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Dietary regulation of mouse intestinal P450 expression and drug metabolism

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Running Title: Dietary regulation of intestinal P450 expression

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Abbreviations: P450, cytochrome P450; CPR, cytochrome P450 reductase; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; WT, wild-type; BA, bile acid; IE, intestinal epithelium; SI, small intestine; SPE, solid phase extraction; LC-MS/MS, liquid chromatography-tandem mass spectrometry; IE-*Cpr*-null, intestinal epithelium-specific *Cpr*-null; PXR, pregnane X receptor; FXR, farnesoid-X-receptor; MDZ, midazolam; OH-MDZ, hydroxymidazolam; T-CA taurocholic acid; d4-CA, cholic-2,2,4,4-d4-acid; MCA, muricholic acid; HPLC; high-performance liquid chromatography.

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Abstract

The study was originally designed to test the hypothesis that the compensatory increase in intestinal P450 expression in the intestinal epithelium-specific P450 reductase (CPR) knockout (IE-*Cpr*-null) mice was due to decreased metabolism of putative P450 inducers present in the diet. Thus, we determined the impact of a dietary change from regular rodent chow to a synthetic diet devoid of phytochemicals on the expression of P450 enzymes in the small intestine (SI) and liver of wild-type (WT) and IE-*Cpr*-null mice. The dietary change diminished expression of CYP1A, 2B, 2C, and 3A in SI and CYP2B, 2C, and 3A in liver of both WT and IE-*Cpr*-null mice. However, the compensatory increase in SI P450 expression still occurred in IE-*Cpr*-null, compared to WT, mice, on the synthetic diet. The diet-change-induced decrease in P450 expression was accompanied by decreases in microsomal midazolam-hydroxylase activity in vitro and first-pass clearance of midazolam in vivo in WT mice. Further studies showed that the dietary change, but not *Cpr* deletion, caused large decreases in bile acid (BA) levels in plasma, liver, SI, and intestinal content, and that treatment of WT mice on the synthetic diet with GW4064, a FXR agonist, restored the levels of CYP3A expression in both liver and SI to those seen in mice fed with regular chow. Taken together, these results highlight the vital role of diet in maintaining adequate expression of major drug-metabolizing P450s and their associated drug-metabolizing activities in the digestive tract, and suggest potential involvement of BA signaling in the regulatory mechanisms.

Introduction

Orally administered xenobiotics, including nutrients and therapeutic drugs, are potentially subject to first-pass metabolism before reaching systemic circulation. The liver and small intestine (SI) are major organs for this first-pass metabolism. Many biotransformation enzymes are expressed in the liver and SI, the most prominent of which are the cytochrome P450 (P450) monooxygenases (Nelson et al., 2004). P450-mediated metabolism in both liver and SI could have a major impact on the bioavailability of a given drug, consequently affecting its therapeutic efficacy and/or the risks of chemical toxicity or adverse drug-drug interactions (Thummel et al., 1997; Doherty and Charman, 2002; Kaminsky and Zhang, 2003).

P450-mediated metabolism also regulates the homeostasis of many endogenous compounds, such as cholesterol and bile acids (BAs). BAs are synthesized from cholesterol in the liver and transported to SI through bile. In the SI, the BAs facilitate the absorption of lipid and fat-soluble vitamins. Most of the BAs in the intestine are reabsorbed by intestinal enterocytes, and then released into portal circulation. Once taken up by hepatocytes, BAs are excreted into bile again to complete the enterohepatic circulation (Chiang, 1998). Excess BAs can be toxic, so BA metabolism is highly regulated. BAs are also signaling molecules, which regulate gene expression through nuclear receptors, including farnesoid-X-receptor (FXR) and pregnane-X-receptor (PXR) (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999; Xie et al., 2001). PXR, which regulates the expression of several CYPs, can be activated by FXR (Jung et al., 2006); the latter plays a key role in the regulation of BA homeostasis (Goodwin et al., 2000; Kim et al., 2007).

The regular laboratory chow diet contains crude plant-derived ingredients and phytochemicals, some of which may induce P450 expression. In wild-type (WT) mice, replacing

a standard laboratory chow with a semisynthetic diet led to reduced hepatic expression of CYP2C29, 2C55, 3A11 and 3A25 mRNAs, as well as decreased liver microsomal activity toward midazolam 1'-hydroxylation (van Waterschoot, R. A. B., et al., 2009); however, such dietary effects on expression of mouse SI P450 have not been examined. In an intestinal epithelium (IE)-specific cytochrome P450 reductase (CPR) knockout mouse model (IE-*Cpr*-null), in which the activities of all microsomal P450s are suppressed in the IE cells due to deletion of the *Cpr* gene, we observed large increases in the expression of multiple P450s (Zhang et al., 2009). This compensatory increase in P450 expression in the SI of the IE-*Cpr*-null mice may be partly explained by a decreased local metabolism and elimination of dietary P450 inducers, although it may also be due to decreased local metabolism of endogenous P450 inducers.

In the present study, we determined whether dietary changes (from regular chow to a synthetic diet) can have a major effect on the expression of various P450 enzymes in the SI, as well as in liver, of WT mice, and whether such expression changes can lead to significant changes in the in vivo disposition of an oral drug (midazolam). We also investigated whether the compensatory increase in P450 expression seen previously in the SI of the IE-*Cpr*-null mice was dependent on dietary exposure to the regular chow, by comparing CYP expression levels between IE-*Cpr*-null and WT mice that are on a synthetic diet. Toward understanding the mechanisms underlying the effects of dietary changes and/or SI *Cpr* deletion on SI P450 expression, we further determined whether the dietary change causes changes in BA homeostasis, and whether diet-change induced alterations in SI P450 expression can be reversed by treatment of mice with GW4064, a FXR agonist.

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Materials and Methods

Materials. AIN-93G synthetic rodent diet was purchased from Dyets (Bethlehem, PA). Prolab® RMH 3500 regular diet was from LabDiet (Hudson, NH). Ingredients of the two diets are described at the manufacturers' websites. Midazolam (MDZ), diazepam, 1'-hydroxymidazolam (1'-OH-MDZ), 4-hydroxymidazolam (4-OH-MDZ), t-butyl-dimethylsilyl-trifluoroacetamide, cholic acid, taurocholic acid (T-CA), and GW4064 were purchased from Sigma-Aldrich (St. Louis, MO). Cholic-2,2,4,4-d4-acid (d4-CA) was obtained from C/D/N Isotopes (Pointe-Claire, Quebec, Canada); α- and β-muricholic acid (MCA) and tauro β-muricholic acid (T-β-MCA) sodium salt were obtained from Steraloids (Newport, RI). All solvents (acetonitrile, methanol, and water) were of high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Houston, TX).

Animals and treatments. Adult male and female IE-*Cpr*-null (Zhang et al., 2009) mice (2- to 3-month-old and congenic on the C57BL/6 background) and age-matched WT littermates were used. Animals were given food and water ad libitum. For dietary studies, mice, raised on regular rodent diet (Prolab RMH 3500), were fed with synthetic diet (AIN-93G) for 3 consecutive weeks. MDZ (30 mg/kg, dissolved in 0.01% hydrochloric acid) was given to mice via oral gavage for pharmacokinetics analysis. GW4064 was administered (once) at 100 mg/kg (in polyethylene glycol 400:Tween 80; 4:1 (v/v)) via oral gavage, and control animals received the vehicle only; all animals were killed 18 h later for tissue isolation and microsome preparation. All animal studies were approved by the Institutional Animal Care and Use Committee of the Wadsworth Center.

Microsome preparation and immunoblot analysis. Microsomal samples were prepared as previously described (Zhang et al., 2009). Proteins were resolved on 10% NuPAGE Bis-Tris-

gels (Invitrogen, Carlsbad, CA) and then transferred to nitrocellulose membranes. For immunodetection, polyclonal rabbit anti-rat CYP1A1 (Millipore, San Diego, CA), anti-CYP3A1 (Biomol Research Laboratories, Plymouth Meeting, PA), goat anti-rat CYP2B, or CYP2C, (BD Gentest, Woburn, MA), and rabbit anti-calnexin (loading control) (Abcam Inc, Cambridge, MA) were used. Peroxidase-labeled goat anti-rabbit IgG or rabbit anti-goat IgG (Sigma-Aldrich) was used as secondary antibodies. The signal was detected with an enhanced chemiluminescence kit (GE Healthcare, Piscataway, NJ); immunoblot quantification was carried out using a Bio-Rad GS-710 Calibrated Imaging Densitometer or a Bio-Rad ChemiDoc XRS+ System (Hercules, CA).

Assay for in vitro metabolism of MDZ. The assay was performed essentially as described (Granvil et al., 2003). Incubations were carried out in a total volume of 200 μl containing 0.1 M phosphate buffer, pH 7.4, either 0.05 mg/ml liver microsomes or 0.5 mg/ml SI microsomes, and 3 μM of MDZ. After a 5-min preincubation at 37°C, the reactions were initiated by the addition of NADPH (1.0 mM) and then allowed to proceed for 5 min before they were terminated by the addition of 50 μl sodium hydroxide solution (100 mM) and 1 ml of an ether/hexane mixture (70/30, v/v). An internal standard (0.5 μg/ml diazepam, in 5 μl methanol) was added to the reaction mixture before extraction; the mixtures were shaken for 10 min, and then centrifuged for 10 min at 4°C and 2,000 g. After being kept for 1 h at -30°C, the supernatant was transferred to a new tube and evaporated under a stream of nitrogen at 40°C. Samples were reconstituted in 50 μl of 20% (v/v) t-butyl-dimethylsilyl-trifluoroacetamide in acetonitrile. Each sample was heated in a 200-μl injection vial for 2 h at 70°C, and 2 μl was injected for gas chromatography-mass spectrometry (GC/MS) analysis.

GC/MS analysis of MDZ and MDZ metabolites. GC/MS analysis was carried out according to previously reported methods (Thummel et al., 1994; Eeckhoudt et al., 1998). The GC/MS system consisted of an Agilent 7890A GC system and a 5975C inert XL EI/CI MSD with Triple-Axis Detector (Agilent Technologies, Santa Clara, CA), a 7693 autoinjector and an Rxi-5ms fused capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness, RESTEK, Bellefonte, PA). The helium carrier gas was at a head pressure of 10 psi, and injector temperature was 260°C. The column temperature was raised 1 min after injection from the initial 160°C to 280°C at 5°C/min and further to 300°C for 5 min. Then, the column was maintained at 300°C for 4 min before full-speed cooling to 85°C. Under these conditions, the retention time of diazepam, MDZ, t-butyl-dimethylsilyl-4-hydroxy-MDZ and t-butyldimethylsilyl-1'-hydroxy-MDZ was 21.6, 22.5, 24.3 and 24.8 min, respectively. The mass spectrometer inlet probe was kept at 280°C and the ions used for quantification were *m*/*z* 310 for MDZ, 398 for t-butyl-dimethylsilyl-1'-hydroxy-MDZ and t-butyldimethylsilyl-4-hydroxy-MDZ, and 283 for diazepam.

Pharmacokinetic analysis. Blood samples were collected from the tail vein at 15, 30, 60, 120, 240 and 360 min following oral MDZ administration. To a 25-μl whole blood sample, 50 μl of 100 mM sodium hydroxide and 5 μl of internal standard (0.5 μg/ml diazepam, in methanol) were added before extraction, as described in the assay for in vitro metabolism. The plasma concentrations of MDZ, 1'-OH-MDZ, and 4-OH-MDZ were determined by GC/MS, as described above. The concentrations are given as means ± SD (*n* = 4-6, each time point). Pharmacokinetic parameters were calculated using the PK Solver software. Statistical significance of differences between groups was examined with Student's t test.

LC-MS/MS analysis of bile acids. Quantitative analysis of BAs was performed as described previously (Inoue et al., 2004), using an ABI 4000 Q-Trap LC-MS/MS system

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(Applied Biosystem, Foster City, CA), fitted with a 3.5-μm Symmetry-C18 column (2.1 × 150 mm, Waters, Milford, MA). The samples were eluted at a flow rate of 0.25 ml/min, with a mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The column was equilibrated with 60%A:40%B; the solvent gradient consisted of linear increases from 40%B to 100%B, between 2 and 20 min, and then stayed at 100%B for 5 min. The MS was operated in the positive ion mode, using electrospray ionization. The parent/product ion pairs of m/z 373/355 for cholic acid and α-MCA, 391/359 for β-MCA, 516/462 for T-CA and T-β-MCA, and 377/359 for the internal standard d4-CA, were measured in the Multiple Reaction Monitoring (MRM) scan mode. The parameters for the chamber were as follows: curtain gas, 30 psig; heated nebulizer temperature, 350°C; ion spray voltage, 4000 V; nebulizer gas, 50 psig; turbo gas, 50 psig, declustering potential, 50 V; and entrance potential, 10 V.

Liver and SI samples were extracted as described previously (Alnouti et al., 2008). Approximately 50 mg of liver and small intestine were homogenized in 2 vol of 50% methanol. A 150 μl portion of the homogenate from each sample was combined with 5 ng d4-CA, and mixed with 2 ml of ice-cold acetonitrile for 1 h; the mixture was then centrifuged at 11,000 g for 10 min. The supernatant was transferred to a new tube, whereas the precipitant was extracted with another 1 ml of ice-cold acetonitrile. The pooled supernatant fractions were dried, and the residues reconstituted in 50% (v/v) acetonitrile for LC-MS analysis.

For intestinal contents, the extraction of BAs was performed as described (Hagio et al., 2009). Briefly, 100 mg (wet weight) intestinal contents, collected by flushing the intact intestinal tract, were homogenized in 2 vol of 50% (v/v) methanol. One milliliter of ethanol was added to the homogenate for extraction. The samples were subjected to sonication twice (10 s each) and

then heated at 60°C for 30 min. After cooling to room temperature, the samples were heated again at 100°C for 3 min before centrifugation at 3000 g for 10 min at 15°C. The supernatants were collected, whereas the precipitates were extracted with another 1 ml of ethanol. The pooled supernatants were dried, and the residues reconstituted in 50% acetonitrile for LC-MS analysis.

For plasma samples, 50 μl of plasma together with 5 ng of d4-CA were extracted with 0.5 ml acetonitrile, as described previously (Alnouti et al., 2008). The extracted BAs were reconstituted in 50% acetonitrile for LC-MS analysis. Authentic compounds were added to charcoal-stripped bovine serum (Hyclone, Logan, UT) for construction of calibration curves.

Results

Effects of dietary change on P450 expression in WT and IE-*Cpr*-null mice

Dietary effects on SI P450 expression were examined by comparing microsomal CYP1A, 2B, 2C, and 3A protein levels between mice that were always on a regular lab chow diet and mice that have been switched from the regular lab chow diet to a synthetic diet for 3 weeks. As shown in Figure 1A, the dietary change led to large decreases in the intestinal levels of all four isoforms in both WT and IE-*Cpr*-null mice. It should be noted that the anti-CYP antibodies were all polyclonal and are expected to recognize multiple, if not all, members in the CYP2B, 2C or 3A gene subfamily. As we have reported previously (Zhang et al., 2009), CYP expression levels in the null mice were higher than those in WT mice in the regular diet groups. Notably, with exception of CYP1A1, which, under the conditions used, was not detected in mouse SI of either strain on the synthetic diet, the strain difference in SI P450 expression levels was also observed in mice on the synthetic diet, despite the overall decreases in P450 expression in both strains (Fig. 1A/B).

The dietary change also affected hepatic P450 expression (Fig. 1C). Levels of CYP2B, 2C, and 3A proteins were all decreased in mice (of either strain) fed with the synthetic diet, with the decreases in the CYP3A protein levels being most striking. In contrast to the situation in the SI, hepatic expression levels of CYP2B, 2C, and 3A proteins were not different between the IE-*Cpr*-null mice and WT mice, on either regular chow or synthetic diet (Fig. 1C/D).

The effects of the dietary change on the expression of CYP3A in lung and kidney were also examined. In contrast to the large decreases in CYP3A expression seen in SI and liver of mice fed with the synthetic diet, only slight diet-related decreases in CYP3A levels were seen in the lung or kidney (data not shown). As was found in the liver, there was no strain difference in

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CYP3A expression levels in lung and kidney, between WT and IE-*Cpr*-null mice, on either regular chow or synthetic diet.

Impact of dietary change on MDZ metabolism and in vivo clearance in WT and IE-*Cpr*-null mice

The large effect of the dietary changes, from a regular chow to a synthetic diet, on both hepatic and intestinal CYP3A expression prompted us to also examine the impact of this dietary regulation on in vitro microsomal metabolism (Fig. 1E) and in vivo clearance (Table 1 and *Supplemental* Figures 1 and 2) of MDZ, a CYP3A model substrate, in both WT and IE-*Cpr*-null mice. As shown in Figure 1E, the dietary change led to significant decreases in rates of microsomal MDZ metabolism (formation of both 1'-OH-MDZ and 4-OH-MDZ) in both liver and SI of WT mice. In the null mice, the dietary change also led to large decreases in rates of hepatic microsomal MDZ metabolism, whereas the activity in SI microsomes were barely detectable with either diet, reflecting the tissue-specific absence of CPR in the SI. In pharmacokinetic studies (*Supplemental* Figures 1 and Table 1), the dietary change led to a ~2-fold increase in AUC value for orally administered MDZ (at 30 mg/kg) in WT mice. However, a diet-related change in MDZ clearance in the IE-*Cpr*-null mice was much less noticeable (with only an ~35% increase in AUC value), which is due largely to the fact that MDZ clearance was already decreased in the null mice by the loss of SI CPR, in the regular diet groups (Table 1 and *Supplemental* Figure 2). In experiments not shown, the diet change-induced decrease in MDZ clearance in WT mice was also associated with significant decreases in plasma levels of 1'-OH-MDZ (2.7-fold in AUC value).

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Effects of dietary change on systemic and intestinal BA homeostasis in WT and IE-*Cpr*-null mice

The persistence of up-regulation of SI CYP expression in IE-*Cpr*-null mice, relative to WT mice, on the synthetic diet (Fig. 1) suggested that factors in addition to dietary P450 inducers may be involved in the regulation of SI P450 expression. Therefore, we next examined whether the homeostasis of BAs, which are abundant in the SI and known to activate pertinent nuclear receptors such as FXR (Hylemon et al., 2009; Gnerre et al., 2004), is altered by the loss of SI CPR, when mice are fed with either regular or synthetic diet. The levels of the five major murine BAs (cholic acid, T-CA, α-MCA, β-MCA and T-β-MCA) were measured in liver, SI, plasma, and intestinal contents from both WT and IE-*Cpr*-null mice. In both WT (Fig. 2) and IE-*Cpr*-null (*Supplemental* Fig. 3) mice, the abundance of all five BAs was significantly decreased by the change from regular chow to the synthetic diet, in liver, SI, plasma, and intestinal contents. However, there was no significant difference between WT and IE-*Cpr*-null mice in the levels of any of the BAs determined when the mice were fed with either regular or synthetic diet (data not shown).

Effects of FXR agonist GW4064 on SI CYP3A expression in WT mice fed with the synthetic diet

It is currently unclear how the dietary change led to decreases in BA levels; however, the downstream link between decreases in BA levels and reductions in SI as well as liver CYP3A expression may be explained by a reduced activation of FXR and/or PXR by BAs, which are known ligands for these receptors. In that connection, treatment of mice on the synthetic diet with GW4064, a FXR agonist, restored the levels of CYP3A expression in both liver and SI to

those seen in mice fed with regular chow (Fig. 3). This result suggests that a decreased activation of FXR by BAs is likely involved in the dietary change-induced suppression of CYP3A expression in the liver and SI.

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Discussion

In this study, we have determined the effects of dietary change on the expression of both intestinal and hepatic CYP2B, 2C, and 3A enzymes, as well as the consequences for systemic drug clearance, using MDZ as a model drug. Furthermore, we found that the previously described up-regulation of CYP2B, 2C, and 3A expression in the SI of the IE-*Cpr*-null mice (Zhang et al., 2009) does not require exposure of the mice to dietary P450 inducers present in regular chow. The effects of a dietary change on hepatic (but not intestinal) P450 expression have also been examined in a previous study (van Waterschoot et al., 2009), where expression of CYP2C29, 2C55, 3A11 and 3A25 mRNAs was found to be decreased when WT FVB mice were changed from a standard lab chow (AM-II; Hope Farms) to a semi-synthetic diet (20% casein, 4068.02; Hope Farms). Collectively, these studies indicate that daily diets can play a vital role in maintaining adequate expression of major drug metabolizing enzymes in the digestive tract, and point to the need to pay greater attention to potential adverse drug-drug interactions involving diet-induced alteration of drug biotransformation in patients.

Much remains to be determined about the mechanism underlying the changes in SI P450 expression that result from either the diet switch or the *Cpr* deletion. Based on results from the present study and literature, it appears that ingredients uniquely present in the regular rodent chow sustain “normal” P450 expression in the SI by maintaining circulating as well as intestinal tissue levels of BAs. BAs are well-known activators for both FXR and PXR (Hylemon et al., 2009; Gnerre et al., 2004, Staudinger et al., 2001; Xie et al., 2001). FXR can also up-regulate PXR expression (Jung et al. 2006). Thus, an increase in BA can activate FXR and PXR in the SI (as well as in liver), ultimately stimulating the expression of CYP3A and other P450s that are regulated by PXR. Conversely, with synthetic diet, BA levels are reduced, leading to less

activation of FXR and PXR, and decreased P450 expression. The effects of a synthetic diet on cholesterol metabolism and BA synthesis have been reported previously (McNamara et al., 1982).

Dietary P450 inducers (Mandlekar et al., 2006) may also directly activate nuclear receptors such as PXR and CAR (constitutive androstane receptor) in the SI, leading to increased expression of their target P450 genes (e.g., *Cyp2b*, *2c*, and *3a*), as was found for the dietary regulation of AhR-mediated *Cyp1a1* expression in the SI and extra-gut organs (Ito et al., 2007; Zhang et al., 2009). Nonetheless, the fact that activation of FXR by GW4046 led to restoration of CYP3A expression in mice fed with the synthetic diet suggests that dietary P450 inducers (e.g., plant-derived PXR agonists) are not absolutely required for normal P450 expression when FXR is activated. Furthermore, since the dietary change caused decreased expression of CYP2B, 2C and 3A in both WT and IE-*Cpr*-null mice, it seems reasonable to conclude that intestinal P450 activity is not responsible for, or otherwise involved in, the observed dietary regulation of these *Cyp* genes in SI and liver.

It remains to be determined how the diet change led to changes in BA homeostasis. Although the synthetic diet and the regular rodent chow used in this study are both nutritionally adequate, the two diets have major differences in the presence or absence of plant-derived raw material as well as subtle differences in chemical composition. For example, although the total protein or fiber content (percentage) in the two diets is similar, the types of proteins and fibers differ by diet. Previous studies have shown that changes in dietary constituents, including fiber contents (Kritchersky, 1978) and protein sources (Liaset et al., 2011), can influence size of the BA pool. Here we further demonstrated, for the first time, that the change from the regular chow to the synthetic diet causes large decreases in all five major murine BAs in plasma, liver, SI, and intestinal content.

Changes in P450 expression or activity in the SI may affect the metabolism and consequently local (SI) and/or systemic bioavailability of dietary P450 inducers. Studies on mice with intestine-specific deletion of the aryl hydrocarbon receptor nuclear translocator (*Arnt*) or the *Cpr* gene revealed the important role of intestinal P450-mediated metabolism in regulating bioavailability of dietary CYP1A1 inducer (Ito et al., 2007; Zhang et al., 2009), and provided the basis for the hypothesis that the loss of P450-mediated metabolism in the intestine leads to increased supply of dietary CYP1A1 inducers not only to the intestine, but also to extra-gut organs. The latter hypothesis was also supported by our more recent study of the IE-*Cpr*-null mice, which demonstrated a pivotal role of intestinal P450, most likely CYP1A1, in controlling the systemic exposure to orally administered benzo(a)pyrene, a prototypical CYP1A1 inducer (Fang and Zhang, 2010). In the current study, we have further demonstrated that feeding mice with a synthetic diet (devoid of plant-derived ingredient) not only abrogated the expression of CYP1A1 in the intestine of both WT and IE-*Cpr*-null mice (Fig. 1A), but also eliminated the induction of CYP1A1 in the lung, an extra-gut organ, by the loss of intestinal CPR (data not shown).

SI CYP-mediated metabolism also seems to control systemic bioavailability of dietary inducers for other P450 enzymes. Germline deletion of all mouse *Cyp3a* genes led to upregulation of *Cyp2c55* and several other *Cyp* genes (but not *Cyp3a*, which could not be examined) in the liver, presumably due to reduced disposition of dietary PXR and/or CAR ligands; the latter hypothesis was supported by the mitigating effects of hepatic or SI transgenic expression of human CYP3A4, and by a reduction in the extent of the “compensatory” increase in CYP expression seen when mice were fed a semi-synthetic diet (van Waterschoot, R. A. B., et al., 2009). However, no change in the expression of CYP2B, 2C, or 3A were observed in the

livers of the IE-*Cpr*-null mice, compared to WT mice, on either regular or synthetic diet (Zhang et al., 2009; this study), suggesting that any role of SI P450 in metabolizing dietary PXR and/or CAR ligands may only become critical when hepatic CYP3A is compromised (or vice versa).

We still do not fully understand how the tissue-specific *Cpr* deletion in the SI leads to up-regulation of the intestinal expression of multiple P450 genes. Our recent study of the genomic and metabolomic changes in the SI of the IE-*Cpr*-null mice revealed remarkable changes in the sterol biosynthesis and metabolism pathways (D'Agostino et al., 2012). It will be worthwhile to determine whether the latter changes lead to activation of nuclear receptors, such as CAR, in the SI; the accumulation of endogenous CAR activators that are intermediate metabolites in the sterol biosynthesis pathway has been proposed previously to explain the up-regulation of P450 expression in the livers of the liver-*Cpr*-null mice (Weng et al., 2005).

In summary, we have shown that a dietary change from a regular rodent chow to a synthetic diet devoid of phytochemicals can cause large decreases in the expression of major drug-metabolizing P450 enzymes in the SI, as well as in liver, with consequent decreases in the metabolic disposition of an oral drug. Our initial efforts toward identification of the underlying mechanisms uncovered the potential involvement of BA signalling. Furthermore, our study of the IE-*Cpr*-null mice provided additional insights to the previously proposed role of SI P450-mediated metabolism of dietary P450 inducers in physiological regulation of P450 expression.

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Authorship Contributions

Participated in research design: P. Zhang, K. Jia, C. Fang, X. Ding, and Q.-Y. Zhang

Conducted experiments: P. Zhang, K. Jia, C. Fang, X. Zhou

Contributed new reagents or analytic tools: None

Performed data analysis: P. Zhang, K. Jia, C. Fang, Qing-Yu Zhang

Wrote or contributed to the writing of the manuscript: P. Zhang, K. Jia, C. Fang, X. Ding, and Q.-Y. Zhang

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Footnotes

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Figure Legends

Fig. 1. Effects of dietary change on P450 expression and microsomal MDZ metabolism. Microsomes were prepared from adult, male WT and IE-*Cpr*-null mice that were either always on a regular lab chow diet (Prolab® RMH 3500) or were switched from the regular diet to a synthetic diet (AIN-93G) for 3 weeks. **A**-**D**, Immunoblot analysis of P450 expression. Each microsomal sample was pooled from 3 mice, and analyzed in duplicate. Antibodies used are described in *Materials and Methods*. The results represent 2-3 independent experiments. **A**, Expression of CYP1A, 2B, 2C, and 3A proteins in mouse SI. Each lane contained 20 (Synthetic/SI samples) or 10 (all others) μg total protein. Calnexin was also analyzed as a loading control. **B**, Densitometry analysis of relative SI CYP2B, 2C, and 3A protein levels in WT and IE-*Cpr*-null mice. WT values from regular lab chow group were set as 1. M ± S.D., (n=3), **, $P < 0.01$, compared with the corresponding *Regular diet* group. **C**, Expression of CYP2B, 2C, and 3A proteins in mouse liver. Each lane contained 10 μg total protein. **D**, Densitometry analysis of relative hepatic CYP2B, 2C, and 3A protein levels in WT and IE-*Cpr*-null mice. WT values from regular lab chow group were set as 1. M ± S.D., (n=3), **, $P < 0.01$, *, $P < 0.05$, compared with the corresponding *Regular diet* group. **E**, In vitro metabolism of MDZ by liver or SI microsomes. Rates of formation of 1'-OH-MDZ and 4-OH-MDZ were determined. Reaction mixtures contained phosphate buffer, pH 7.4, 3 μM MDZ, and 0.05 mg/ml liver microsomes or 0.5 mg/ml SI microsomes, in a total volume of 200 μl. The values reported are means ± S.D. (n = 6). **, $P < 0.01$, compared with the corresponding *Regular diet* group; #, $P < 0.01$, compared with the WT SI in the *Regular diet* group, (Student's t test).

Fig. 2. BA levels in plasma, liver, SI epithelium, and intestinal contents of adult male WT mice fed with regular or synthetic diet. Five different BAs were determined using LC-MS/MS as described in *Materials and Methods*. Results are shown as means ± S.D (n = 6). *, $P < 0.05$; **, $P < 0.01$, compared with the corresponding *Regular diet* group. Corresponding data for IE-*Cpr*-null mice are shown in *Supplemental* Figure 3. CA, cholic acid.

Fig. 3. Effect of GW4064 treatment on liver and SI CYP3A expression in mice fed with synthetic diet. Adult male WT mice fed with the synthetic diet for 3 weeks were treated once with GW4064 by oral gavage or with the vehicle alone, and tissues were obtained 18 h later for microsome preparation. Age-matched male WT mice fed with regular chow were also included for comparison. A. Immunoblot analysis of CYP3A expression was performed as described in Figure 1. Microsomes were prepared from pooled enterocytes of 2 mice in each group and analyzed in duplicates. Calnexin was analyzed as a loading control. Each lane contained 10 μg total protein. The results represent three independent experiments. B. Results of densitometry analysis for relative CYP3A protein levels. M ± S.D., (n=3), **, $P < 0.01$, *, $P < 0.05$, compared with the *Regular diet* group.

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TABLE 1

Dietary effects on pharmacokinetic parameters for orally administered MDZ in WT and IE-*Cpr*-null mice

WT and IE-*Cpr*-null mice (2- to 4-month-old male) from the regular diet or synthetic diet groups were given a single oral dose of MDZ (30 mg/kg). Blood samples were collected from individual mice at 0.25 to 6 h after MDZ administration. Concentrations of MDZ in the plasma were determined using GC-MS, as described in *Materials and Methods*, and the plasma concentration-time curves are shown in *Supplemental* Figures 1 and 2. Values shown represent means ± S.D. (*n* = 4-6).

Strain	Diet	Tmax	Cmax	$t_{1/2}$	$AUC_{0\text{-}6h}$
		h	*nmo/ml*	*h*	*nmol.h/ml*
WT	Regular	0.5	1.9 ± 0.1	4.6 ± 0.5	5.8 ± 0.5
WT	Synthetic	0.5	3.2 ± 0.3[a]	3.2 ± 0.1	10.7 ± 0.4[a]
IE-*Cpr*-null	Regular	0.5	3.0 ± 0.5[b]	2.9 ± 0.2[b]	8.4 ± 0.7[b]
IE-*Cpr*-null	Synthetic	0.25	3.0 ± 0.3	3.1 ± 0.3	11.4 ± 0.6[c]

[a] $P < 0.01$, compared with the WT/Regular diet group.

[b] $P < 0.01$, compared with the WT/Regular diet group.

[c] $P < 0.01$, compared with the IE-*Cpr*-null/Regular diet group

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Fig. 1

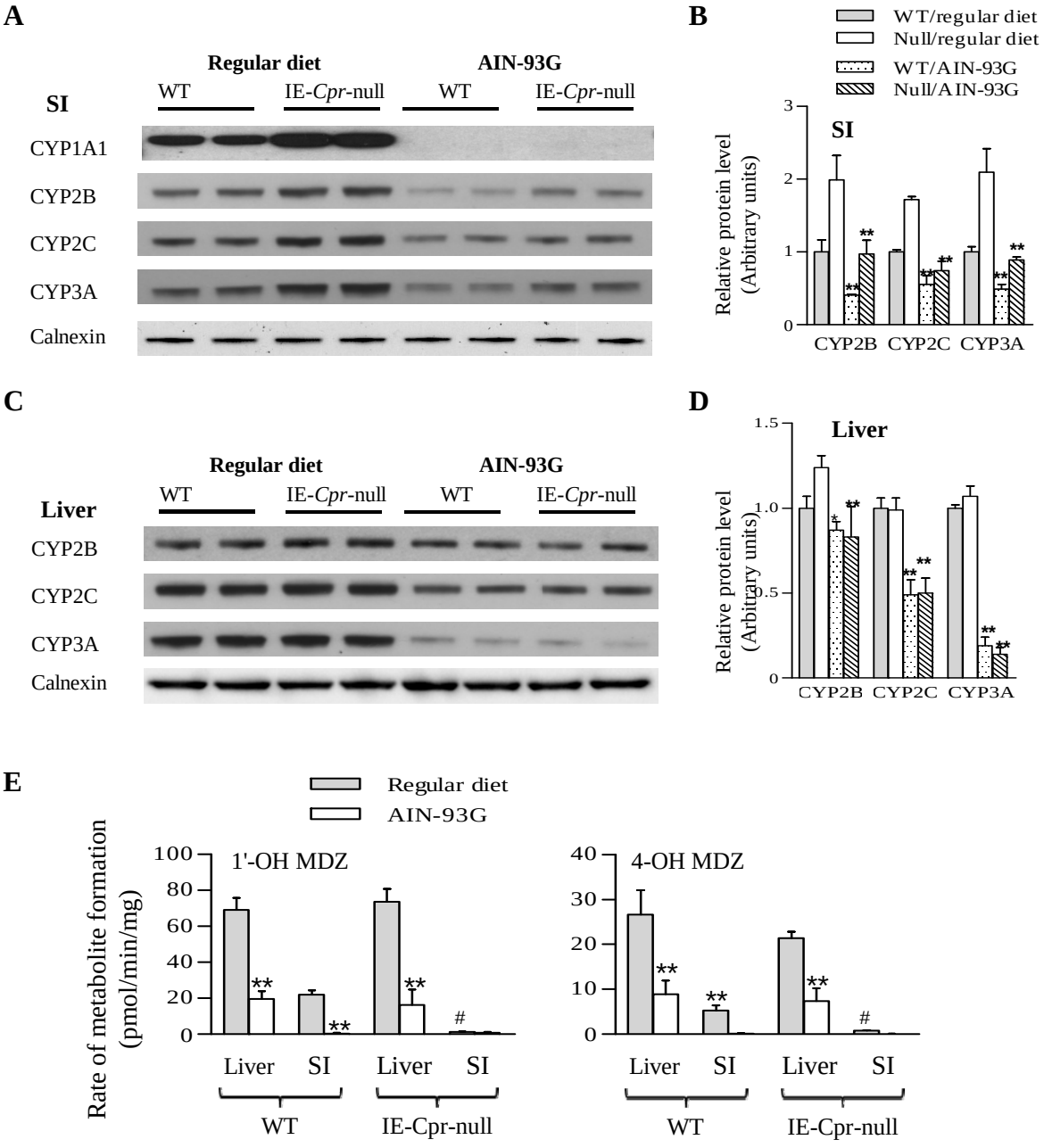

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Fig. 2

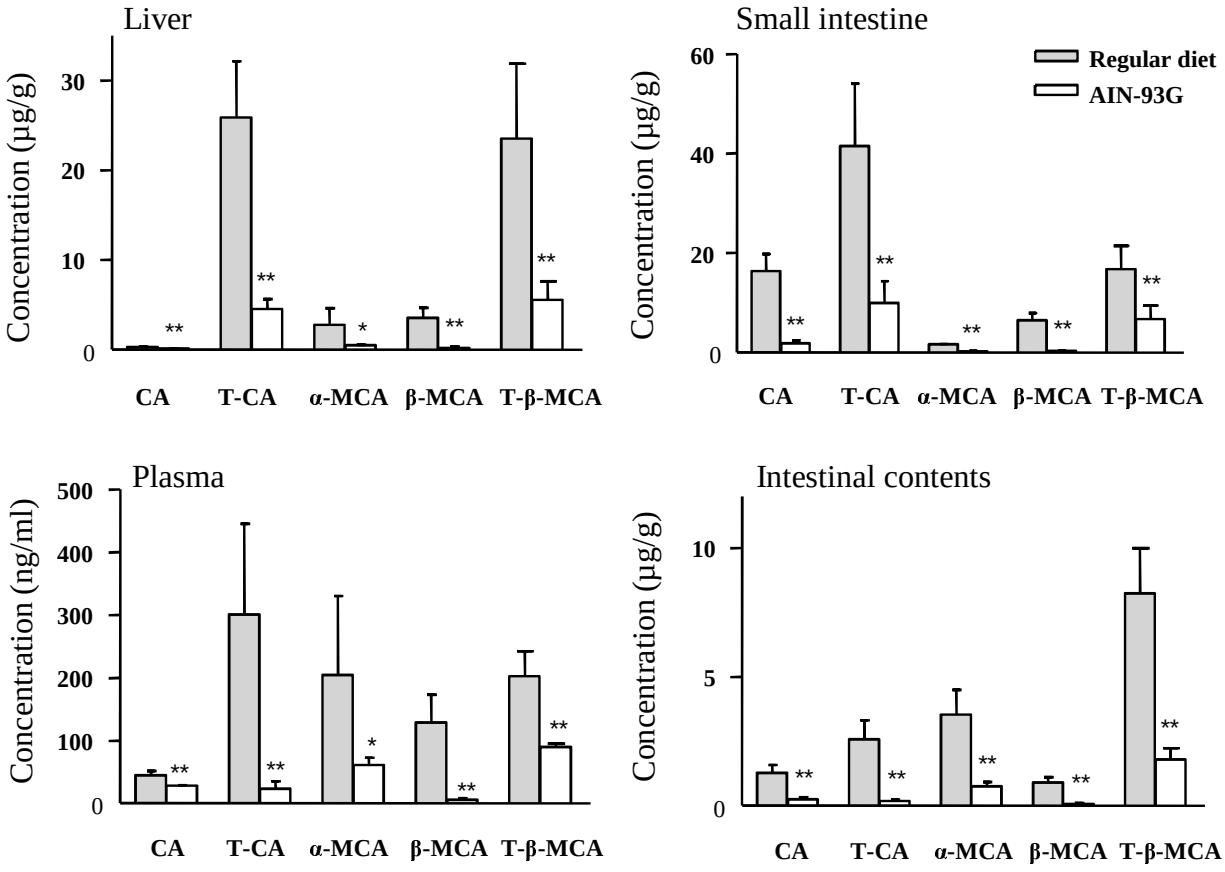

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Fig. 3