels of CYP mRNAs were normalized for RNA loading by taking the ratio of 18S and expressed as fold induction over untreated slices. Results are mean of ratios of individual slices (n=3 per treatment) ± SEM. Values significantly different from control: *p < 0.05.

Induction by βNF in duodenum slices, but not quantifiable because of lack of signal from untreated slices. See text for comments.

Not detectable
While analyzing the mRNA from rat intestinal slices, we found that CYP1A1 levels from untreated (control) slices after 6h were only 10% ± 3% (n=6 ± SEM) of the levels measured directly after preparation of the slices. For CYP2B1, this was 60% ± 15% (n=6 ± SEM) of initial values, and CYP1A2 mRNA level was only detectable after βNF induction.

To confirm the mRNA data, rat liver slices were also examined for CYP mediated enzyme activities. βNF resulted in 16-fold induction of CYP1A-dependent 7-ethoxyresorufin O-deethylase (EROD) activity (Fig. 4).

**Figure 4.** Induction of EROD (CYP1A1/1A2) activity in rat liver slices exposed to βNF (50 µM), PB (1 mM) and Dex (100 µM) for 48 hours. The results are expressed as mean ± SEM of two different experiments, n=6 (from individual experiment, n=3). Values significantly different from control: *p < 0.05.

DEX demonstrated a 4-fold increase in CYP3A mediated testosterone 6β-hydroxylase activity (Table 2). The formation of 16α-, 2α-hydroxytestosterone and androstenedione was not significantly affected.

**Table 2.** Effect of βNF, PB and DEX on the hydroxylation of testosterone in rat liver slices exposed for 48 h. Testosterone hydroxylation (OH-T; hydroxytestosterone) is expressed as pmol/min/mg protein. Results are means ± SEM (n=6) of two separate experiments. Values significantly different from control: * p< 0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6βOH-T</th>
<th>16αOH-T</th>
<th>2αOH-T</th>
<th>Androstenedione</th>
</tr>
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<tr>
<td>Control</td>
<td>39.6 ± 6.5</td>
<td>34.0 ± 2.2</td>
<td>24.5 ± 2.4</td>
<td>95.0 ± 13.5</td>
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<tr>
<td>βNF</td>
<td>24.7 ± 2.5</td>
<td>23.6 ± 4.2</td>
<td>12.2 ± 1.6</td>
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<tr>
<td>PB</td>
<td>22.0 ± 4.1</td>
<td>26.3 ± 3.1</td>
<td>15.6 ± 1.5</td>
<td>67.8 ± 4.5</td>
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<tr>
<td>DEX</td>
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<td>48.4 ± 3.9</td>
<td>37.0 ± 3.7</td>
<td>88.2 ± 8.5</td>
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Discussion

Xenobiotics, including drugs, can influence CYP activity by upregulating the transcription of CYP genes. This is a non-desirable feature for a drug or drug candidate as it might cause variability in pharmacokinetics, as well as drug-drug interactions, or even therapeutic failure. Although clinical induction may still be debatable [1], profound therapeutic consequences have been observed when it occurs [22,23]. For pharmaceutical industries, knowledge of possible CYP-induction of potential drug candidates in drug discovery or early development may help in preventing industries from investing in the wrong compounds.

Despite the existence of species differences in CYP induction, making extrapolation from animals to humans difficult or even impossible in some cases, it is still common that drug regulatory agencies request information on CYP induction of candidate drugs obtained from animal studies. In addition, information on induction potential of new chemical entities in pharmacological animal models can be useful as it might explain time dependent pharmacokinetics (auto-induction).

However, in late discovery or early drug development, these animal studies are rather difficult to carry out because of the large number of animals needed. Therefore, there is a need for simple and reproducible in vitro models to study CYP-inductions.

The present study describes the usefulness of freshly prepared liver and intestinal slices in combination with rapid evaluation of CYP gene expression using real-time quantitative RT-PCR, as a tool to investigate CYP induction. In addition, this study demonstrates that, in general, the in vitro data is representative for the in vivo situation.

The three compounds included in this study are: βNF, PB and DEX. These compounds were selected based on the fact that significant literature is available and that they cover a range of CYP isoforms. To illustrate better the relationship between CYP induction observed in vivo and in vitro, the data are presented in Table format (Tables 3 and 4).

Although the induction intervals are selected arbitrarily, it is clear that there is a quite good concordance between the in vitro and in vivo data for both rat liver and intestine.

Table 3. A schematic comparison of induction (qualitative and quantitative) observed in vivo and in vitro in rat liver after exposure to βNF, PB and DEX.

<table>
<thead>
<tr>
<th></th>
<th>CYP1A1</th>
<th>CYP1A2</th>
<th>CYP2B1</th>
<th>CYP3A1</th>
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<td>vitro</td>
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<td>vitro</td>
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<tr>
<td>βNF</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
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<tr>
<td>DEX</td>
<td>-</td>
<td>-</td>
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</table>

- no significant induction
+ < 25 fold induction
++ 25-100 induction
+++ > 100 fold induction
Table 4. A schematic comparison of induction (qualitative and quantitative) observed in vivo and in vitro in rat duodenum after exposure to βNF, PB and DEX.

<table>
<thead>
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<th>CYP2B1</th>
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<tr>
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<td>DEX</td>
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<td>-</td>
<td>+</td>
<td>a</td>
<td>-</td>
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</tbody>
</table>

- no significant induction
+ < 25 fold induction
++ 25-100 induction
+++ > 100 fold induction

a The induction level has been classified as +, even though there is no detection of mRNA expression in untreated slices.

Generally, our results are in agreement with the literature on enzyme activities of microsomes after induction [20,24-26]. A direct comparison of folds mRNA induction is hampered by differences in the experimental conditions. Results, as depicted in Fig. 3, were found to agree with earlier reports on strong CYP1A1 induction by βNF and weaker induction by PB [27]. A strong induction of CYP1A2 in intestinal slices by βNF is in agreement with PCR analyses of intestine after in vivo treatment [26]. Also, CYP2B1 induction by PB as reported here is in agreement with earlier in vitro results on rat liver slices [4].

There are contrasting reports on CYP3A1 induction in intestinal tissue. Here, we find an induction of CYP3A1 by DEX in vivo but not in vitro. CYP3A1 was not found to be induced after an i.p. dose of 100 mg/kg/day for 3 days [28], however a 8 to 30 fold induction of CYP3A1 has been reported after an oral DEX administration of 1 and 20 mg/kg/day for 3 days [29]. Apart from the differences of exposure to DEX, it is yet unclear why we observed here an intestinal induction of CYP3A1 by DEX in vivo, but not in vitro.

All three compounds tested showed qualitatively similar induction in vivo and in vitro towards the CYP isoforms tested, except for βNF towards CYP2B1 in liver and intestine and DEX towards CYP1A2 in liver (where induction was found in vitro, but not in vivo) and CYP3A1 in intestine (that was not detected in vitro, even after treatment). Also quantitatively, the induction was remarkably similar, and only induction of CYP2B1 by DEX in liver and CYP1A1 by βNF in intestine demonstrated to be more pronounced in vitro when compared to in vivo.

Relatively few quantitative/qualitative differences that were observed between in vitro and in vivo may be explained by several reasons. First of all, the different incubation/exposure times in comparison to animal treatment may explain some changes for CYP mRNA expression, which is time dependent [27,30]. In addition, we found a decrease of mRNA expression in control intestinal slices during incubation similar to in vitro results on hepatocytes [31] that may also contribute to differences between in vitro and in vivo.
The use of rat liver slices as a tool to identify CYP induction has been described by several authors [4,11,32], although in these studies no direct comparison with in vivo data was made, apart from one recent study where comparable results were observed for the rat [27]. Moreover, as far as we are aware of, this is the first description of CYP induction in rat intestinal slices.

Thus, the current study underlines the potential of both liver and intestinal slices, to study CYP induction in vitro. In this study, intestinal slices from rats only were used, but analogous to liver slices, we expect that also intestine from other species including human can be used to prepare intestinal slices, using the very same procedure [14]. This may further expand the potential use of intestinal slices.

In summary, the RT-PCR method here described is a rapid and suitable procedure to assess changes in CYP mRNA expression in whole liver and intestine, and also from liver slices and intestinal tissue slices from rats. In addition, the induction profiles (qualitative and quantitative) observed in vitro are very similar compared to in vivo. All together, those data demonstrate that liver and intestinal slices are a useful tool to study CYP induction in vitro.
CYP induction in rat liver and intestine slices

References

CYP induction in rat liver and intestine slices


