Expression and Induction of CYP2C P450 Enzymes in Primary Cultures of Human Hepatocytes

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ABSTRACT

Although CYP2C8, CYP2C9, and CYP2C19 play an important role in drug biotransformation, factors influencing the expression and activity of these CYP2C P450s in human liver remain largely undefined. We used primary cultures of human hepatocytes from 15 subjects to assess the inducibility of CYP2C enzyme expression by prototypical inducer agents, including rifampicin, dexamethasone, and phenobarbital. After culture for 72 h in serum-free medium on collagen, Western blotting revealed that CYP2C9 was the only CYP2C enzyme expressed at appreciable levels in untreated hepatocytes. Subsequent treatment with 25 μM rifampicin for 48 h elicited marked increases in CYP2C8 (700 ± 761%), CYP2C19 (854%), and CYP2C9 (209 ± 176%) protein content versus a 550 ± 170% enhancement of CYP3A4 enzyme levels. Parallel increases in CYP2C mRNA, measured by Northern blotting and/or RNase protection, were found in rifampicin-treated hepatocytes, with CYP2C8, CYP2C9, and CYP2C19 transcripts exhibiting increases of 688 ± 635, 207 ± 49, and 230 ± 60%, respectively, versus an 8.8-fold enhancement of CYP3A4 mRNA levels. Dexamethasone (10 μM) treatment enhanced CYP2C8 mRNA (360 ± 100%) and protein (274%) content, although this steroid had less effect on CYP2C9 and CYP2C19 transcripts (23 ± 21% and 21 ± 36%, respectively) and enzyme levels (55 and 143%, respectively). Phenobarbital (100 μM) was a powerful inducer of CYP2C9 (850%) and CYP2C19 (735%) mRNA content, and also increased CYP2C8 (610%) and CYP3A4 (205%) transcripts. Our results show that CYP2C enzyme expression in human hepatocytes is highly inducible by rifampicin, dexamethasone, and phenobarbital. Because these xenobiotics are ligands and/or activators of the pregnane X receptor and/or constitutive androstane receptor, such orphan nuclear receptors and their response elements may partake in regulating CYP2C gene expression in humans.

The human CYP2C gene subfamily consists of at least four distinct genes, namely, CYP2C8, CYP2C9, CYP2C18, and CYP2C19 (Romkes et al., 1991; Goldstein et al., 1994). CYP2C8, CYP2C9, and CYP2C19 are expressed in human liver and comprise 10 to 20% of the total aggregate P450 content found in that organ (de Morais et al., 1994a; Shimada et al., 1994). CYP2C9 and CYP2C19 partake in the oxidative metabolism of many commonly used pharmacological agents, including the antibacterials sulfamethoxazole and dapsone (Barry et al., 1997; Winter et al., 2000), the anticoagulant warfarin (Kaminsky et al., 1993), nonsteroidal anti-inflammatory agents (e.g., diclofenac and ibuprofen) (Leemann et al., 1993), omeprazole, an H2 antagonist (Karam et al., 1996; Yamazaki et al., 1997), diazepam, a benzodiazepine (Jung et al., 1997; McGinnity et al., 1999), and antiepileptics such as phenytoin (Giancarlo et al., 2001). CYP2C9 and CYP2C19 also catalyze oxidation of the prototypic P450 substrates tolbutamide and S-mephénytoin in human liver microsomes (Knodell et al., 1987; Hall et al., 1994; Lasker et al., 1998; Wester et al., 2000). Moreover, recent reports (Lillibrige et al., 1998a,b) indicate that CYP2C19 plays a role in metabolizing the human immunodeficiency virus-protease inhibitor nelfinavir, which is intriguing since most drugs of this class are metabolized solely by CYP3A4 (von Moltke et al., 1998 and references within). CYP2C8 exhibits catalytic properties that are distinct from those of CYP2C9 and CYP2C19, and functions in human liver as the principal catalyst of retinol and retinoic acid oxidation (Leo et al., 1989), paclitaxel (Taxol) hydroxylation (Rahman et al., 1994; Richardson et al., 1995), carbamazepine epoxidegenation (Kerr et al., 1994), and carisoprodol oxidation (Dalen et al., 1996). Exposure to xenobiotics, including currently utilized ther-

ABBREVIATIONS: P450, cytochrome P450; PB, phenobarbital; RIF, rifampicin; PXR, pregnane X receptor; PXRE, PXR response element; CAR, constitutive androstane receptor; DEX, dexamethasone; bp, base pair(s); DMSO, dimethyl sulfoxide.
A drinker is defined as an individual who consumes at least 50 g of ethanol/day.

A smoker is defined as a user of 1 pack of cigarettes/day.

HTN, hypertension; IDDM, insulin-dependent diabetes mellitus; LSD, lysergic acid diethylamide; XTC, 3,4-methylenedioxymethamphetamine; NIDDM, non-insulin dependent diabetes mellitus.

A smoker is defined as a user of 1 pack of cigarettes/day.

A drinker is defined as an individual who consumes at least 50 g of ethanol/day.
CYP2A4 probes; the T7 promoter site was used to prime synthesis of antisense RNA. A human β-actin (125 bp) or cyclophilin (100 bp) antisense probe was added to each RNA sample to normalize the amounts of RNA loaded in each gel lane. Total hepatocyte RNA was hybridized to the 32P-labeled antisense probes, digested with RNase, and applied to acrylamide/urea gels. Hybridization signals on the resultant autoradiograms were then quantified as described above for Northern blots.

**Protein Blot Analysis.** Western blotting of hepatocyte microsomal proteins to nitrocellulose and subsequent immunochemical staining with P450 antibodies were performed as described elsewhere (Lasker et al., 1998). The properties of the CYP2C and CYP3A4 polyclonal antibodies used for these studies have been reported previously (Feierman and Lasker, 1996; Lasker et al., 1998; Wester et al., 2000). Hepatocyte CYP2C and CYP3A4 enzyme levels were quantified by first scanning the blots with an Epson Expression 1600 scanner (Epson America, Torrance, CA), and then integrating immunostaining intensities with Un-Scan-It software.

**Materials.** cDNA probes to human CYP2C8, CYP2C9, CYP2C19, and CYP3A4 were obtained from Puracyp (San Diego, CA), whereas antisense probes to human 18S rRNA, human β-actin, and human cyclophilin (100 bp) were purchased from Ambion. Restriction enzymes were purchased from New England Biolabs (Beverly, MA), and biocinchoninic acid was obtained from Pierce Chemical Co. (Rockford, IL). RIF, PB, and DEX were purchased from Sigma-Aldrich (St. Louis, MO). TRizol reagent was obtained from Invitrogen, RNeasy kits were obtained from QIAGEN, and nylon membranes were purchased from Accelrys (Burlington, MA). All other reagents used have been described elsewhere or were of the highest quality available.

**Data Analysis.** Results are presented as the mean ± standard deviation or standard error in the case of three or more samples or as the average (including the range) when only two specimens were compared.

### Results

Liver samples were obtained from 15 different subjects, 9 of whom were male, ranging in age from 6 to 65 years (Table 1). Hepatocytes isolated from these samples were placed into primary culture under defined conditions and were then used to assess the inductive effects of specific chemical agents on human CYP2C enzymes. Logistics dictated the use of only a single specimen in any given experiment, whereas cell quantity governed the scope of the induction studies that could be performed. As shown in Fig. 1, CYP2C9 was the only CYP2C enzyme expressed at appreciable levels in zero time hepatocytes from subjects HH933, HH940, and HH926 (see lanes marked 0 in Fig. 1, A and B). In contrast, there was little expression of CYP2C8 and CYP2C19 immunoreactive protein in the same hepatocytes, although both of these P450s as well as CYP2C9 were readily detected in human liver microsomes (lanes marked LMx in Fig. 1, A and B) (Lasker et al., 1998; Wester et al., 2000). Hepatocytes from another human subject, HH954, gave similar results. CYP2C9 protein expression was fairly stable in hepatocyte cultures over at least 48 h, with enzyme levels in DMSO-treated cells remaining at 73.8 ± 47% (range 32.6–139%; n = 4) of those found in zero time cells (data not shown). In comparison, cells treated with DMSO for 48 h, like zero time cells, did not contain appreciable amounts of CYP2C8 and CYP2C19 protein (Fig. 1, A and B), although hepatocytes from subjects HH926 and HH954 did exhibit some “constitutive” CYP2C19 expression. Because cells designated as zero time had already been cultured for 72 h (under Experimental Procedures), the results indicate that CYP2C9 enzyme expression is well maintained in culture for up to 120 h, whereas levels of CYP2C8 and CYP2C19 protein decline dramatically during the initial 72 h in culture, at least among the four different subjects studied here.

We first examined the effects of RIF treatment on CYP2C enzyme levels in primary hepatocyte cultures. CYP3A4 was also included here since the agents shown to induce this P450 in human liver have also been described to stimulate expression of one or more hepatic CYP2C enzymes (Gerbal-Chaloin et al., 2001; Rae et al., 2001; Synold et al., 2001). To ensure an adequate CYP2C-inductive response, we treated hepatocytes with a relatively large RIF dose (25 μM) for 48 h. Of the
four subjects analyzed, HH926, HH933, and HH940 exhibited marked induction (260–1580%) of CYP2C8 protein levels in response to RIF, whereas HH954 failed to respond to RIF treatment, with an increase in CYP2C8 expression (Fig. 2). The overall RIF-mediated increase in hepatocyte CYP2C8 levels among these subjects was 525 ± 357% (mean ± S.E.M.; n = 4). In the case of CYP2C9, HH933 and HH940 exhibited a 230 to 450% increase in CYP2C9 levels upon RIF treatment, whereas HH926 and HH954 showed much less (10–60%) of an elevation in this enzyme (Figs. 1 and 2), giving an overall CYP2C9 increase of 209 ± 176% among the subjects. Similar variability in RIF-mediated CYP2C19 induction was noted, as HH940 displayed a very large (1621%) enhancement in immunoreactive CYP2C19 protein, HH926 exhibited a modest (86%) increase, and HH954 did not demonstrate any enhancement in CYP2C19 expression. A fourth subject, HH933, failed to express CYP2C19 protein, either before or after RIF treatment. Overall, the RIF-mediated increase in CYP2C19 levels was 569 ± 527% (n = 3). In contrast, hepatocytes from each of these four subjects responded to RIF with a sizable increase (453 ± 42%; range, 120–816%) in CYP3A4 levels, as shown in Fig. 1C and Fig. 2. In fact, close examination of the immunoblot in Fig. 1C (lanes marked 25 and 50) reveals RIF-mediated induction of CYP3A5 as well as CYP3A4, because the gel system used here is capable of resolving these closely migrating P450s (Feierman and Lasker, 1996).

RNase protection assays were utilized to assess the effects of RIF on CYP2C and CYP3A4 mRNA expression in cultured human hepatocytes. The cells used for this particular experiment differed from those employed in the CYP2C protein induction studies given above and from those used in Northern analyses. As shown in Fig. 3, treatment of cells from subjects HH841, HH845, HH867, and HH840 with 50 μM RIF for 72 h resulted in a striking (688 ± 635%; range 200–1620%) increase in CYP2C8 mRNA content, whereas CYP2C9 and CYP2C19 transcript levels in the former three subjects were found to increase by 207 ± 49% (range 0–300%) and 167 ± 152% (range 150–240%), respectively. CYP3A4 mRNA content in cells from subjects HH841 and HH867 was enhanced 880% (range 280–1480%) by RIF exposure (Fig. 3). The magnitude of these increases in CYP2C8, CYP2C9, and CYP3A4 mRNA paralleled those found with the corresponding proteins, whereas in the case of CYP2C19, this protein was induced by RIF in only two of the four subjects examined. The expression of CYP2C19 is known to be polymorphic, however (de Morais et al., 1994a,b), and those subjects who failed to respond to RIF treatment with an enhancement of CYP2C19 protein expression may have been of the poor metabolizer genotype.

The inductive effects of RIF on hepatic CYP2C and CYP3A4 mRNA expression were examined in greater detail by varying the dose and duration of treatment with this antibiotic. Treatment with 10 μM RIF for 24 h caused a 580% (range 293–867%) increase in CYP2C8 mRNA levels in cells from subjects HH867 and HH903 (Fig. 4), while CYP2C9 and CYP2C19 transcripts were enhanced to a lesser extent (80% (range 70–90%) and 90% (range 60–120%) increases, respectively). Prolonging the duration of 10 μM RIF treatment to 72 h resulted in even larger increases of CYP2C8 mRNA (710%, range 610–810%) and CYP2C19 mRNA (250%, range 90–410%) content, although there was no clear-cut effect of RIF treatment duration on CYP2C9 transcript levels. CYP3A4 mRNA content was elevated 300% and 500%, respec-
DEX, a synthetic adrenocortical steroid, has been reported to stimulate CYP3A4 and CYP3A5 expression in human liver (Schuetz et al., 1996; Pascussi et al., 2000) and may also enhance hepatic CYP2C enzyme levels (Gerbal-Chaloin et al., 2001). We thus assessed the effects of DEX treatment on CYP2C and CYP3A4 mRNA expression in hepatocyte cultures from different subjects (HH840, HH860, HH870, HH899, HH875, and HH954). We then tested whether PB, an established inducer of CYP3A4 as well as a CAR activator, was also capable of stimulating expression of CYP2C enzymes in primary human hepatocyte cultures. Cells from subject HH886 were exposed to PB for 48 h, after which RNA was isolated and subjected to Northern blotting. Treatment with 1 mM PB resulted in an 18-fold increase in CYP2C8 mRNA, a 6-fold enhancement of CYP2C9 mRNA, and a 10-fold increase in CYP2C19 mRNA content in this particular liver sample. Moreover, PB stimulated CYP3A4 mRNA expression 23-fold above control levels (data not shown). Lowering the dose of PB to 100 μM resulted in an even larger enhancement of CYP2C9 and CYP2C19 RNA transcripts (10- and 11.5-fold, respectively) but in a smaller increase of CYP2C8 and CYP3A4 mRNA levels (4- and 3.5-fold, respectively) in this particular subject. The capacity of RIF (10 μM), DEX (10 μM), and PB (100 μM) to modulate CYP2C gene expression was compared in hepatocyte cultures from subjects HH899 and HH886 (see above). Representative Northern blots showing hybridization
of our human CYP2C9, CYP2C8, and 18S rRNA cDNA probes to their corresponding mRNAs are shown in Fig. 6A. As depicted, each probe reacted with only a single RNA species, and none of the treatments was found to influence 18S ribosomal RNA levels. Of the three agents, PB proved to be the most efficacious inducer of CYP2C9 and CYP2C19 gene transcription, stimulating expression of these two mRNAs by 850% (range 720–980%) and 735% (range 620–850%), respectively, compared with control values (Fig. 6B). Similar to results obtained with other hepatocyte samples (Fig. 5), DEX had essentially no effect (range 150–200%) on CYP2C9 mRNA accumulation, although this steroid enhanced CYP2C8, CYP2C19, and CYP3A4 transcripts by 330% (range 230–430%), 285% (range 270–300%), and 420%, respectively. RIF elicited substantial increases in CYP2C9 mRNA accumulation, although this steroid enhanced CYP2C8, CYP2C19, and CYP3A4 transcripts by 330% (range 230–430%), 285% (range 270–300%), and 420%, respectively. RIF elicited substantial increases in CYP2C9 mRNA accumulation, although this steroid enhanced CYP2C8, CYP2C19, and CYP3A4 transcripts by 330% (range 230–430%), 285% (range 270–300%), and 420%, respectively. RIF elicited substantial increases in CYP2C9 mRNA accumulation, although this steroid enhanced CYP2C8, CYP2C19, and CYP3A4 transcripts by 330% (range 230–430%), 285% (range 270–300%), and 420%, respectively. RIF elicited substantial increases in CYP2C9 mRNA accumulation, although this steroid enhanced CYP2C8, CYP2C19, and CYP3A4 transcripts by 330% (range 230–430%), 285% (range 270–300%), and 420%, respectively. RIF elicited substantial increases in CYP2C9 mRNA accumulation, although this steroid enhanced CYP2C8, CYP2C19, and CYP3A4 transcripts by 330% (range 230–430%), 285% (range 270–300%), and 420%, respectively. RIF elicited substantial increases in CYP2C9 mRNA accumulation, although this steroid enhanced CYP2C8, CYP2C19, and CYP3A4 transcripts by 330% (range 230–430%), 285% (range 270–300%), and 420%, respectively.

Primary cultures of human hepatocytes were used in this study to assess the effects of exemplary P450-inducing agents on hepatic expression of the CYP2C subfamily members CYP2C8, CYP2C9, and CYP2C19. We employed cultured hepatocytes since this in situ model system has proven quite useful for predicting the in vivo responses of patients to certain chemical inducers as well as potential drug interactions (Kedderis, 1997; LeCluyse et al., 2000; Hamilton et al., 2001). We found that RIF, DEX, and PB, which are not only commonly used therapeutics, but also PXR and CAR activators and/or ligands, were all capable of markedly enhancing the expression of CYP2C8, CYP2C9, and CYP2C19 enzyme levels in hepatocytes. Evidence that the increased expression of these CYP2C450 proteins stemmed from increased transcription of their corresponding structural genes was provided by the enhanced CYP2C450 mRNA levels found in induced-treated cells (Figs. 3 and 5). Of the three inducers tested, RIF proved the most potent in terms of increasing CYP2C8 expression, whereas PB was more efficacious as an inducer of hepatocyte CYP2C9 and CYP2C19 levels. In comparison, DEX was found to be a relatively weak CYP2C enzyme inducer, although this synthetic steroid nevertheless proved capable of increasing CYP2C8 and CYP2C19 transcripts at least 4-fold (Fig. 6). In contrast, CYP2C9 expression was not markedly enhanced in hepatocytes treated with DEX. These results are consistent with a previous observation (Gerbal-Chaloin et al., 2002) in which DEX exhibited little effect on CYP2C9 transcripts compared with RIF and an earlier study that demonstrated that this glucocorticoid had minimal effect on CYP2C9 expression and catalytic activity despite enhanced mRNA accumulation (Gerbal-Chaloin et al., 2001). Increased abundance of CYP2C enzymes in RIF-, DEX-, and/or PB-treated hepatocytes would likely result in an enhanced cellular capacity to metabolize drugs (e.g., warfarin, diclofenac, paclitaxel, and omeprazole) that are substrates for these P450s.

Cultured human hepatocytes exposed to PB, DEX, and, in particular, RIF were shown here to contain increased CYP3A4 mRNA and protein levels (Figs. 2–4 and 6). Prior studies have established that the highly inducible expression of CYP3A4 is regulated via binding or activation by chemicals, including RIF and PB to PXR and/or CAR, followed by the subsequent binding of the receptor to XRE and/or XRE-M (Goodwin et al., 1999), which ultimately results in CYP3A4 gene transcription (Barwick et al., 1996; Lehmann et al., 1998; Sueyoshi et al., 1999; Jones et al., 2000; Moore et al., 2000; Xie et al., 2000). Because the expression of certain CYP2C enzymes can be stimulated by the same agents (Morel et al., 1990; LeCluyse et al., 2000; Gerbal-Chaloin et al., 2001; Rae et al., 2001), we surmised that regulation of these P450 genes may also be mediated, at least in part, by orphan nuclear receptors. Gerbal-Chaloin et al. (2001) have studied the induction of human CYP2C enzymes by the same chemicals as those employed here and, in most cases, our results are in complete agreement with theirs. One common finding is the extensive degree of variability noted in constitutive...
and inducible CYP2C enzyme expression among the samples, whereas another is the greater RIF-mediated induction of CYP2C8 compared with CYP2C9 and CYP2C19, a phenomenon also described by Rae et al. (2001).

Gerbal-Chaloin et al. (2001) reported that CYP2C8, CYP2C9, and CYP2C19 mRNA and protein were expressed at substantial levels in untreated human hepatocytes cultured for up to 96 h, whereas Runge et al. (2000) described that the latter two P450s were maintained for 48 days in culture. We found, however, that only CYP2C9 protein was highly expressed in untreated hepatocytes after culture for 72 h (Fig. 1), whereas CYP2C8 and CYP2C19 protein levels were poorly maintained (although we were able to detect their corresponding mRNAs). Whether such discrepant results stem from inter-laboratory differences in the culture medium and/or the substratum utilized remains to be explored, although it is well known that the maintenance of P450 enzyme expression in cultured hepatocytes can be fraught with difficulties. The problem is generally more severe with rodent hepatocytes (Brown et al., 1995) than with those from humans (Runge et al., 2000), although we have found that certain human P450 enzymes (e.g., CYP2E1 and CYP4A11) are also not well maintained in culture (J. M. Lasker and J. L. Raucy, unpublished observations). For this study, where human hepatocytes were cultured on collagen-coated plates, CYP2C9 protein expression remained reasonably stable over at least 120 h (72 h pretreatment plus 48 h treatment), with enzyme levels in control (DMSO-treated) cells remaining at nearly 75% of those found in zero time cells (data not shown). However, the same untreated hepatocytes no longer expressed appreciable amounts of immunoreactive CYP2C8 and CYP2C19 protein (Fig. 1, A and B), suggesting that both of these CYP2C P450s decline rapidly in culture, at least under the conditions we used.

Upon RIF treatment, CYP2C8 and CYP2C19 expression increased markedly in cultured cells, although two subjects (HH933 and HH954) failed to respond (Figs. 1 and 2). Our inability to detect CYP2C19 protein in RIF-treated hepatocytes was not due to the inherent instability of the enzyme in culture. Rather, the expression of CYP2C19 is polymorphic in nature (de Morais et al., 1994a,b), and those subjects who did not exhibit enhanced CYP2C19 expression upon RIF treatment may have been of the poor metabolizer genotype. A factor underlying the absence of a CYP2C19-inductive response to RIF may be the presence of polymorphisms also residing in the regulatory region of this gene. The same phenomenon, namely, multiple single-nucleotide polymorphisms in the regulatory region, could explain why one subject (HH954; Fig. 2) failed to respond to RIF treatment with an increase in CYP2C8 content.

Interestingly, we found that the extent of induction of CYP2C9 and CYP2C19 mRNAs in cultured hepatocytes treated with PB was greater than that found with CYP2C8 and CYP3A4 mRNAs (Fig. 6). These results contrast a previous report (Gerbal-Chaloin et al., 2001) indicating that 100 μM PB produced similar induction of CYP2C8 and CYP2C9 mRNA. The reason for this discrepancy is unclear but may relate to variability among hepatocyte samples. In the present investigation, PB-mediated induction of CYP2C9 and CYP2C19 transcripts was much larger than that elicited by either RIF or DEX. Conversely, RIF administration caused a greater enhancement of hepatocyte CYP2C8 and CYP3A4 mRNA levels compared with PB treatment. The similarities between CYP3A4 and CYP2C8 in terms of their response to PB and RIF indeed suggest that PXR may be participating in the induction of both of these P450s (Lehmann et al., 1998). This conclusion is based upon findings here and in a recent report describing paclitaxel-mediated induction of CYP3A4 and CYP2C8 via PXR in human hepatocytes (Synold et al., 2001). That these P450s are coregulated may also explain their similar responses to other inducing agents, such as DEX and omeprazole. In contrast, PB and the PB-like compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene activate CAR and induce CYP3A in rodent hepatocytes, but these agents do not activate PXR (Xie et al., 2000). Conversely, RIF serves as a ligand for PXR but fails to activate CAR (Moore et al., 2000). Therefore, the greater responsiveness of CYP2C9 and CYP2C19 to PB treatment compared with CYP3A4 and CYP2C8 indicates that CAR may represent an important transcription factor in terms of the regulation of the former CYP2C enzymes.

In summary, primary cultures of human hepatocytes were employed to demonstrate that the CYP2C enzymes CYP2C8, CYP2C9, and CYP2C19 are inducible by therapeutic agents that also function as ligands and/or activators for the orphan nuclear receptors PXR and CAR. CYP2C9 is well maintained when hepatocytes are cultured in a serum-free, chemically defined medium on collagen-coated plastic dishes, whereas CYP2C8 and CYP2C19 are rapidly lost under these culture conditions. CYP2C8 is the CYP2C P450 inducible to the greatest extent by RIF, PB, and/or DEX treatment, whereas CYP2C9 and CYP2C19 expression is more responsive to PB treatment. DEX is a comparatively weak inducer of all three P450s. These different patterns of CYP2C enzyme induction suggest distinct mechanisms of regulation, and the similarities between the inductive responses of CYP2C9 and CYP2C19 to all three xenobiotics raises the possibility of their coregulation. On the other hand, the expression of CYP2C9 was enhanced by RIF and DEX in a manner very similar to that of CYP3A4, indicating similar modes of regulation. If that is indeed the case, the 5′-upstream region of the human CYP2C9 gene may contain a xenobiotic response element capable of binding a ligand complexed to a PXR/RXR heterodimer.

References