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Role of CYP1A2 and CYP2E1 in the pentoxifylline ciprofloxacin drug interaction

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Abstract

In this study the drug interaction between ciprofloxacin (CIPRO) and pentoxifylline (PTX) was investigated and the role of CYP1A2 in the drug interaction was determined with the aid of a selective CYP1A2 inhibitor, furafylline (FURA), and the *Cyp*1A2 knockout mouse. Serum concentrations of PTX (83.4 ± 1 μmol/l) and metabolite-1 (M-1) (13.7 ± 2.8 μmol/l) following a single injection of PTX (100 mg/kg i.p.) were significantly higher ($P < 0.05$) in mice treated with CIPRO (25 mg/kg i.p. 9 days) compared to serum concentrations of PTX (46.3 ± 0.5 μmol/l) and M-1 (6.4 ± 1.1 μmol/l) in mice administered saline. Murine hepatic microsomes were incubated with PTX alone or the combination of PTX and CIPRO. The metabolism of PTX in the murine hepatic microsomes containing both CIPRO and PTX was significantly decreased compared to microsomes incubated with PTX alone, suggesting that CIPRO may inhibit the metabolism of PTX. To further clarify the role of CYP1A2 in the metabolism of PTX in mice, the effect of a selective CYP1A2 mechanism based inhibitor, FURA, on the metabolism of PTX was investigated and our results indicate that FURA inhibited metabolism of PTX. We then investigated PTX elimination in the *Cyp*1A2 knockout mouse. Blood levels of PTX were assessed at 2 and 20 min following a single injection of PTX (32 mg/kg i.v). Serum concentration of PTX was determined in *Cyp*1A2 knockout mice compared to *Cyp*1A2 wild type control mice. The serum concentration of PTX in *Cyp*1A2 wild type mice ($n = 9$) was 22.2 ± 3.2 μmol/l at 20 min following injection of PTX. The serum concentration of PTX in *Cyp*1A2 knockout mice ($n = 11$) was significantly elevated at 20 min following injection of PTX compared to *Cyp*1A2 wild type mice. These results clearly indicate that inhibition of CYP1A2 catalytic activity that occurs in the *Cyp*1A2 knockout mice is sufficient to alter metabolism of PTX and result in markedly elevated levels in serum of *Cyp*1A2 knockout mice. The results of Western analysis in murine microsomes suggest that CYP1A2 protein levels were not altered by CIPRO indicating that CIPRO did not downregulate *Cyp*1A2. The results of Western analysis also indicated that CIPRO treatment increased CYP2E1 in mouse microsomes and the implications of these will be discussed.

Keywords: PTX; CIPRO; *Cyp*1A2 knockout; CYP1A2; FURA; CYP2E1; Proliferation

1. Introduction

Pentoxifylline (PTX), a cytokine antagonist, has been used to treat peripheral vascular disorders and has been identified as a potential therapeutic agent in the treatment of liver fibrosis [1]. A drug interaction between the methylxanthine derivative PTX (structure shown in Fig. 1) and the fluoroquinolone antibiotic ciprofloxacin (CIPRO, structure shown in Fig. 1) has been reported to occur in humans resulting in significantly elevated serum concentrations of PTX and its metabolite-1 (1-(5-hydroxyhexyl)-3,7-dimethylxanthine, structure shown in Fig. 1) [2]. We have reported that both PTX and its metabolite-1 (M-1) inhibit fibroproliferation and collagen synthesis, two of the hallmarks in hepatic fibrosis [3]. An increase in serum concentration of parent drug and active metabolite would likely provide enhanced therapeutic, antifibrotic benefit. The mechanism for the drug interaction between CIPRO and PTX was investigated in vivo in the mouse. An in vitro PTX metabolism assay was also developed in hepatic

Abbreviations: CIPRO, ciprofloxacin; PTX, pentoxifylline; FURA, furafylline; EROD, ethoxyresorufin-*o*-deethylase; AHH, arylhydrocarbon hydroxylase

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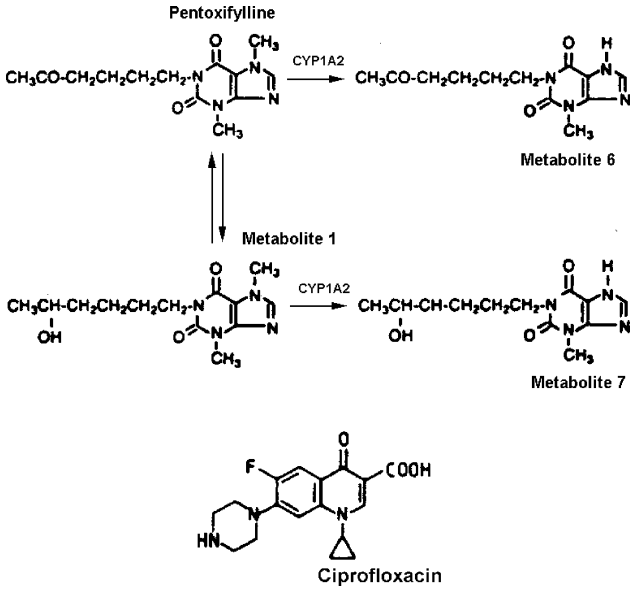

Fig. 1. Structures of pentoxifylline, metabolite-1 and ciprofloxacin. The pathways showing the metabolism of pentoxifylline to metabolite-1 and the metabolism of pentoxifylline to metabolite-6 and the metabolism of metabolite-1 to metabolite-7 are shown. The metabolism of pentoxifylline to metabolite-6 and the metabolism of metabolite-1 to metabolite-7 are proposed to be mediated by CYP1A2 and thus an inhibition of CYP1A2 would result in elevated sera levels of pentoxifylline and metabolite-1.

microsomes to determine the mechanism by which CIPRO alters the metabolism of PTX using murine microsomes. To further clarify the role of CYP1A2 in the metabolism of PTX we investigated the effect of a selective CYP1A2 inhibitor [4], furafylline (FURA), on the metabolism of PTX. PTX elimination was also investigated in the *Cyp*1A2 knockout mouse [5] compared to *Cyp*1A2 wild type control mice to assess whether inhibition of CYP1A2 and/or downregulation of *Cyp*1A2 in vivo was sufficient to alter PTX kinetics and support the in vitro results obtained using FURA. Western analysis was done to verify the effect of CIPRO on CYP1A2.

2. Methods

2.1. In vivo studies

We investigated the CIPRO/PTX interaction in mice (male, 25 g, CD1, Charles River Labs) and investigated the mechanism behind the drug interaction. In the in vivo experiment, mice ($n = 12$) were treated with CIPRO (Bayer) (25 mg/kg i.p. 9 days) and compared to control mice ($n = 12$) which received saline injection. PTX (Sigma Chemical Co.) (100 mg/kg i.p.) was administered to CIPRO treated and control mice and blood samples were obtained. PTX concentrations in serum were determined using HPLC. Samples were centrifuged and the serum was collected and extracted using solid phase extraction columns (JT Baker C18 cartridge) and 30 mg/ml phenacetin (Sigma Chemical Co.) was added as internal standard. The extracted samples were evaporated using a nitrogen evaporator and resuspended in mobile phase (70% Millipore water, 30% acetonitrile) 100 μl total volume.

2.2. HPLC analysis of PTX

PTX is analyzed routinely by HPLC analysis [6,7] using the maximal absorbance wavelength of 273 nm. The HPLC column is a 25 cm × 4.6 mm C18 reversed phase Beckman Ultrasphere (i.d. 5 μm). All samples were run on HPLC using a mobile phase 70% Millipore water, 30% acetonitrile, and flow rate of 2 ml/min. Concentrations of PTX in serum were determined using the standard curve for PTX and referenced to the internal standard phenacetin.

2.3. PTX metabolism in Cyp1A2 knockout mice

The *Cyp*1A2 knockout mouse is deficient in the *Cyp*1A2 gene, lacks the CYP1A2 protein and appears to be quite robust and have a normal growth rate [5]. Thus the *Cyp*1A2 knockout mouse provides a good model to assess the role of CYP1A2 in PTX metabolism. We assessed PTX blood levels in mice (4 months old, average weight 30 g) following intravenous injection of PTX (32 mg/kg). PTX blood levels were determined in homozygous *Cyp*1A2 knockout mice and compared to *Cyp*1A2 wild type mice. Blood was collected at two time periods following injection. At 2 min post PTX injection, a small blood sample was collected from the tail vein to verify the baseline concentration of PTX in serum. At 20 min, the mice were sacrificed and blood was obtained by cardiac puncture to assess PTX metabolism in the *Cyp*1A2 knockout mice and also to assess fibroproliferation (as described below). The i.v. route was chosen and the dose reduced to approximately 1/3 of the dose given i.p. to CD1 mice.

2.4. In vitro assay of PTX metabolism by murine microsomes

The in vitro metabolism of PTX was carried out in microsomes. Microsomes were prepared using the method of ElDefrawry et al. [8]and protein assessed by the method of Bradford [9]. The assay was a modification of one we have previously published for the metabolism of theophylline in vitro by microsomes [10] by adapting this method to measure metabolism of PTX in microsomes [11]. The incubation mixture contained Tris, $MgCl_2$, D-glucose-6-phosphate, NADP, glucose-6-phosphate dehydrogenase as the NADPH generating system (Gentest) 500 μM PTX with and without CIPRO or other inhibitor as required. CIPRO has been tested at 500 μM [12]. Following 2 h incubation at 37 °C, the internal standard phenacetin was added. The incubation mixture was then extracted with chloroform:isopropanol (90:10) and the extracts were evaporated.

2.5. Inhibition of CYP1A2 by selective isozyme inhibitor

To selectively inhibit CYP1A2, the specific isozyme inhibitor FURA (Gentest) (10 μM) was used in vitro in microsome assays [4] according to Newton et al. [13].

2.6. Enzyme assays

The method of Nebert and Gelboin [14] was used to assay the arylhydrocarbon hydroxylase (AHH) activity of the microsomes. The method used to measure ethoxyresorufin *o*-deethylase (EROD) activity of the microsomes was that described by Burke and Mayer [15].

2.7. Fibroproliferation assay

Fibroproliferation was assessed by modification of the tritiated thymidine incorporation method of Dohlman et al. [16] using fibroblasts as reported by Peterson [17]. Briefly, normal skin fibroblasts were resuspended in Dulbecco's (DMEM, Gibco), antibiotic/antimycotic (AA, Gibco) and 0.5% fetal calf serum (FCS). Two hundred microliter aliquots of cell suspension (8×10^3 cells) were added to 0.32 cm^2 flat bottom wells of 96-well microtiter plates and incubated for 24 h at 37 °C in 5% CO_2 in air. The media was replaced by 200 μl of DMEM supplemented with factors incubated as above for a further 22 h. DMEM supplemented with FCS (10%) was used to stimulate proliferation of fibroblasts in the presence or absence of sera obtained from *Cyp*1A2 wild type and *Cyp*1A2 knockout mice 20 min following a single injection with PTX (32 mg/kg i.p., Sigma Chemical Co.). Methyl-^{3}H-thymidine 0.5 μCi (Amersham) was added to each well, incubated for an additional 2 h and then the cells were harvested by aspiration onto glass fiber filters using a Brandel Cell Harvester (Xymotech Biosystems) and the radioactivity was determined by liquid scintillation. All samples were tested in quadruplicate. The ^{3}H-thymidine uptake assay was validated by manual cell counts and dimethylthiazol diphenyltetrazolium bromide (MTT) assay [18] as a measure of fibroblast number following treatment with either proliferative or antiproliferative agents [19].

2.8. Western analysis

Liver microsomes were prepared as described above and lysates were prepared in Laemmli sample buffer, boiled 5 min, and protein resolved on 10% SDS-polyacrylamide gel electrophoresis. The proteins were transferred to membranes and probed with antibodies to CYP1A2 (Gentest) and CYP2E1 (Gentest) and the protein band was visualized using LumiGlo (New England Biolabs). Protein was measured using a BioRad detergent compatible protein assay kit (Bio-Rad), and equal loading was confirmed by Coomassie staining of blots.

2.9. Statistical analysis

A one-way ANOVA was performed using SigmaStat version 2.0 software ($P < 0.05$ for significance). Student–Newman–Keuls test was used to examine differences between treatment groups ($P < 0.05$ for significance). Results are expressed as mean ± S.E.

3. Results

3.1. CIPRO and PTX interaction in vivo

In the in vivo experiments serum concentrations of PTX (83.4 ± 1 μmol/l) were significantly elevated ($P < 0.05$) in the CIPRO treated mice compared to saline controls (46.3 ± 0.5 μmol/l) indicating that a drug interaction between CIPRO and PTX occurs in the mouse when animals receive the CIPRO for 9 days prior to assessment of PTX elimination (Fig. 2).

3.2. Generation of M-1 in the presence and absence of CIPRO in vivo

Assessment of M-1 by HPLC in sera obtained from animals treated with CIPRO plus PTX was compared to animals treated with PTX alone. In this experiment, animals were pretreated with CIPRO for 9 days followed by a single injection of PTX or treated with saline for 9 days followed by a single injection with PTX. Twenty minutes following injection of PTX (100 mg/kg i.p.), a blood sample was taken for HPLC analysis and M-1 (13.7 ± 2.8 μmol/l) was significantly higher ($P < 0.05$) in mice treated with CIPRO (25 mg/kg i.p. 9 days) compared to serum concentrations of M-1 (6.4 ± 1.1 μmol/l) in mice administered saline. Results indicate that animals

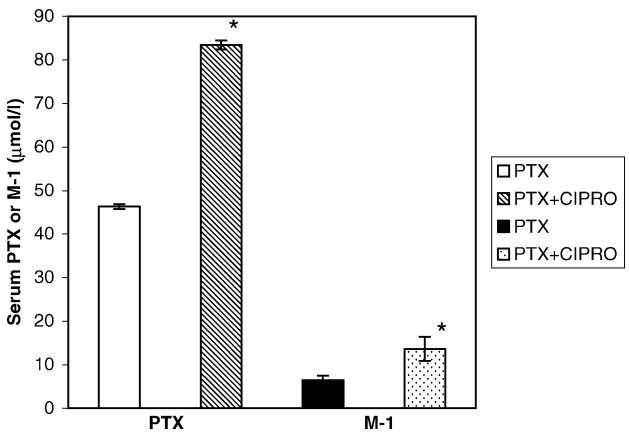

Fig. 2. Assessment of PTX and M-1 by HPLC in sera obtained from animals treated with CIPRO plus PTX was compared to animals treated with PTX alone. Sera was obtained from mice 20 min following a single injection of PTX (100 mg/kg). Mice had been treated with CIPRO (25 mg/kg i.p.) or saline for 9 days. Each bar represents the mean ± S.E. (*) Significantly different $P < 0.05$ compared to saline treated animals.

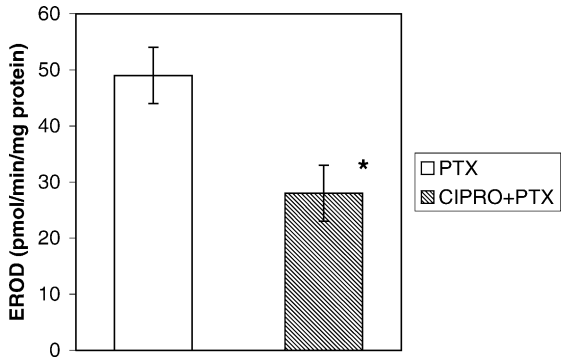

Fig. 3. The ethoxyresorufin-*o*-deethylase (EROD) activity in microsomal preparations obtained from animals treated with CIPRO for 9 days prior to treatment with PTX compared to animals treated with saline for 9 days prior to treatment with PTX. Each bar represents the mean ± S.E. (*) Significantly different $P < 0.05$ compared to saline treated animals.

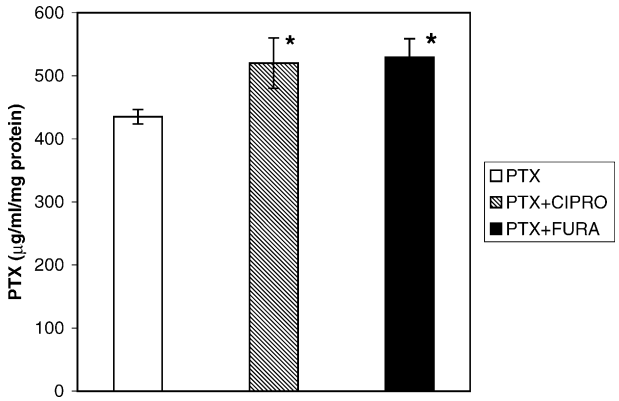

Fig. 4. Effect of CIPRO and FURA on PTX metabolism in vitro. The metabolism of PTX in microsome preparations containing PTX alone was compared to preparations containing PTX plus CIPRO or PTX plus FURA. Each bar represents the mean ± S.E. (*) Significantly different $P < 0.05$ compared to control microsomes.

pretreated for 9 days with CIPRO had a 113% increase in blood levels of M-1 compared to saline treated controls (Fig. 2).

3.3. Hepatic enzyme activity in the presence and absence of CIPRO in vivo

The EROD activity was assessed in microsomal preparations obtained from animals treated with CIPRO for 9 days prior to treatment with PTX compared to animals treated with saline for 9 days prior to treatment with PTX (Fig. 3). The results indicate that 9 days pretreatment with CIPRO resulted in a 43% decrease in EROD activity in microsomal preparations. Assessment of arylhydrocarbon hydroxylase activity indicated no difference between AHH activity in animals pretreated for 9 days with CIPRO compared to saline treated animals (results not shown).

3.4. CIPRO and PTX interaction in vitro

In the in vitro experiments, hepatic microsomes were prepared and the metabolism of PTX was assessed in microsome preparations which included PTX alone, PTX plus CIPRO, and PTX plus FURA. Following incubation the samples were extracted and PTX concentration was determined by HPLC. Under the conditions described, microsome preparations containing PTX (500 μM) plus CIPRO (500 μM) had significantly higher levels of PTX after 2 h of incubation compared to microsome preparations containing PTX alone. These results indicate that CIPRO inhibits the metabolism of PTX over a 2 h period in isolated hepatic microsomes suggesting that the drug interaction occurs in mouse microsomal preparations (Fig. 4). Treatment of microsomes with FURA, a selective mechanism based CYP1A2 inhibitor also resulted in elevated levels of PTX (Fig. 4).

The rate of metabolism of PTX was determined by assessing PTX levels at 0 time and at 2 h following incubation with microsomes. This was determined in microsomal preparations containing PTX alone or PTX plus CIPRO. The results indicate that the presence of CIPRO for 2 h in the incubation mixture resulted in a greater than 50% decrease in the rate of metabolism of PTX (Fig. 5).

3.5. PTX elimination in the Cyp1A2 wild type and Cyp1A2 knockout mice

Serum concentrations of PTX were determined by HPLC as detailed in Section 2. Twenty minutes following an intravenous injection of PTX (32 mg/kg), PTX blood levels were 22.2 ± 3.2 μmol/l in wild type *Cyp*1A2 mice ($n = 9$) (Fig. 6). PTX blood levels in *Cyp*1A2 knockout mice 20 min following intravenous injection were significantly ($P = 0.03$) elevated 44% above levels obtained in *Cyp*1A2 wild type animals such that the 20 min PTX blood level in *Cyp*1A2 knockout mice was 31.9 ± 2.7 μmol/l ($n = 11$). These results suggest that in animals lacking the *Cyp*1A2 gene, PTX metabolism is significantly reduced resulting in an accumulation of PTX in the serum.

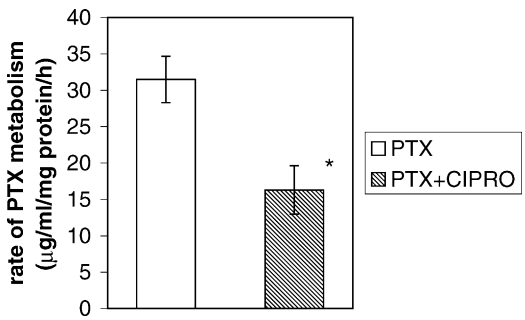

Fig. 5. The rate of metabolism of pentoxifylline in microsomal preparations containing pentoxifylline alone or pentoxifylline plus ciprofloxacin. Each bar represents the mean ± S.E. (*) Significantly different $P < 0.05$ compared to control.

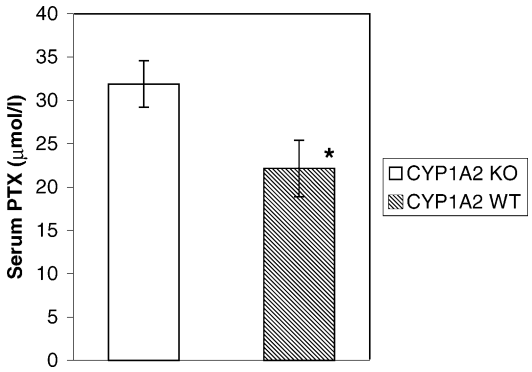

Fig. 6. Assessment of PTX in the sera of *Cyp*1A2 wild type and *Cyp*1A2 knockout mice. Serum was obtained from mice 20 min following injection of PTX (32 mg/kg i.v.). Each bar represents the mean ± S.E. (*) Significantly different $P < 0.05$ compared to *Cyp*1A2 wild type mice.

3.6. Effect of sera containing PTX on fibroproliferation

We have previously reported that PTX reduces fibroproliferation in a concentration-related manner in an in vitro assay [18]. In this study we assessed the inhibitory effect of sera obtained from *Cyp*1A2 knockout mice which had received PTX 20 min prior to sera sampling and compared this to sera obtained from wild type *Cyp*1A2 mice which had received PTX 20 min prior to sera sampling. Fibroproliferation was assessed by tritiated thymidine uptake in fibroblasts stimulated to proliferate using FCS (10%) with and without the sera from wild type or *Cyp*1A2 knockout mice (Fig. 7). The proliferation of fibroblasts stimulated with 10% FCS was 18,579 ± 753 cpm. Treatment of fibroblasts with sera obtained from wild type *Cyp*1A2 mice that had received PTX 20 min prior to sampling reduced fibroproliferation by 37%. This significant reduction in fibroproliferation suggests that PTX, which was easily detectable by HPLC, was able to inhibit fibroproliferation due to its presence at 20 min in the sera of wild type *Cyp*1A2 mice. The PTX level in this sample as determined by HPLC was 10.76 μmol/l. Sera obtained from *Cyp*1A2 knockout mice 20 min following injection of PTX decreased fibroproliferation 73% compared to FCS. The PTX concentration in this sample as determined by HPLC was 20.11 μmol/l, i.e., significantly higher sera concentration of PTX in *Cyp*1A2 knockout mice compared to *Cyp*1A2 wild type mice. The sera obtained from *Cyp*1A2 knockout mice 20 min following PTX injection had a greater inhibitory effect (58%) on fibroproliferation than sera obtained from wild type *Cyp*1A2 mice that had received PTX.

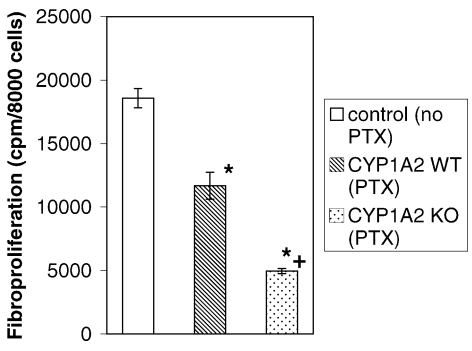

Fig. 7. Fibroproliferation was assessed by tritiated thymidine uptake in normal skin fibroblasts stimulated to proliferate using FCS (10%) with and without the sera from wild type or *Cyp*1A2 knockout mice. Each bar represents the mean ± S.E. (*) Significantly different $P < 0.05$ compared to control fibroproliferation (without addition of sera from either wild type or *Cyp*1A2 knockout mice). (+) significantly different $P < 0.05$ compared to *Cyp*1A2 wild type mice.

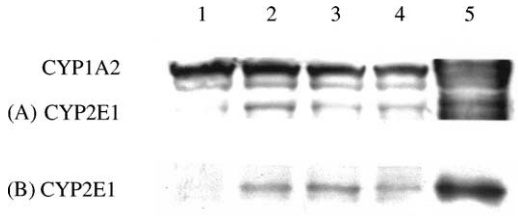

Fig. 8. CYP1A2 and CYP2E1 in hepatic microsomes following treatment with CIPRO. A representative Western blot of lysates prepared from hepatic microsomes obtained from mice treated with CIPRO (25 mg/kg i.p. for 9 days) compared to saline treated controls. Saline control (lane 1), CIPRO (lanes 2, 4), CIPRO + PTX (lane 3), manufacturer's positive antibody control 3-MC treated (lane 5). CYP1A2 antibody (panel A), CYP2E1 antibody (panel B).

3.7. Western analysis of CYP1A2 and CYP2E1

Lysates were prepared from liver microsomes obtained from mice treated with CIPRO (25 mg/kg i.p.) for 9 days ($n = 4$), CIPRO (25 mg/kg i.p.) for 9 days plus single injection of PTX 20 min prior to sacrifice ($n = 4$) and compared to saline treated controls ($n = 4$) and Westerns were assessed with selective antibodies to CYP1A2 (Fig. 8A) and CYP2E1 (Fig. 8B). The representative results shown in Fig. 8A indicate that treatment of mice with CIPRO for 9 days did not alter CYP1A2 but did appear to increase CYP2E1. The increase in CYP2E1 following 9 days of treatment with CIPRO was confirmed using a selective antibody to CYP2E1 (Fig. 8B).

4. Discussion

The drug interaction between PTX and CIPRO was reproduced in vivo in mice and also in vitro using murine liver microsomes, where we demonstrated that CIPRO inhibits the metabolism of PTX in vitro. Our in vivo results indicate that administration of CIPRO to mice results in a two-fold increase in blood levels of PTX and M-1 following PTX administration. PTX is metabolized to seven metabolites in mammals [20–22]. The first five metabolites are dimethylxanthines with substitutions at the N1 position. Metabolite-6 and metabolite-7 are 3-methylxanthines having been de-methylated at the 7 position from PTX and

M-1 [1-(5-hydroxyhexyl)-3,7-dimethylxanthine], respectively [23]. The major metabolites of PTX are M-1 and metabolite-5 [24]. M-1 has been shown to have similar pharmacological properties to PTX in peripheral vascular disease [25,26]. Our results suggest that PTX and M-1 are equally effective in inhibiting fibroproliferation stimulated by PDGF [27]. PTX metabolism and the levels of M-1 are altered by CIPRO [2], smoking [28]and liver disease [29]. Coadministration of CIPRO with PTX increased plasma levels of PTX and M-1 in subjects [2]and we report a similar increase in mice. The interaction between CIPRO and PTX likely occurs via inhibition of cytochrome P4501A2 by reducing the *N*-demethylation of PTX and M-1 to metabolite-6 and metabolite-7, respectively as shown in the metabolic profile (Fig. 1) [12,30,31].

Our results obtained in isolated microsome preparations suggest that the interaction between CIPRO and PTX likely involves inhibition of CYP1A2. This was illustrated by a greater than 40% decrease in EROD activity, a selective CYP1A2 enzyme, in microsomes prepared from animals treated for 9 days with CIPRO compared to animals treated with saline. CIPRO did not affect AHH activity, which is also a CYP1A enzyme but more selective for CYP1A1. The in vitro assessment of PTX metabolism in microsomal preparations also suggests that presence of CIPRO in the incubation mixture for the 2 h incubation period resulted in a greater than 50% decrease in the rate of metabolism of PTX during the 2 h incubation period, suggesting that CIPRO markedly inhibits the metabolism of PTX in vitro. We also demonstrated in vitro that the metabolism of PTX was inhibited by the selective mechanism based CYP1A2 inhibitor, FURA, further suggesting a role for CYP1A2 in the metabolism of PTX.

Cytochrome P4501A2 (CYP1A2) is a member of the cytochrome P450 family and is involved in metabolism of many xenobiotics as well as endogenous chemicals and drugs. The CYP1A2 enzyme is one of the CYP1A enzymes and is constitutively expressed in liver and can be induced by exposure to polycyclic aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The *Cyp*1A2 knockout was developed as a targeted mutation in the *Cyp*1A2 gene [5]. Animals lacked expression of *Cyp*1A2, lack the CYP1A2 protein and otherwise appeared to be normal and were able to reproduce. The mice used in the current studies were offspring from these *Cyp*1A2 knockout mice. To further define the role of CYP1A2 in the metabolism of PTX, we used the *Cyp*1A2 knockout mouse and assessed the blood levels of PTX by HPLC following injection of PTX (32 mg/kg i.v.). *Cyp*1A2 knockout mice ($n = 11$) were compared to *Cyp*1A2 wild type controls ($n = 9$). The results of these studies indicate that CYP1A2 deficiency is enough to alter PTX kinetics and increase blood levels of PTX. Taken together these results suggest that *Cyp*1A2 knockout mice, due to the absence of the *Cyp*1A2 gene and CYP1A2 enzyme activity, have a reduced ability to metabolize PTX resulting in elevated levels of PTX and possibly other active metabolites of PTX resulting in a more pronounced inhibitory effect on proliferation. Together these results point to the important role that cytochrome P4501A2 plays in PTX metabolism and lends further support to the role of CYP1A2 in the CIPRO PTX drug interaction and further supports the in vitro results obtained using the specific mechanism based CYP1A2 inhibitor FURA.

Results obtained using the fibroproliferation assay suggest that the inhibitory effect of sera obtained *Cyp*1A2 knockout mice 20 min following injection of PTX was greater than the effect of sera obtained from *Cyp*1A2 wild type mice further supporting the premise that an inhibition of CYP1A2 produces higher levels of PTX and M-1. The results obtained in the *Cyp*1A2 knockout mouse supports a mechanism whereby the enzyme is inhibited but also could suggest that the drug interaction of ciprofloxacin and pentoxifylline may involve downregulation of *Cyp*1A2 by ciprofloxacin. The results obtained with Western analysis clearly indicate that ciprofloxacin treatment does not downregulate *Cyp*1A2. The Western analysis also revealed a novel additional protein band which was detectable using the CYP1A2 antibody (Fig. 8A) and that this protein was consistently increased in livers of animals treated with ciprofloxacin. The CYP1A2 antibody used was found to cross react with CYP2E1. Repeating the Western analysis with a specific antibody to CYP2E1 (Fig. 8B), confirmed that CYP2E1 was increased in livers following ciprofloxacin treatment. This novel effect of ciprofloxacin on CYP2E1 has several important implications. CYP2E1 inhibition decreases the hydroxylation of theophylline [32] and thus the increase in CYP2E1 observed following CIPRO treatment may increase the formation of the hydroxylated M-1 from PTX. In addition to producing higher sera levels of PTX and M-1, the drug interaction between ciprofloxacin and pentoxifylline has been reported to produce a novel metabolite, i.e., the *R*-enantiomer of M-1 (M-1R) which is 800-fold more potent than PTX [33], but the mechanism for the generation of the *R*-enantiomer of M-1 in the CIPRO-PTX drug interaction is not known. A recent report suggests that CYP2E1 is capable of stereochemical discrimination and that CYP2E1 displays preferential production of *R*-enantiomers [34]. Together with our results indicating that CIPRO increases CYP2E1, it is tempting to speculate that CYP2E1 may mediate the production of the *R*-enantiomer of M-1 in the drug interaction between CIPRO and PTX. The increase in CYP2E1 by ciprofloxacin has another important implication and may explain the drug interaction between ciprofloxacin and phenytoin [35,36].

Interestingly, CYP1A2 activity is preferentially decreased in patients with cirrhosis [37]and this likely explains why PTX and M-1 plasma levels are markedly elevated in cirrhotic patients compared to normal subjects [29]. Other members of the CYP1A family of enzymes including arylhydrocarbon hydroxylase activity are decreased in

patients with liver disease [38,39]and in the pig model of hepatic fibrosis [40]. These reports together with our results on the equipotent effect of PTX and M-1 on fibroproliferation [19] suggests that the antifibroproliferative effect of PTX in patients with liver cirrhosis may be due to the combined effect of (a) the additional biological activity derived from M-1 and (b) the elevated blood levels of PTX and M-1 resulting from the inhibitory effect of cirrhosis on CYP1A2. One implication of this altered drug metabolism due to a drug-disease (PTX-liver disease) interaction may be highly beneficial because the target population of patients for new antifibroproliferative drugs (such as PTX) are those patients with liver disease who may demonstrate this altered PTX metabolism. If PTX is used as an antifibrotic drug, the reduced CYP1A2 activity reported to occur in liver disease patients may be beneficial.

In summary, we have established the CIPRO-PTX drug interaction in mice such that PTX and M-1 levels increase in mice receiving CIPRO and PTX compared to mice receiving PTX alone. Our results suggest an important role for CYP1A2 in the metabolism of PTX and suggest that inhibition of CYP1A2 would enhance the antifibrotic effect of PTX. Our results also show that CIPRO upregulates CYP2E1 and this could enhance the production of the hydroxylated M-1 from PTX, may provide a mechanism to explain the generation of the M-1*R* enantiomer and provides a mechanism to explain another reported drug interaction between CIPRO and phenytoin.

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