REGULATION OF CYTOCHROME P4501A1 EXPRESSION IN RAT SMALL INTESTINE

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ABSTRACT:

The predominant inducible cytochrome P450 (CYP) in rat small intestine is CYP1A1, which, when induced to elevated levels by xenobiotics or dietary constituents, has the potential to metabolize and consequently reduce the systemic uptake of low concentrations of orally ingested, bioactivatable polycyclic aromatic hydrocarbons and heterocyclic aromatic amines. We investigated the regulation of small intestinal CYP1A1 in an effort to develop its anticancer potential. The time courses of hepatic and intestinal CYP1A1 induction by β-naphthoflavone (BNF) were compared quantitatively at the protein and mRNA levels by immunoblot and competitive RNA-polymerase chain reaction analyses. CYP1A1 mRNA levels in both organs increased sharply and were maximal at ~6 hr and returned to near basal levels by 12 hr after BNF treatment. In contrast, hepatic CYP1A2 mRNA levels increased much more gradually. Small intestinal CYP1A2 mRNA concentrations were insufficient to support translation of detectable protein. Maximal levels of intestinal and hepatic CYP1A1 protein occurred between 12 and 24 hr, and 24 and 48 hr, respectively, after BNF. Intestinal CYP1A1 protein was detectable earlier and for a shorter duration than hepatic CYP1A1. CYP1A1 induction was first detected in crypt cells 3 hr before the appearance of activity in villous cells, and maximal levels of activity were reached in crypt cells 12 to 18 hr before maximal and 1.5-fold (per mg protein) higher responses in villous cells—induction thus occurs in both villous and crypt cells. Previously detected decreases in CYP1A1 inducibility from duodenum to ileum correlated with decreases in immunoblot determined-Ah receptor levels. Intestinal CYP1A1 induction does not involve the glucocorticoid receptor in contrast to hepatic induction. These studies have revealed several novel features of small intestinal CYP1A1 regulation.

Gene products of the P450 family gene superfamily are reasonably well represented in rat small intestinal epithelial cells—the enterocytes. Of the 33 P450 forms in families 1 to 3 identified to date in the rat (1), at least six forms have been demonstrated to be expressed in the small intestine, including CYP1A1, CYP2B1, CYP2C6, CYP2C11, CYP2D, and CYP3A1 (2–4).

Enterocytes provide the first metabolic machinery for orally ingested xenobiotics and are the site for xenobiotic first-pass metabolism in the small intestine. Since some of the small intestinal P450s—such as CYP1A1, CYP2B1, and CYP3A1—are inducible (2), in some instances by constituents of a normal diet (5), these P450s can regulate, to variable extents depending on the level of induction of the small intestinal P450s, the metabolic fate of many ingested xenobiotics. In addition, the short life of enterocytes, 2 days in the rat (6), confers a correspondingly short life on small intestinal P450-activated adducts to DNA and other enterocyte macromolecules. A potential benefit is to diminish the carcinogenic consequences of ingested bioactivatable procarcinogens.

In the rat small intestine, the most prominent inducible form of P450 is CYP1A1 (4). The inducibility of CYP1A1-related activity in rat small intestine was first reported over 20 years ago (7, 8). This form probably does not occur constitutively, but is inducible by constituents of commonly used rat chow and is, consequently, usually detected in small intestinal preparations in the absence of administration of known inducing agents to the rats (3, 9–11). Inducing agents, such as BNF, however, readily induce small intestinal CYP1A1 by up to 17-fold (2, 4) in rats on a normal chow diet. The induction of CYP1A1 mRNA in rat enterocytes by BNF has also recently been reported (12).

Many PAH procarcinogens are metabolized by CYP1A1 and in this process are bioactivated to putative carcinogens (13). The potential of small intestinal cells containing CYP1A1 to reduce the systemic uptake of orally ingested PAHs by bioactivating them is under investigation in this laboratory. PAHs entering the small intestine can be: 1) bioactivated and covalently bound to enterocyte macromolecules and removed by sloughing off the enterocytes within 2 days; 2) metabolized and excreted back into the lumen and eliminated in the feces; and/or 3) metabolized and the stable metabolites absorbed systemically. All three possibilities could diminish the carcinogenicity of orally ingested carcinogens, in the latter case because the small intestinal PAH metabolites are unlikely to undergo further metabolic bioactivation in what would otherwise be target organs.

In an effort to incorporate the potential of the inducible small intestinal CYP1A1 to metabolize PAHs into cancer chemoprevention strategies, we have investigated aspects of its regulation, and these are reported herein.

Materials and Methods

BNF, DEX, peroxidase-conjugated rabbit anti-goat IgG, and peroxidase-conjugated goat anti-rabbit IgG were from Sigma Chemical Co. (St. Louis, MO). The BCA protein assay kit was obtained from Pierce Chemical Co. (Rockford, IL). The ECL kit was purchased from Amersham (Arlington Heights, IL). The TRI reagent was from Molecular Research Center, Inc.
for the preparation of CYP1A1 and 1A2 competitors. For CYP1A1 and CYP1A2 were made from cDNA as described by Förster
primers have been described earlier (15). Preparation of exogenous competitor solutions just before use. Upon standing, very dilute solu-
tions of total RNA and 50 pmol of oligo d(T) 16 primer (Perkin-Elmer) in a total volume of 20 µl. The reaction mixtures were incubated at 25°C for 5 min, followed by incubation at 42°C for 50 min and, for enzyme inactivation, at 95°C for 5 min. RNA:DNA heteroduplexes were destroyed by incubation with 2 units of RNase H (Gibco BRL) at 37°C for 20 min, followed by enzyme inactivation at 95°C for 5 min. In control reactions, the reverse transcriptase and RNA samples were substituted with water. All RNA preparations were reverse-transcribed at the same time using a master mix containing all components except the RNA.

PCR reactions were performed using the RNA-PCR Core Kit from Perkin-
Elmer. Reaction mixtures contained 2 µl of the first-strand cDNA, or the buffer mixture from the control reverse transcriptase reaction, as well as different amounts of exogenous CYP1A1 or CYP1A2 competitor templates in a final volume of 100 µl. Dilutions of the competitors were made in water from concentrated stock solutions just before use. Upon standing, very dilute solutions showed decreased competitor concentrations, presumably due to absorption to surfaces. The reactions were conducted for 35 cycles in an Applied Biosystems 7900 thermal cycler with a 15-sec incubation at 98°C, a 15-sec incubation at 65°C, and a 30-sec incubation at 72°C. The sequences of the PCR primers have been described earlier (15). Preparation of exogenous competitor templates for CYP1A1 and CYP1A2 were made from cDNA as described by Förster (16). cDNA from the livers of rats treated with BNF was used as the template for the preparation of CYP1A1 and 1A2 competitors.

Forward primer for CYP1A1 (948–968): 5'-TGAACCTCTTTGGAAGCTCGTGGT
Reverse primer for CYP1A1 (1352–1370): 5'-CCAATGACTTCTTCGTCTGC
Linker primer for CYP1A1: 5'-TTTGCCTGGTTCTCAGAATGGCCACGTGA
Target sequence for CYP1A1 is 422 bp; competitor sequence is 405 bp.
Forward primer for CYP1A2 (930–950): 5'-TGAACCTCTTTGGAAGCTCGTGGT
Reverse primer for CYP1A2 (1475–1493): 5'-TTCATGGTCAGCCCATAGC
Linker primer for CYP1A2: 5'-AGGCCACACGCGTGAACATGGTTC
Target sequence for CYP1A2 is 563 bp; competitor sequence is 407 bp.
after BNF administration and at 0.1% of the maximal small intestinal CYP1A1 mRNA level. There is a gradient of epithelial cell differentiation along the crypt-villous axis of the adult rat small intestine. Studies were undertaken to determine whether BNF induction of CYP1A1 occurs in mature villous cells or in undifferentiated crypt cells. The rates of metabolism of $R$-warfarin to $R$-6- and $R$-8-hydroxywarfarin catalyzed by the microsomal preparations from villous cell or crypt cell fractions as a function of time after a single dose of BNF are shown in fig. 3. Rat CYP1A1 catalyzes the formation of both of these warfarin metabolites. Induction of CYP1A1 was evident in both crypt cell and villous cell fractions, but the time course of induction differed in the two populations, with a $\sim$3-hr lag in the initial response and a 12- to 18-hr lag in the maximal response between the induction in the crypt and the villous cells. The extent of CYP1A1 induction was much greater in villous cells than in crypt cells, when determined by both $R$-6- (fig. 3A) and $R$-8-hydroxywarfarin (fig. 3B) formation rates.

As reported in our previous study (2), the extent of CYP1A1 induction by BNF decreased markedly along the length of the small intestine from the duodenum to the ileum (fig. 4, inset). Because the initial event of induction by BNF is its binding to the cytosolic AhR, we determined the quantitative distribution of AhR along the length of the small intestine to explore the mechanism of the decreasing inducibility of CYP1A1 down the length of the small intestine. The entire length of the small intestine was equally divided into seven segments, with segment 1 being closest to the pylorus and segment 7 nearest the cecum. The immunoblot analysis of cytosolic preparations from the different intestinal segments of BNF-treated rats is shown in fig. 4. The level of AhR was highest in segment 1 and decreased distally. This gradient distribution of AhR along the length of the small intestine correlated very well with the pattern of CYP1A1 induction, suggesting that the gradient of CYP1A1 induction is at least partly due to the gradient distribution of AhR.

The synergistic enhancement of BNF induction of hepatic CYP1A1 by coadministration of DEX is a well-established factor in the regulation of this hepatic P450. We examined the effects of DEX on CYP1A1 induction by BNF in the rat liver and small intestine to determine if DEX also synergistically induces CYP1A1 in the small intestine. The rates of formation of $R$-warfarin metabolites catalyzed
by intestinal or liver microsomal preparations of rats treated with BNF, DEX, or both inducers together are shown in fig. 5. Although BNF significantly induced CYP1A1 in both the liver and small intestine, DEX alone had very little effect on CYP1A1 levels at the concentration used. However, when DEX was coadministered with BNF, CYP1A1 induction by BNF in rat liver was enhanced (1.5-fold). This synergism was previously found to be even more dramatic with adrenalectomized rats (22). In contrast, DEX treatment produced no significant effect on BNF-induced CYP1A1 expression in rat small intestine. Cotreatment with BNF and DEX at higher DEX concentrations caused toxic effects, even though DEX alone can be given at much higher doses to rats (data not shown).

Discussion

The properties of small intestinal CYP1A1 to metabolize ingested PAH and heterocyclic aromatic amine procarcinogens, and to be inducible, make this enzyme an ideal candidate for participation in a cancer chemoprevention strategy. Elevation of levels of this P450 by dietary constituents will diminish uptake of orally ingested PAH and heterocyclic aromatic amine procarcinogens, and thereby diminish carcinogenic consequences of their systemic uptake. Chemopreventative agents should exhibit minimal toxicity and high efficacy, be amenable to oral administration, have a known mechanism of action, and be reasonably priced (23). Dietary constituents capable of inducing small intestinal CYP1A1 would meet all of these criteria. The current study was undertaken to gain insight into mechanistic aspects of small intestinal induction for application to the development of the chemoprevention strategy. CYP1A1 is also an inducible constituent of human small intestine (24), which adds relevance to the current study.

Studies on the time course of small intestinal CYP1A1 induction demonstrated that this P450 is more rapidly responsive to induction in the small intestine than it is in the liver. This applies even when the inducing agent is administered intraperitoneally, which presumably precludes early exposure to the inducing agent as a causative factor in the earlier response. Induced levels of small intestinal CYP1A1 were maintained for shorter periods than in the liver, which is probably a consequence of the short life span of the enterocytes. These results suggest that, whereas the short response time for induction of small intestinal CYP1A1 will facilitate its use in blocking procarcinogen uptake, the short maintenance of induced levels will require frequent use of dietary inducing agents. This study (fig. 1, A and C) confirmed earlier reports that CYP1A2 protein is inducible in the liver, but is not detectable in the small intestine (2). This is despite observations that CYP1A2 mRNA has been detected in the small intestine by using RNA-PCR techniques.

Quantitative RNA-PCR studies revealed why CYP1A2 protein is not detected in the small intestine. CYP1A1 mRNA levels in the liver and small intestine reached similar levels per total RNA, with a similar time course. Comparisons of the CYP1A1 immunoblots, based on the same quantities of microsomal protein with the corresponding mRNA levels, suggest that CYP1A1 translatable efficiency is higher, or that CYP1A1 protein is more stable in the liver than in the small intestine. In addition, comparison of CYP1A1 and CYP1A2 mRNA levels in liver with the corresponding immunoblot data suggests that CYP1A1 and CYP1A2 are translated with similar efficiencies in this

Fig. 3. Time course of BNF induction of CYP1A1 in microsomes from crypt cells (●) and villous cells (△) monitored by R-warfarin 6- and 8-hydroxylase (OH) activities.

Fig. 4. Distribution of CYP1A1 protein (inset) and AhR protein along the length of the rat small intestine.
Rats were treated with BNF (40 mg/kg), DEX (5 mg/kg), or BNF plus DEX in corn oil in a single intraperitoneally administration. Rats were killed 24 hr after administration of the inducers, and microsomes were prepared from the liver (A) or small intestine (B) of both untreated (CONT) or treated animals. The rate of formation of R-6- and R-8-hydroxywarfarin was determined as described in the legend to fig. 3. Each bar represents the mean ± SD (N = 3).

organ. When these observations are taken into consideration, the absence of any detectable CYP1A2 protein by immunoblots of small intestinal epithelial cells is a consequence of the low levels of CYP1A2 mRNA expressed in these cells. Previously reported non-quantitative RNA-PCR studies (2) were deceptive, in that small intestinal CYP1A1 and CYP1A2 mRNA levels seemed to be at comparable concentrations. Current quantitative data clearly demonstrate that small intestinal CYP1A2 mRNA levels are only at 0.1% of the CYP1A1 mRNA levels. The discrepancy must be a consequence of highly efficient amplification of CYP1A2 cDNA relative to CYP1A1 cDNA.

The question of where induction of CYP1A1 occurs in the small intestine is controversial, in the undifferentiated crypt cells or in the mature villous cells. One study has reported no induction by BNF in the crypts (25), whereas another study has revealed induction of CYP1A1 in both crypt and villous cells (4). Despite claims that differential chelation-elution can separate villous tips, midvillous cells, and crypt cells (26), we have previously demonstrated that separation of villous cells into subfractions is not feasible by these techniques (4). Separation of villous cells and crypt cells can readily be achieved, however, and we have used these fractions in an attempt to assess where induction of small intestinal CYP1A1 occurs. The results indicate that the BNF induction of CYP1A1 occurs initially in the crypt cells; and by 12 hr after BNF induction, CYP1A1 activity levels in crypt cells were maximal. Induction of CYP1A1 activity in villous cells lagged that in the crypt cells, which suggests that induction occurs in the crypt cells, and that the induced levels of CYP1A1 are carried with migrating and differentiating cells up the villus. However, the higher levels of CYP1A1/mg microsomal protein at-

tained in the villous cells vs. the crypt cells are not consistent with this conclusion. Studies suggest that induction of CYP1A1 occurs initially in the crypt cells and that subsequent induction also occurs in the mature villous cells.

We have previously determined that the inducibility of CYP1A1 diminishes as a function of the distance from the pyloric valve along the length of the rat small intestine (2). Because the first step in CYP1A1 induction involves binding of the inducing agent, BNF in this study, to the AhR, we determined the levels of the receptor by immunoblot analysis along the length of the small intestine. The levels of AhR did decrease in a comparable manner to the induced levels of CYP1A1, which suggests that the basis for the fall-off in CYP1A1 inducibility is at least partially due to the fall-off in AhR levels.

Studies on the synergistic enhancement by DEX of hepatic CYP1A1 induction by BNF have been interpreted to indicate that the glucocorticoid receptor can play a role in CYP1A1 induction (21). Our studies have revealed that, in the context of this synergistic induction, small intestinal and hepatic CYP1A1 exhibit differences in their mechanisms of induction. In the small intestine, the glucocorticoid receptor does not apparently participate in CYP1A1 induction, despite its presence in enterocytes (27).

In summary, we have determined several aspects of the regulation of small intestinal CYP1A1, including the time course of CYP1A1 mRNA and protein induction, the role of crypt and villous cells in CYP1A1 induction, the role of AhR levels in the diminishing inducibility of CYP1A1 along the length of the small intestine, and the lack of a role for the glucocorticoid receptor in CYP1A1 induction.

References


