CYP2A5/CYP2A6 EXPRESSION IN MOUSE AND HUMAN HEPATOCYTES TREATED WITH VARIOUS IN VIVO INDUCERS

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ABSTRACT:

Induction of coumarin 7-hydroxylation, catalyzed by CYP2A5 in mice and CYP2A6 in humans by various known in vivo murine inducers and modifiers, was compared in human and mouse hepatocytes in culture. Phenobarbital and rifampicin were efficient inducers (up to 10-fold induction) after 48-h treatment in murine cultured hepatocytes, whereas the enzyme activity in human hepatocytes was much more refractory to induction. However, a prolongation of incubation time to 72 h in human hepatocytes led to a modest restoration of inducibility by phenobarbital. Of the three porphyrinogenic inducers studied, griseofulvin induced the murine enzyme efficiently, but not the human enzyme, whereas aminotriazole and thioacetamide had no effect on either species. Pyrazole produced substantial induction in both human and murine hepatoctyes, whereas cobalt chloride, which is also an in vivo inducer of the mouse enzyme, had no effect. Clofibrate, an in vivo depressor of coumarin 7-hydroxylase, also depressed hepatocyte activities. In both murine and human hepatocytes, changes in CYP2A5/6 mRNA levels correlated roughly with enzyme changes, except in the case of cobalt chloride, which increased mRNA levels despite a lack of effect on enzyme activity. In general, human and mouse hepatocytes gave a similar response to CYP2A inducers. However, some differences were found, which means that, although CYP2A isozymes are probably regulated in a similar manner in both species, it is necessary to be cautious before extrapolating to human the results found in mouse models.

Coumarin 7-hydroxylase activity is catalyzed almost solely by CYP2A5 and CYP2A6 in mouse and human livers, respectively (Pelkonen et al., 1997). Studies on the inducibility of coumarin 7-hydroxylase in mouse liver in vivo have demonstrated that the activity is inducible by a large variety of compounds, including the classic inducers (phenobarbital, rifampicin), pyrazole and its derivatives and porphyrinogenic substances (thioacetamide, aminotriazole, griseofulvin, cobalt chloride). CYP2A5 activity is decreased by clofibrate, a representative peroxisome proliferator in rodent liver (Kojo et al., 1996).

Much less is known about the inducibility of CYP2A6 in humans. Coumarin has proven to serve as an excellent probe for the in vivo characterization of CYP2A6-associated metabolism (Rautio et al., 1992). Antiepileptic therapy with carbamazepine, clonazepam, phenobarbital, and phenytoin was associated in humans with an increased capacity to form 7-hydroxycoumarin from coumarin, thus indicating CYP2A6 induction in vivo (Sotaniemi et al., 1995). In human cultured hepatocytes, at least phenobarbital and rifampicin are capable of inducing CYP2A6 (Dalet-Beluche et al., 1992).

A mouse primary hepatocyte model was used to study induction and regulation of CYP2A5 on a chemically defined, controllable environment (Salonpää et al., 1994). Earlier studies have demonstrated that phenobarbital and some other in vivo inducers also increased CYP2A5 in hepatocytes in vitro, but several other efficient in vivo inducers did not increase CYP2A5 expression in primary hepatocytes, which indicates profound differences in the way the induction is controlled in vivo and in isolated hepatocytes (Salonpää et al., 1997).

To uncover the potential inducing effects of several of the above-mentioned substances on human CYP2A6-associated coumarin 7-hydroxylase activity, we utilized human primary hepatocytes in culture and compared the inducibility of coumarin 7-hydroxylase in human and murine hepatocytes. This comparison revealed that there are at least quantitative, if not qualitative, differences between human and murine hepatocytes in their responses to murine in vivo inducers.

Experimental Procedures

Materials. William’s medium E, insulin-transferrin-selenate media supplement (ITS), dexamethasone, bovine serum albumin, and coumarin were obtained from Sigma Chemical Co. (St. Louis, MO). Insulin was from Lilly (Indianapolis, IN), collagenase was from Worthington Biochemical (Freehold, NJ). Fetal calf serum (FCS), newborn calf serum, Ham F-12 and Leibovitz

1 Abbreviations used are: ITS, insulin-transferrin-selenate media supplement; CYP, cytochrome P-450; COH, coumarin 7-hydroxylase; FCS, fetal calf serum; MTT, 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide; PCR, polymerase chain reaction.
L-15 culture media, and gentamicin were from Life Technologies, Inc. (Paisley, Scotland).

**Isolation and Culture of Hepatocytes.** Human hepatocytes were obtained from surgical liver biopsies (weighing 1–3 g) of patients undergoing cholecystectomy after informed consent was obtained. Patients had no known liver pathology, and they did not receive any medication during the weeks before surgery. The patients were not habitual consumers of alcohol or other drugs. A total of 16 liver biopsies (8 males and 8 females) were used (Table 1). The ages of the patients ranged from 32 to 82 years. Hepatocytes were isolated using a two-step microperfusion technique of the tissue as described elsewhere (Gómez-Lechón et al., 1997). Cellular viability, estimated by the dye exclusion test with 0.4% trypan blue in saline, was higher than 90%. Hepatocytes were seeded on fibronectin-coated plastic dishes (3.5 μg/cm²) at a density of 8 × 10⁶ viable cells/cm² and cultured in Ham’s F-12/Lebovitz L-15 (1:1) medium supplemented with 2% newborn calf serum, 10 mM glucose, 50 μM/ml penicillin, 50 μM/ml streptomycin, 0.2% bovine serum albumin, and 10 nM insulin. One hour later the medium was changed, and after 24 h the cells were shifted to serum-free, hormone-supplemented medium (10 nM insulin and 10 nM dexamethasone). The medium was changed daily. Under these culture conditions the cells are metabolically competent (Donato et al., 1995; Gómez-Lechón et al., 1997).

Mouse hepatocytes were obtained from male DBA/2 mice, aged 7 to 10 weeks. To isolate hepatocytes, mouse livers were perfused with collagenase solution as described previously (Salonpää et al., 1994, 1997). After filtration and centrifugation (30g, 2 min), the isolated hepatocytes were dispersed in William’s medium E containing dexamethasone (20 ng/ml), ITS (insulin 5 nM), 10% FCS at a density of 9 × 10⁶ cells/90-mm plasma-treated plastic dish (Falcon 3003). The cultures were maintained at 37°C in a humidified incubator. After 2 to 3 h, nonattached cells were discarded by aspiration, followed by changing the medium to William’s E without FCS.

**Incubation of Hepatocytes with Potential Inducers.** Dose-response curves for cytotoxicity and enzymatic activity induction were obtained by incubating hepatocytes with increasing concentrations of the compounds up to the highest nontoxic concentration. Treatment of human hepatocytes was started at 24 h of culture, and cells were incubated in the presence of inducers for 48 h (in a few experiments up to 72 h). In mouse hepatocytes, compounds were added to the culture medium just after the cells were attached to the plates, which occurred 2 to 3 h after plating. Cells were then incubated for 48 h. The cytotoxicity of the studied compounds in hepatocytes was estimated as follows. Human hepatocytes were seeded on 96-well microtiter plates and treated with a wide range of concentrations of the compounds. Cellular viability was assessed by the MTT test in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method in as described in detail elsewhere (Borenfreund et al., 1988). The maximal noncytotoxic concentration was estimated from the dose-response curves. Cytotoxicity on mouse hepatocytes was estimated earlier (Salonpää et al., 1997; Vittala, 1997). The cytotoxicity of the studied compounds was usually caused by roughly similar concentrations. In the case of aminotriazole and thioacetamide, murine hepatocytes seemed to be about twice as sensitive as human hepatocytes.

For CYP2A6 mRNA determination by reverse transcriptase-polymerase chain reaction (PCR), treatment was started at 24 h, and cells were incubated only for the following 24 h. Control cultures were treated with the same final concentration of the solvents. For mRNA analysis by the Northern technique in mouse hepatocytes, treatment with studied compounds began 2 to 3 h after plating, when the cells were attached, and lasted for 48 h.

**Enzyme Activity Assay.** Coumarin 7-hydroxylase (COH) was determined directly in living human hepatocytes cultured on 96-well plates as previously described (Donato et al., 1998a). In brief, monolayers were washed twice with phosphate-buffered saline, 20 mM, pH 7.4, to remove detached cells. Hepatocytes were incubated with culture medium containing 50 μM coumarin for 1 h at 37°C, and 7-hydroxycoumarin was enzymatically deconjugated and quantified fluorimetrically in aliquots of culture medium. Cellular protein was determined by the Lowry method (1951) adapted to 96-well plates in our laboratory.

Coumarin 7-hydroxylase in mouse hepatocytes was assayed as follows. After 48 h in culture, mouse hepatocytes were scraped, washed, suspended in 200 μl of phosphate-buffered saline and sonicated. Cellular protein contents were determined according to Bradford (1976). COH activity was measured as described previously (Aitio, 1978) using 100 μM coumarin as substrate. Because the absolute values of enzyme catalytic activities varied from one batch of cells to another, activities are given relative to the respective control values, which were normalized to 100%.

**Analysis for Specific mRNA.** In the case of human hepatocytes, RNA purification and cDNA synthesis were performed as described by Jover et al. (1996). Forward and reverse primers selected for human CYP2A6 and β-actin and/or glucose-6-phosphate dehydrogenase as an internal control for data normalization are presented in Table 2. A sample of the appropriately diluted cDNA was added to 27 μl of the reaction mixture (final concentrations: 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 60 μM each deoxynucleotide triphosphate, 1 U of Taq DNA polymerase, and 0.2 μM each primer). Amplification was programmed for an initial denaturation of 4 min at 94°C, followed by 30 cycles of 45 s at 94°C, 45 s at 60°C, 1 min at 72°C, and a final extension of 5 min at 72°C. Appropriate dilutions were employed for each cDNA to ensure that the resulting PCR products derived only from the exponential phase of the amplification. Under these conditions, the yield of the PCR product was found to be proportional to the input cDNA (Jover et al., 1997). For quantitative analysis, aliquots of the PCR reaction were subjected to electrophoresis on 1.2% agarose gel, and the products were visualized by ethidium bromide staining. The gel image was digitalized, and the band intensity was quantified.

To measure CYP2A5 mRNA, adherent mouse hepatocytes were scraped, washed, and homogenized in 400 μl of extraction buffer. mRNA was purified by Quick-Prep Micro mRNA purification kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Purified mRNA was separated on a 1% agarose gel and transferred to QuantiBlot nylon membrane (Qiagen, Chatsworth, CA). RNA was fixed by UV-crosslinking in UV Stratalinker 1800 (Stratagene, San Diego, CA), and the membrane was hybridized to a [32P]dCTP-labeled CYP2A5 cDNA fragment. Radioactivity was measured with PhosphorImager SI equipment (Molecular Dynamics, Sunnyvale, CA).

**Statistical Analysis.** Each assay was carried out in at least three different cell preparations, and each determination was done in four plates (eight wells for cytotoxicity experiment) from each culture. The results shown are the mean ± S.E.M. Data were analyzed with the Student’s t test. Values of P < .05 were considered significant.

**Results**

**Basal Activity Values in Human and Murine Hepatocytes.** Coumarin 7-hydroxylase activities in basal conditions were measured for both human and murine hepatocytes. To measure coumarin 7-hydroxylase activity and inducibility in human hepatocytes, cells obtained from 16 different individuals were studied. The human values shown in Table 1 correspond to basal activity at 72 h of culture. It can be

<table>
<thead>
<tr>
<th>Biopsy Number</th>
<th>Sex</th>
<th>Age</th>
<th>Viability</th>
<th>COH Activity a</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH334</td>
<td>Female</td>
<td>67</td>
<td>95</td>
<td>9.9 ± 1.0</td>
</tr>
<tr>
<td>BH338</td>
<td>Female</td>
<td>33</td>
<td>99</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>BH339</td>
<td>Male</td>
<td>75</td>
<td>92</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>BH340</td>
<td>Female</td>
<td>56</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>BH342</td>
<td>Male</td>
<td>62</td>
<td>89</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>BH346</td>
<td>Male</td>
<td>36</td>
<td>95</td>
<td>14.5 ± 2.8</td>
</tr>
<tr>
<td>BH348</td>
<td>Female</td>
<td>67</td>
<td>97</td>
<td>20.1 ± 1.2</td>
</tr>
<tr>
<td>BH350</td>
<td>Male</td>
<td>68</td>
<td>97</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>BH351</td>
<td>Female</td>
<td>82</td>
<td>92</td>
<td>4.0 ± 1.9</td>
</tr>
<tr>
<td>BH352</td>
<td>Male</td>
<td>78</td>
<td>91</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>BH354</td>
<td>Female</td>
<td>35</td>
<td>98</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>BH357</td>
<td>Male</td>
<td>72</td>
<td>95</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>BH358</td>
<td>Female</td>
<td>62</td>
<td>89</td>
<td>9.6 ± 2.6</td>
</tr>
<tr>
<td>BH370</td>
<td>Male</td>
<td>32</td>
<td>90</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>BH373</td>
<td>Male</td>
<td>56</td>
<td>89</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>BH378</td>
<td>Female</td>
<td>37</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

a COH activity was measured at 72 h of culture.
observed that the basal coumarin 7-hydroxylase activity exhibited a rather large variability, from 1 to about 20 pmol/mg of cellular protein per minute, i.e., a 20-fold variation (Table 1). Basal activity at 24 h of culture ranged from 1.7 to 26.8 pmol/mg/min.

Similar coumarin 7-hydroxylase activity levels were found in male DBA/2 mouse cultured hepatocytes. In murine hepatocytes, a 72-h incubation tends to lead in many cases to an apparent decrease in viability and a rather drastic decrease in coumarin 7-hydroxylase activity and inducibility, and consequently the 24- and 48-h values are given: at 24 h in culture the average value for eight different hepatocyte preparations was 4.9 pmol/mg/min (with a range of 1.4–8.8), and after 48 h, the average value of coumarin 7-hydroxylase activity was 3.1 pmol/mg/min (ranging between 1.3 and 8.7).

**Effect of Murine in Vivo Inducers/Depressors on Coumarin 7-Hydroxylase Activity in Human and Mouse Hepatocytes.** The inducibility of coumarin 7-hydroxylase in human and murine hepatocytes in cultures after exposure of the cells for 48 h (in some cases for 72 h in human hepatocytes) to the studied substances is summarized in Table 3. The figures in Table 3 show the highest concentration of the compound that could be used without overt cytotoxicity. In principle, similar concentration ranges in human and murine hepatocytes were studied.

Two classical inducers, phenobarbital and rifampicin, were incubated with both human and mouse hepatocytes. In murine hepatocytes, phenobarbital induced a severalfold increase in coumarin 7-hydroxylase activity (3.8-fold on average; maximum 10-fold) (Table 3). Instead, a tendency to decreased activities was observed. In principle, similar concentration ranges in human and murine hepatocytes were studied.

With rifampicin as an inducer, up to 6.1-fold induction was seen in murine hepatocytes, whereas no induction was observed in human hepatocytes (one or more concentration in five individual cultures; BH346, -348, -350, -352, and -373). Prolongation of treatment time to 72 h did not result in an increase in human hepatocytes (BH370). Prolongation of treatment to 72 h resulted in a modest increase of enzyme activity (1.40–1.8-fold) at concentrations between 50 and 200 μM (Table 3).

Thioacetamide did not induce the enzyme in either human (three individual cultures; BH346, -348, and -352) or murine hepatocytes (Table 3). Instead, a tendency to decreased activities was observed. Prolongation of treatment to 72 h did not result in an increase of activity.

In vivo clofibrate acid causes a drastic decrease in coumarin 7-hydroxylase activity in mouse liver (Nakata et al., 1996). In hepatocytes in culture, clofibrate acid tended to decrease coumarin 7-hydroxylase activity in both human (four individual cultures; BH346, -348, -350, and -352) and murine hepatocytes in culture (Table 3). No increased effect was observed after a longer treatment time.

**Effects of Modifiers on CYP2A5/6 mRNA in Human and Mouse Hepatocytes.** The effects of the different compounds on CYP2A5 and CYP2A6 mRNA levels were also examined in cultured murine and human hepatocytes, respectively. As seen in Fig. 3, treatment of mouse hepatocytes with fenobarbital, rifampicin, pyrazole, cobalt chloride, and griseofulvin produced marked increases in the amount of specific CYP2A6 mRNA. These effects correspond to increases in the catalytic activity (Figs. 1 and 2; Table 3). A noteworthy exception was cobalt chloride, which was unable to induce the catalytic activity but increased the amount of mRNA. An explanation might be that the up-regulation of the CYP2A5 enzyme by cobalt chloride is somehow blocked after the increase in mRNA.

A good correlation between the effects on CYP2A6 mRNA levels and coumarin 7-hydroxylase activity was also found in human hepatocytes (Figs. 1–3; Table 3). Comparison of the results obtained in primary cultured hepatocytes from both species revealed that exposure of cells to the different compounds produced, in general, lower effects on human hepatocytes than on mouse hepatocytes.

**Discussion**

Most studies on the effects of chemical inducers on CYP isozymes have been done on laboratory animals. In particular, regulation of
coumarin 7-hydroxylase catalyzed by CYP2A5 in mouse liver has been extensively characterized, but comparison with what happens to the orthologous CYP2A6 enzyme in human liver has been lacking. Human hepatocyte cultures provide a valuable experimental model for studying the mechanisms involved in the regulation of CYP isozymes in humans. Cultured hepatocytes from different donors exhibit a rather wide variability in coumarin 7-hydroxylase activity (about 20-fold, Table 1) that can be related to the marked interindividual differences in CYP2A6 protein levels and activity observed in human liver (Yun et al., 1991; Rautio et al., 1992). It has been suggested that these large variations differences might be due to CYP2A6 inducibility by certain drugs or environmental and nutritional factors (Cashman et al., 1992) or to genetic polymorphism (Fenandez-Salguero et al., 1995; Pelkonen et al., 1997). In the present study, we show that a number of compounds, including classic CYP inducers, metal ions, and porphyrinogenic agents, increased CYP2A6 activity at mRNA levels in human hepatocytes in primary culture, and these results suggest that those compounds could act as inducers of

TABLE 3
Effect of murine in vivo inducers/depressors on COH activity in human and mouse hepatocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Human Hepatocytes</th>
<th>Marine Hepatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Concentration</td>
<td>Activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>h % of control %</td>
<td></td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>48</td>
<td>1 mM</td>
<td>84 ± 7</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>2 mM</td>
<td>104 ± 11</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>48</td>
<td>50 µM</td>
<td>82 ± 18</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>25 µM</td>
<td>114 ± 11</td>
</tr>
<tr>
<td>Pyrazole</td>
<td>48</td>
<td>10 nM</td>
<td>760 ± 46'</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200 µM</td>
<td>63</td>
</tr>
<tr>
<td>Cobalt chloride</td>
<td>48</td>
<td>100 µM</td>
<td>90 ± 22</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200 µM</td>
<td>188 ± 6</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>48</td>
<td>200 µM</td>
<td>182 ± 28'</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>100 µM</td>
<td>156 ± 32</td>
</tr>
<tr>
<td>Aminotriazole</td>
<td>48</td>
<td>200 µM</td>
<td>117 ± 13</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200 µM</td>
<td>171</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>48</td>
<td>50 µM</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>500 µM</td>
<td>85 ± 12</td>
</tr>
</tbody>
</table>

*Concentrations close to the highest nontoxic concentration of the compound.

**Concentrations below the highest nontoxic concentration of the compound.

*P < .05.

**ND, not determined.

Fig. 1. The effect of pyrazole on coumarin 7-hydroxylase activity in cultured primary human and mouse hepatocytes.

Human (circles) and mouse (squares) cultured hepatocytes were exposed to increasing concentrations of pyrazole, and COH activity was assayed 48 h later as described under Experimental Procedures. Values correspond to mean ± S.E. of three different hepatocyte cultures. Results are expressed relative to COH activity in control cells. *P < .05.

Fig. 2. The effect of griseofulvin on coumarin 7-hydroxylase activity in cultured primary human and mouse hepatocytes.

Human (circles) and mouse (squares) cultured hepatocytes were exposed to increasing concentrations of griseofulvin, and COH activity was assayed 48 h later as described under Experimental Procedures. Values correspond to mean ± S.E. of three to five different hepatocyte cultures. Results are expressed relative to COH activity in control cells. *P < .05.
CYP2A6 in human liver. Comparative studies in human and mouse hepatocytes showed that effects on CYP2A5 and CYP2A6 were similar, which means that the two isozymes might be regulated in a similar manner in both species. Only rifampicin displayed a different action in both species; it induces CYP2A5 enzyme in murine hepatocytes but seems to have no effect on human cells.

As we have observed earlier with some inducers in mouse in vivo and in hepatocytes, increases in the amount of the specific CYP2A5 mRNA corresponded roughly to increases in the catalytic activity (Hahnemann et al., 1992; Salonpää et al., 1995, 1997; Kojo et al., 1998). Whether this increase is transcriptional or post-transcriptional is not known in most cases. There is some evidence that phenobarbital is a transcriptional inducer (Aida and Negishi, 1991; Hahnemann et al., 1992), whereas pyrazole might act post-transcriptionally (Geneste et al., 1996; Tilloy-Ellul et al., 1999). In the present study a noteworthy exception was cobalt chloride, which was unable to induce the catalytic activity but increased the amount of mRNA severalfold. This finding is in contradiction with our earlier in vivo study in mice, where cobalt chloride increased both comarin 7-hydroxylase activity and CYP2A5 mRNA (Kocer et al., 1991; Hahnemann et al., 1992). A possible explanation might be that in murine hepatocytes the up-regulation of CYP2A5 enzyme by cobalt chloride is somehow blocked after the increase in mRNA.

Most inducers of CYP2A5 in murine hepatocytes were also efficient inducers of CYP2A6 in human hepatocytes, although CYP2A6 seemed to be much more refractory to the induction than CYP2A5. In particular, after 48-h treatment with phenobarbital, comarin 7-hydroxylase remained at the control level in human hepatocytes, whereas it was increased at least 3-fold, and even 10-fold in some cultures, in murine hepatocytes. Phenobarbital is, however, intrinsically able to induce CYP enzymes in human hepatocytes, because: 1) an induction in 7-benzoyloxyresorufin O-dealkylase activity and CYP2B6 mRNA levels was demonstrated at 48 h (Rodriguez-Antona et al., 2000); 2) incubation of human hepatocytes for 72 h in the presence of phenobarbital increased comarin 7-hydroxylase activity about 2-fold; and 3) increased levels of CYP2A6 mRNA were found. The mechanism by which phenobarbital induces murine CYP2A5 is insufficiently known, but studies from our laboratory point to a possible role of CAMP-mediated signal transduction (Salonpää et al., 1994, 1997). It may be that culturing human hepatocytes lead to a loss of or a decrease in an important mediator for CYP2A6 induction by phenobarbital, which, however, is not necessary for the phenobarbital induction of other responsive CYPs. The loss is relative, because a longer incubation leads at least to a partial restoration of inducibility.

In the case of rifampicin, strong differences between human and mouse hepatocytes were found. Rifampicin is an efficient in vitro inducer of CYP2A5, but it does not produce any changes in CYP2A6 activity and mRNA levels. In contrast, treatment of cultured human hepatocytes with rifampicin resulted in increased CYP3A4 activity that correlates with an induction of CYP3A4 protein and specific mRNA levels (Donato et al., 1998b; Rodriguez-Antona et al., 2000), which indicates that human hepatocytes are able to respond to rifampicin treatment.

In conclusion, both similarities and differences in regulation of CYP2A enzymes in mouse and human hepatocytes were demonstrated. As such, this conclusion is not surprising considering the large species differences in CYP enzymes between, for example, rodent and human. However, as reviewed more extensively elsewhere (Fernandez-Salgueiro et al., 1995; Fernandez-Salgueiro and Gonzalez, 1995; Pelkonen et al., 1997), human and mouse CYP2A-associated comarin 7-hydroxylases display relatively similar characteristics in terms of both enzymatic and regulatory properties. The pharmacological and toxicological consequences of this induction of human CYP2A6 induction can be clinically relevant. Inducers can influence the metabolism of therapeutic agents simultaneously or subsequently administered, and this can alter their efficacy or increase their toxicity. Human CYP2A6 is involved in the oxidation of drugs, including comarin and nicotine (Yun et al., 1991; Cashman et al., 1992), as well as in the metabolic activation of certain carcinogenic and toxic chemicals (Yun et al., 1991). Ultimately, it is the balance between bioactivation, detoxication, and defense mechanisms that determines the susceptibility of an organism to drugs and other xenobiotics.

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References


