IN VIVO AND IN VITRO INDUCTION OF CYTOCHROME P450 ENZYMES IN BEAGLE DOGS

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ABSTRACT:
The aim of this study was to determine the in vitro and in vivo effects of several prototypical inducers, namely β-naphthoflavone, 3-methylcholanthrene, phenobarbital, isoniazid, rifampin, and clofibric acid, on the expression of cytochrome P450 (P450) enzymes in beagle dogs. For the in vitro induction study, primary cultures of dog hepatocytes were treated with enzyme inducers for 3 days, after which microsomes were prepared and analyzed for P450 activities. For the in vivo induction study, male and female beagle dogs were treated with enzyme inducers for 4 days (with the exception of phenobarbital, which was given for 14 days), after which the livers were removed and microsomal P450 activities were determined ex vivo. Treatment of male beagle dog hepatocyte cultures (n = 3) with β-naphthoflavone or 3-methylcholanthrene resulted in up to a 75-fold increase in microsomal 7-ethoxyresorufin O-dealkylase (CYP1A1/2) activity, whereas in vivo treatment of male and female beagle dogs with β-naphthoflavone followed by ex vivo analysis resulted in up to a 24-fold increase. Phenobarbital caused a 13-fold increase in 7-benzyloxyresorufin O-dealkylase (CYP2B11) activity in vitro and up to a 9.9-fold increase in vivo. Isoniazid had little or no effect on 4-nitrophenol hydroxylase activity in vitro. Rifampin caused a 13-fold induction of testosterone 6β-hydroxylase (CYP3A12) activity in vitro and up to a 4.5-fold increase in vivo. Treatment of dogs in vivo or dog hepatocytes in vitro with clofibric acid appeared to have no effect on CYP4A activity as determined by the 12-hydroxylation of lauric acid. In general, the absolute rates (picomoles per minute per milligram of microsomal protein) of P450 reactions catalyzed by microsomes from cultured hepatocytes (i.e., in vitro rates) were considerably lower than those catalyzed by microsomes from dog liver (i.e., ex vivo rates). These results suggest that beagle dogs have CYP1A, CYP2B, CYP2E, and CYP3A enzymes and that the induction profile resembles the profile observed in humans more than in rats.

Enzyme induction enables some xenobiotics to accelerate their own biotransformation (auto-induction) or the biotransformation and elimination of other drugs. Drugs and new molecular entities are often screened for their ability to induce P4501 and other phase I and phase II enzymes with the aim of predicting or explaining drug-drug interactions in humans, and increases in liver weight and/or proliferation of the endoplasmic reticulum or peroxisomes, pharmacokinetic tolerance, and/or formation of liver and thyroid tumors in rodents. Enzyme induction is often evaluated by an ex vivo procedure whereby the xenobiotic is administered to rats and mice (or other laboratory animals) in vivo, followed by an evaluation of changes in the levels of liver microsomal P450 and other enzymes in vitro (Parkinson, 2001).

A number of P450 enzymes in human and/or rodent liver microsomes are inducible, including various members of the CYP1A, CYP2A, CYP2B, CYP2C, CYP2E, CYP3A, and CYP4A subfamilies (Parkinson, 2001). To date, eight P450 genes have been sequenced in the dog (compared with up to 60 sequenced genes in the rat) including CYP1A1, CYP1A2, CYP2B11, CYP2C11, CYP2C41, CYP2D15, CYP3A12, and CYP3A26 (http://www.icgeb.trieste.it/~P450srv/genesperspecies.html). However, for a species that is widely used by the pharmaceutical industry to assess the safety of drugs under development, relatively little information is available in the literature on the inducibility of P450 enzymes in the dog. Several studies have been performed in which a particular P450 cDNA has been cloned and the sequence compared with related enzymes in other species (Ohta et al., 1989; Graves et al., 1990; Ciaccio et al., 1991; Sakamoto et al., 1995; Fraser et al., 1997; Roussel et al., 1998; Lankford et al., 2000). However, much remains to be elucidated about the structure, function, and regulation of dog P450 enzymes. Two CYP1A enzymes, P450 D2 and P450 D3, have been purified from the liver of polychlorinated biphenyl-treated female beagle dogs (Ohta et al., 1989). Catalytic and structural properties of both proteins were shown to be similar to rat CYP1A2, although P450 D3 exhibits spectral properties similar to those of rat CYP1A1. It is not known whether either of these proteins is constitutively expressed in dog liver (Ohta et al., 1989).

Dog liver microsomes contain CYP2B11 (also called PBD-2), a constitutively expressed and phenobarbital-inducible enzyme with high metabolic activity toward 2,4,5,2',4',5'-hexachlorobiphenyl.

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(Duignan et al., 1988). Like the rat CYP2B1, dog CYP2B11 (PDB-2) catalyzes both the 16α- and 16β-hydroxylation of testosterone. Another testosterone 16α-hydroxylase has been purified from dog liver and identified as a member of the CYP2C subfamily (Uchida et al., 1990). Whereas rat CYP2B1 catalyzes the 16α- and 16β-hydroxylation of testosterone at roughly equal rates, dog CYP2B11 preferentially catalyzes the 16α-hydroxylation of testosterone at 13 to 15 times the rate of testosterone 16β-hydroxylation (Coulter et al., 1993; Ohmori et al., 1993). Another CYP2 protein, CYP2D15, has been cloned by Sakamoto et al. (1995). Recently, CYP2E1 cDNA has been cloned from beagle dogs followed by characterization and expression of the encoded protein (Lankford et al., 2000). Interestingly, the amino acid sequence of dog CYP2E1 exhibits 77% identity to the human ortholog, which is slightly higher than the identity to the rodent or rabbit sequence (75–76%). Characterization of the expressed CYP2E1 protein indicated that dog CYP2E1 has a lower affinity for chloroxazone than does human CYP2E1 (Lankford et al., 2000).

Dog liver is thought to express multiple forms of CYP3A, as has been shown in rat and human. PB1-D, a CYP3A enzyme, was purified from phenobarbital-treated dog liver but also appears to be expressed constitutively (Ciaccio and Halpert, 1989). Molecular and immunoochemical analyses indicate the presence of at least one other CYP3A enzyme in dog liver (Ciaccio and Halpert, 1989; Ciaccio et al., 1991). In contrast to rats, there are no marked sex differences in CYP3A activity in dog liver microsomes. Like the corresponding rat enzyme, the dog CYP3A enzyme, CYP3A12 catalyzes the 6β-hydroxylation of testosterone (Ciaccio and Halpert, 1989). In addition, CYP3A12 catalyzes the 16β-hydroxylation of testosterone, which is also catalyzed in part by CYP2B11. Recently, a cDNA encoding a protein exhibiting 95.6% amino acid identity with CYP3A12 was isolated from phenobarbital-induced dogs (Fraser et al., 1997). This enzyme, called CYP3A26, is not as prominent as CYP3A12 in hydroxylating steroids.

A CYP4A protein has yet to be identified in dog liver (Adas et al., 1999). DUT-1, purified from liver microsomes of untreated male beagle dogs, catalyzes the 12-hydroxylation of lauric acid, but the N-terminal sequence of this protein is different from any other P450 characterized to date (Shiraga et al., 1994). Due to the lack of information on the inducibility of P450 enzymes in the dog, we examined the induction profile of several P450 enzymes in cultured male dog hepatocytes (in vitro) and in microsomes prepared from the livers of male and female dogs that were treated with prototypical P450 inducers (in vivo). These inducers included β-naphthoflavone, 3-methylcholanthrene, phenobarbital, isoniazid, rifampin, and clofibric acid. In each case, several marker substrate reactions and Western immunoblotting were used to assess P450 enzyme induction.

Materials and Methods

Chemicals and Reagents. Insulin, Dulbecco's modified Eagle's medium, GlutaMAX-1 (dipeptide α-alanyl-L-glutamine 200 mM supplied in 0.85% NaCl), modified Chee's medium, minimal essential medium nonessential amino acids, and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA). Matrigel and ITS (M), isoniazid (100 μM), 3-methylcholanthrene (10 μM), phenobarbital (250 μM), rifampin (50 μM), or clofibric acid (100 μM). Prototypical inducers were dissolved in DMSO, except isoniazid, which was dissolved in saline. At the end of the treatment period, the hepatocytes were harvested, and microsomes were prepared as described previously (Madan et al., 1999). The microsomal samples were stored at −80°C for later analysis of P450 activities. The protein concentration in the microsomal samples was determined with a BCA Protein Assay Kit, according to Technical Bulletin 23225X from Pierce Chemical Co. (Smith et al., 1985; Wielchelman et al., 1987)

Treatment of Dogs in Vivo and Preparation of Microsomes. Male and female beagle dogs (7–18 months old; Ridglan Farms, Mt. Horeb, WI) were treated with subcutaneous injection with corn oil vehicle (two males and two females), salin vehicle (two males and two females), 10 mg/kg/day β-naphthoflavone (two males and one female), 10 mg/kg/day rifampin (two males and two females), 10 mg/kg/day clofibric acid (two males and one female), or phenobarbital (two males and two females). Animals treated with phenobarbital were treated for 14 consecutive days with dosing escalations from 10 mg/kg/day (days 0 and 1) to 20 mg/kg/day (days 2 through 5) to 30 mg/kg/day (days 6 through 13). Dogs treated with vehicle or enzyme inducers (other than phenobarbital) were treated for 4 consecutive days. After completion of the dosing regimen, the dogs were euthanized by intravenous injection with sodium pentobarbital (5 ml per dog) followed by excision of the livers, which were subsequently perfused with chilled saline. After perfusion, the livers were snap-frozen in liquid nitrogen and stored at −70°C. Microsomes were prepared as described previously (Pearce et al., 1996). This experimental design was reviewed and approved by the Institutional Animal Care and Use Committee of WIL Research Laboratories, Inc. (Ashland, OH).

Enzyme Assays. The O-dealkylation of 7-ethoxyresorufin and 7-benzoyloxyresorufin, the 6β-, 16α-, and 16β-hydroxylation of testosterone, the 4-hydroxylation of nitrophenol, and the 12-hydroxylation of lauric acid were determined by methods described previously (Burke and Mayer, 1974; Wood et al., 1983; Koop, 1986; Sorderfan et al., 1987; Romano et al., 1988; Sorderfan and Parkinson, 1988; Giera and Van Lier, 1991; Tierney et al., 1992; Burke et al., 1994; Pearce et al., 1996). The incubation conditions for each of the assays are given in Table 1.

Western Immunoblotting. Microsomal samples were analyzed by Western immunoblotting to determine levels of immunoreactive CYP1A, 2B, 3A, and 4A. Microsomes were subjected to SDS-polyacrylamide gel electrophoresis, based on the method originally described by Laemmli (1970). Briefly, microsomes were mixed in a 1:1 ratio with NuPage sample dilution buffer (pH = 8.5) containing 1.09 M glycerol, 141 mM Tris-base, 106 mM Tris-HCl, 73 mM SDS, 0.51 mM EDTA, 0.22 mM Serva Blue G250, and 0.175 mM phenol red and heated at 100°C for 2 to 5 min. The denatured proteins (up to 10 μg per lane, as specified in figure legends) were subjected to electrophoresis on precast 4 to 12% NuPage bis-Tris gels (pH 6.4; constant voltage of 200 V; electrophoresis time ~55 min) (Novex). Proteins were transferred electrolytically to polyvinylidene difluoride membranes and subjected to immu-
noblotted, based on the method by Towbin et al. (1979), with a Blot Module from Novex. Membranes were incubated in blocking buffer containing 10% (w/v) Carnation nonfat dry milk and 0.05% (v/v) Tween 20 in Tris-buffered saline (10 mM Tris-HCl and 150 mM NaCl, pH 7.4) and then probed with polyclonal antibodies raised against purified rat liver microsomal CYP1A1, CYP2B1, CYP3A1 (Parkinson and Gemzik, 1991), or CYP4A (Affinity Bioreagents, Golden, CO) at final concentrations ranging from 0.25 μg/ml to 10 μg/ml. The secondary antibody was affinity-purified goat-anti-rabbit IgG (H + L) conjugated with alkaline phosphatase from Kirkegaard and Perry Laboratories, which was diluted in blocking buffer to a final concentration of 0.25 μg/ml. Membranes were washed three times with Tris-buffered saline, and the proteins were visualized by incubation with BCIP/NBT phosphatase substrate.

**Statistical Analysis.** For analysis of microsomes from the in vitro cultures, data are mean ± standard deviation of three preparations. An asterisk (*) indicates a statistically significant (p < 0.05) difference from control as determined by a one-way repeated measures analysis of variance test followed by a Dunnett’s post hoc test. For ex vivo analysis of microsomes, data are duplicate measurements of a pool of microsomes from two dog livers (with the exception of the β-naphthoflavone and clofibric acid females, in which case, only a single dog liver was available).

**Results and Discussion**

There are several reports on the cloning and sequencing of cDNAs encoding dog P450 enzymes, and on the characterization of the catalytic activity of dog P450 enzymes (Gee et al., 1984; Duignan et al., 1987; Ciaccio and Halpert, 1989; Ciaccio et al., 1991; Graves et al., 1990; Nicolas et al., 1995; Sakamoto et al., 1995; Ekins et al., 1996; Fraser et al., 1997; Nishibe et al., 1998; Roussel et al., 1998; Adas et al., 1999; Lankford et al., 2000; Hewitt et al., 2001). Several studies have shown that a select compound can cause induction of one or more P450 enzymes in dog (McKillop and Pickup, 1991; Robertson et al., 1995; Nishibe and Hirata, 1995; McKillop et al., 1998; Ma et al., 1998). However, little information is available on the profile of P450 enzymes induced by prototypical P450 enzyme inducers, namely, those that cause CYP1A, CYP2B, CYP2E, CYP3A, or CYP4A induction in other mammalian species (McKillop et al., 1998; Nishibe and Hirata, 1993; Nishibe and Hirata, 1995; Nishibe et al., 1998). Along with enzyme induction, a large number of chemically diverse compounds have been shown to cause hepatic hyperplasia and proliferation of the endoplasmic reticulum and peroxisomes in rodents. Such compounds also cause formation liver tumors in rodents after chronic administration (Reddy and Rao, 1977; Reddy and Qureshi, 1979; Fitzgerald et al., 1981). On the other hand, nonrodent species, including dog, have been reported to be much less sensitive to peroxisome proliferation (Foxworthy et al., 1990).

**Evaluation of Dog Hepatocytes.** Viability of the final preparation of hepatocytes (after Percoll gradient centrifugation) was greater than 70% for each of the three preparations of dog hepatocytes. Dog
hepatocytes attached to collagen-coated culture dishes. After 6 days in culture, representative culture dishes seeded with freshly isolated hepatocytes were photographed under light microscopy (Fig. 1). Hepatocytes exhibited morphology traits consistent with normal cells: the cells were cuboidal and contained granular cytoplasm with one or two centrally located nuclei (Fig. 1). Interestingly, the cellular morphology of the cultured dog hepatocytes closely resembled that of cultured human hepatocytes but not rat hepatocytes (LeCluyse et al., 1999, 2000). Unlike rat hepatocyte cultures, dog hepatocyte cultures tended to be completely confluent, covering nearly 100% of the culture dish. Even though the collagen substratum and Matrigel overlay caused cells to spread and flatten to a certain degree, the hepatocytes retained a high degree of three-dimensional architecture.

**Induction of Cytochrome P450 Content in Dogs in Vivo.** The effects of treating male and female dogs in vivo with β-naphthoflavone, phenobarbital, rifampin, or clofibrate acid on P450 content are shown in Table 1. Treatment with β-naphthoflavone resulted in up to a 1.5-fold increase in P450 content, whereas treatment with phenobarbital or rifampin resulted in up to 2.0- and 3.1-fold increases, respectively. On the other hand, treatment with clofibrate acid resulted in up to a 32% decrease in P450 content. The determination of P450 content requires relatively large amounts of microsomal protein (approximately 1 mg per assay); therefore, this assay was not performed with microsomal samples from cultured hepatocytes.

**Induction of EROD (CYP1A1/2) Activity.** Although EROD activity has not been shown to be specific for dog CYP1A as it has for the rat, there is indirect evidence suggesting that dog CYP1A catalyzes EROD. McKillop (1985) reported that EROD activity increases 3- to 5-fold in dogs treated with the CYP1A inducer β-naphthoflavone but not with the CYP2B inducer phenobarbital. CYP1A1 and CYP1A2 mRNAs are expressed at low levels in the liver of untreated dogs, but the levels increase after treatment with the polychlorinated biphenyl mixture, Kameklor KC-500 (Uchida et al., 1990). Treatment of cultured dog hepatocytes with β-naphthoflavone has been shown to increase EROD activity by 25-fold (Nishibe and Hirata, 1993).

The effects of treating dog hepatocyte cultures (n = 3) with β-naphthoflavone and 3-methylcholanthrene, phenobarbital, isoniazid, rifampin, or clofibrate acid on EROD activity are shown in Fig. 2A. Treatment of cultured hepatocytes with β-naphthoflavone or 3-methylcholanthrene resulted in a 75-fold induction of EROD (CYP1A1/2) activity, whereas the other inducers had little or no effect (Fig. 2A). Both the absolute rates (expressed as picomoles per minute per milligram of microsomal protein) and fold induction of CYP1A1/2 by β-naphthoflavone were reproducible (<25% relative standard deviation) among three preparations of cultured dog hepatocytes. The effects of treating male or female beagle dogs in vivo with β-naphthoflavone, phenobarbital, rifampin, or clofibrate acid, followed by ex vivo analysis of the liver microsomal samples, are shown in Fig. 2B. Treatment of dogs with β-naphthoflavone resulted in up to a 24-fold increase in EROD activity. Western immunoblotting confirmed that treatment of male or female dogs in vivo or in vitro with β-naphthoflavone (or 3-methylcholanthrene; in vitro only), but not phenobarbital, rifampin or clofibrate acid, caused a marked increase in immunoreactive CYP1A1/2 (Fig. 2C).

There are two notable differences between the CYP1A1 induction in vitro and ex vivo. First, EROD activity (picomoles per minute per milligram of microsomal protein) in vitro was approximately one-tenth of that observed ex vivo. Second, Western immunoblotting revealed two immunoreactive proteins (tentatively identified as CYP1A1 and CYP1A2) in microsomal samples from dogs treated with β-naphthoflavone in vivo, whereas the same treatment in vitro appeared to induce a single immunoreactive protein (Fig. 2C). The exact identity of these immunoreactive proteins is not known; however, since the lower band appeared in untreated male and female dogs, it would seem, by analogy with most other species (with the exception of guinea pig), that the lower band is CYP1A2 (Thomas et al., 1984). By a similar comparison, it appears that only CYP1A1 induction was detected in vitro.

**Induction of BROD (CYP2B11) Activity.** 7-Benzoyloxyresorufin

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**Fig. 2.** The effect of treating dog hepatocytes or subcutaneous injection of dogs with prototypical P450 inducers on 7-ethoxyresorufin O-dealkylase (CYP1A) activity.

Fresly isolated dog hepatocytes were isolated and cultured for up to 6 days, and beagle dogs were treated by subcutaneous injection as described under Materials and Methods. A, in vitro cultures treated with prototypical inducers. B, male and female beagle dogs were treated with prototypical inducers. C, microsomes were analyzed by Western immunoblotting. Gels were loaded with 10 μg per lane (except for Rat BNF samples, which were 0.05, 0.10, and 0.20 μg per lane for ex vivo samples and 0.1 μg per lane for in vitro samples). Samples of rat liver microsomes prepared from rats treated with one of four prototypical inducers were also analyzed as reference standards. Asterisk (★) indicates p < 0.05 as described under Materials and Methods. UT, untreated; BNF, β-naphthoflavone; 3-MC, 3-methylcholanthrene; PB, phenobarbital; RIF, rifampin; INH, isoniazid; CLOF, clofibrate acid; CO, corn oil.
O-dealkylation has been shown to be a specific substrate for dog CYP2B11 (Klekotka and Halpert, 1995). Like the corresponding rat enzyme, dog CYP2B11 (PDB-2) catalyzes both the 16α- and 16β-hydroxylation of testosterone; however, dog CYP2B11 preferentially catalyzes the 16α-hydroxylation of testosterone at 13 to 15 times the rate of testosterone 16β-hydroxylation (Coulter et al., 1993; Ohmori et al., 1993).

Treatment of cultured dog hepatocytes with phenobarbital resulted in a 13-fold induction of BROD (CYP2B11) activity, whereas the other inducers tested had little or no effect (Fig. 3A). Both the absolute rates (expressed as picomoles per minute per microgram of microsomal protein) and fold induction of BROD by phenobarbital were reproducible (<25% relative standard deviation) among three preparations of cultured dog hepatocytes. Treatment of dogs with phenobarbital resulted in up to a 9.9-fold increase in BROD activity (Fig. 3B). Western immunoblotting confirmed that treatment of male or female dogs with phenobarbital, but not β-naphthoflavone, rifampin, or clofibric acid, caused a marked increase in immunoreactive CYP2B11 (Fig. 3C). Induction of CYP2B11 in cultured hepatocytes was also measured by testosterone 16α-hydroxylase activity (Duignan et al., 1987). The pattern of induction of testosterone 16α-hydroxylase activity (Table 1) was similar to that shown in Fig. 3, A and B, for BROD activity.

It should be noted that the “induced” level of CYP2B11 (in vitro) on the Western immunoblot appeared to be the same as the “control” level in microsomes prepared from untreated male beagle dog, an observation that is consistent with the BROD results (Fig. 3, A and B). These data suggest a marked difference in the degree to which CYP2B11 is expressed in vitro versus ex vivo.

Induction of 4-Nitrophenol Hydroxylase (CYP2E1) Activity. Lankford et al. (2000) have isolated and characterized a full-length CYP2E1 cDNA from a beagle liver cDNA library. The deduced amino acid sequence shares 77% identity to rat, rabbit, and human CYP2E1. In rodents, CYP2E1 catalyzes the hydroxylation of 4-nitrophenol (Koop, 1986). Treatment of cultured hepatocytes with the prototypical enzyme inducers had little or no effect on 4-nitrophenol hydroxylase activity (Fig. 4). Induction of CYP2E1 was also measured by chlorzoxazone 6-hydroxylase activity, a measure of human CYP2E1 activity. In agreement with the data shown in Fig. 4 for 4-nitrophenol hydroxylation, treatment with prototypical P450 inducers had little or no effect on chlorzoxazone 6-hydroxylase activity (data not shown). Western immunoblotting for detection of the CYP2E1 isozyme was not performed. Consistent with our results, Jayyosi et al. (1996) reported that treatment of dogs with isoniazid had only a slight effect on chlorzoxazone 6-hydroxylase activity and immunoreactive CYP2E1 levels.

Induction of Testosterone 6β-Hydroxylase (CYP3A12) Activity. Treatment of cultured hepatocytes with phenobarbital or rifampin resulted in a 7.3- and 13-fold induction of testosterone 6β-hydroxylase (CYP3A12) activity, respectively, whereas the other inducers examined had little or no effect (Fig. 5A). Western immunoblotting
confirmed that treatment of cultured hepatocytes with phenobarbital and rifampin, but not β-naphthoflavone or 3-methylcholanthrene, isoniazid, or clofibric acid, caused a marked increase in immunoreactive CYP3A12 (Fig. 5C). It should be noted that the induced levels of CYP3A12 on the Western immunoblot indicated a level of protein expression comparable with that observed in microsomes prepared from untreated male beagle dog, an observation that is consistent with the testosterone 6β-hydroxylase results (Fig. 5, A and B). These data also suggest a marked difference in the degree to which CYP3A12 is expressed in vitro versus ex vivo.

Treatment of dogs with phenobarbital or rifampin resulted in up to a 2.6- and 4.5-fold induction of testosterone 6β-hydroxylase (CYP3A12) activity, respectively, whereas the other inducers examined had little or no effect (Fig. 5B). Western immunoblotting confirmed that treatment of male or female dogs with phenobarbital or rifampin resulted in up to a 2.6- and 4.5-fold induction of testosterone 6β-hydroxylase (CYP3A12) activity, respectively, whereas the other inducers examined had little or no effect (Fig. 5B).
rifampin, but not β-naphthoflavone or clotfibric acid, caused a marked increase in immunoreactive CYP3A12 (Fig. 5C). As noted previously for CYP1A and CYP2B, the CYP3A12 activity in vitro was substantially lower (∼1/5) than that observed ex vivo. However, in the case of CYP3A12, the fold induction was greater in vitro, which is attributable to the higher control CYP3A12 activity ex vivo. These results are in agreement with a study that reported a 5-fold induction of testosterone 6β-hydroxylation in dog hepatocytes followed by treatment with 30 μM rifampin (Nishibe and Hirata, 1995).

**Induction of Lauric Acid 12-Hydroxylase (CYP4A) Activity.** Treatment of freshly isolated hepatocytes with clofibric acid had little or no effect on lauric acid 12-hydroxylase activity (CYP4A); however, phenobarbital and rifampin appeared to increase this activity by 2-fold (Fig. 6A). Treatment of dogs in vivo with the prototypic inducers examined had little or no effect on lauric acid 12-hydroxylase activity (Fig. 6B). The lack of CYP4A4 induction by clofibric acid was confirmed by Western immunoblotting (Fig. 6C); however, the 2-fold increase in activity by phenobarbital and rifampin was not associated with a 2-fold increase in CYP4A4 levels. As in other cases, the rate of lauric acid 12-hydroxylation was substantially lower in vitro compared with that observed ex vivo.

Treatment of rats with the peroxisome proliferator, clotfibric acid, results in induction of lauric acid 12-hydroxylase activity, a marker of CYP4A1–3. In contrast to the rat, human CYP4A does not appear to be an inducible enzyme (Butterworth et al., 1989). DUT-1, purified from liver microsomes of untreated male beagle dogs, catalyzes the 12-hydroxylation of lauric acid, but the NH2-terminal sequence of this protein is different from that of any other P450 characterized to date (Shiraga et al., 1994). Treatment of dog hepatocytes with clotfibric acid and subcutaneous injection of male and female beagle dogs with clotfibric acid had no effect on lauric acid 12-hydroxylase activity. Consistent with this finding, clotfibric acid did not induce immunoreactive CYP4A either in vitro or in vivo. Since DUT-1 does not bear amino acid sequence homology with CYP4A proteins (Shiraga et al., 1994), it is possible that DUT-1 (or another enzyme that catalyzes 12-hydroxylation of lauric acid, but the NH2-terminal sequence of this protein is different from that of any other P450 characterized to date (Shiraga et al., 1994)).

**References**


