Bile Acid Induction of Cytokine Expression by Macrophages Correlates with Repression of Hepatic Cholesterol 7α-Hydroxylase*

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In the studies reported herein, we show that two complementary experimental models—inbred strains of mice (i.e. C57BL/6 and C3H/HeJ), and a differentiated line of rat hepatoma cells (i.e. L35 cells), require the activation of cytokines by monocyte/macrophages to display bile acid negative feedback repression of cholesterol 7α-hydroxylase (CYP7A1). Feeding a bile acid-containing atherogenic diet for 3 weeks to C57BL/6 mice led to a 70% reduction in the expression of hepatic CYP7A1 mRNA, whereas no reduction was observed in C3H/HeJ mice. The strain-specific response to repression of CYP7A1 paralleled the activation of hepatic cytokine expression. Studies using cultured THP-1 monocyte/macrophages showed that the hydrophobic bile acid chenodeoxycholate, a well established potent repressor of CYP7A1, induced the expression of mRNAs encoding interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-α). In contrast, the hydrophilic bile acid ursodeoxycholate, which does not repress CYP7A1, did not induce cytokine mRNA expression by THP-1 cells. Chenodeoxycholate activation of cytokines by THP-1 cells was blocked by the peroxisome proliferator-activated receptor γ agonist rosiglitazone. The expression of cytokines (e.g. IL-1 and TNF-α) by THP-1 cells paralleled with the ability of these cells to produce conditioned medium that when added to rat L35 hepatoma cells, repressed CYP7A1. Moreover, rosiglitazone, which blocks cytokine activation by macrophages, also blocked the repression of CYP7A1 normally exhibited by C57BL/6 mice fed the bile acid-containing atherogenic diet. The combined data indicate that the activation of cytokines may mediate CYP7A1 repression caused by feeding mice an atherogenic diet containing bile acids.

Bile acids, the major metabolites produced from cholesterol, are amphipathic steroid detergents necessary for the digestion and absorption of fat soluble nutrients from the intestine (1–9). The conversion of cholesterol to bile acids is regulated by the expression of cholesterol 7α-hydroxylase (CYP7A1), a cytochrome P450 enzyme unique to the liver parenchymal cell (4–6). Bile acid synthesis exhibits negative feedback regulation (7, 8) by decreasing the enzymatic activity of CYP7A1 (9). It is generally accepted that bile acids can inhibit the transcription of the CYP7A1 gene (1–3).

Many different experimental models have been used to examine bile acid negative feedback regulation of CYP7A1 and some have yielded conflicting results. Bile acid negative feedback repression of CYP7A1 has been experimentally demonstrated by infusing bile acids into the intestine of bile fistulae rats (10) and hamsters (11). The ability of different bile acids to repress CYP7A1 expression correlates with the hydrophobic index of the infused bile acid; CDCA is a potent repressor, whereas UDCA is not (12). The finding that infusing taurocholate into the portal vein of bile fistulae mice was unable to repress CYP7A1 led to the conclusion that a factor produced within the enterohepatic circulation may be required to repress CYP7A1 (10).

Bile acid repression of CYP7A1 has been demonstrated using primary cultured rat hepatocytes (13) and human hepatoma HepG2 cells (14–16). Data from these cultured cell studies suggest that multiple mechanisms exist in regard to bile acid repression of CYP7A1 expression. These mechanisms include: “bile acid response” elements (BARE) (17), activation of protein kinase C (18), and activation of the farnesoid X receptor (FXR) (16, 19).

L35 is a stable line of rat hepatoma cells that have been used for studies examining the expression of CYP7A1 (20–22). L35 cells express CYP7A1 at levels equal to that of rat liver, which is 10-fold greater than the levels expressed by either HepG2 cells or primary rat hepatocytes (20). Moreover, with the one notable exception of resistance to repression by bile acids, the expression of CYP7A1 by L35 cells responded normally to essentially all the effectors established to alter CYP7A1 expression in vivo (20–22). The inability of bile acids to repress CYP7A1 expression by L35 cells led to the proposal that they are missing factors necessary to mediate this repression (21).

In the studies reported herein, we show that these factors are cytokines produced by macrophages.

MATERIALS AND METHODS

Mouse Studies—Female C3H/HeJ and C57BL/6 mice 10–12 weeks old were obtained from Jackson Laboratory, Bar Harbor, ME. The mice were housed in a room with a normal light cycle (lights on from 6 a.m. to 6 p.m.) were fed either normal Purina breeder chow or ground Purina breeder chow supplemented with 20% olive oil, 2% cholesterol, and 0.5% taurocholic acid (bile acid-containing atherogenic diet) and water ad libitum. Mice were maintained on the above diets for 3 weeks.

In the experiments examining the effect of rosiglitazone on CYP7A1

1 The abbreviations used are: CYP7A1, cholesterol 7α-hydroxylase; BSA, bovine serum albumin; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; FXR, farnesoid X receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL, high density lipoprotein; IL-1, interleukin 1; IFN-γ, interferon gamma; PPARY, peroxisome proliferator-activated receptor γ; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; BARE, bile acid response element.
expression, C57BL/6 mice fed the Chow diet and the bile acid-containing atherogenic diet were divided into two groups. Half the mice in each diet group were given either vehicle (0.25% Tween 80, 1% carboxymethylcellulose) alone or vehicle containing 1 mg/ml of rosiglitazone daily by oral gavage. Mice were sacrificed at 9 a.m., and blood was obtained for subsequent analysis.

Hepatic cytokine mRNAs were quantitated using RNase protection assays. In vitro transcribed [α-32P]UTP-labeled antisense cytokine probes were generated using cytokine multiprobe template kits: mouse mCK-2 (catalog number 45002P) and mouse mCK-3 (catalog number 45003P; PharMingen International) and a MAXIscript in vitro transcription kit (catalog number 1314) using T7 RNA polymerase per the manufacturer’s instructions. The content of human cytokines mRNAs was also quantitated by RNase protection assays except human template kits were used (human hCK-2, catalog number 45032P and human hCK-3, catalog number 45033P; PharMingen International).

Cultured Cell Studies—Rat L35 cells were cultured in DMEM (21, 22). THP-1 cells were cultured in RPMI medium 1640 plus 10% FBS containing: 0.1% BSA, 0.1% BSA with and without CDCA (100 μμg), 0.1% BSA containing UDCA (100 μμg) and rosiglitazone (as indicated in Fig. 2). Cells were harvested, and poly(A) RNA was isolated, as described previously (21, 22). Statistical significance was determined by Student’s t test using double-tailed p values.

RESULTS AND DISCUSSION

When fed the bile acid-containing atherogenic diet, C57BL/6 mice display repression of CYP7A1, whereas C3H/HeJ mice do not (23–25). Quantitative trait loci analysis of C3H/HeJ and C57BL/6 mice shows that marked phenotypic differences exist in regard to displaying inflammation in response to consuming the bile-acid-containing atherogenic diet (26, 27). C3H/HeJ mice display essentially a complete resistance to hepatic inflammation, whereas C57BL/6 mice display a remarkable susceptibility (26, 27). We examined if strain-specific differences in cytokine activation might be the basis for the strain-specific differences in CYP7A1 repression. On the normal Chow diet, the expression of CYP7A1 mRNA expression was similar in C57BL/6 and C3H/HeJ mice (Fig. 1, A and B). In contrast, the bile acid-containing atherogenic diet caused marked differences in the expression of CYP7A1 by the two strains of mice. While C3H/HeJ mice displayed no significant change in CYP7A1 expression, C57BL/6 mice showed a marked 70% decrease, p < 0.01 (Fig. 1, A and B).

The individual strains also displayed distinct differences in the response of hepatic cytokine expression to the bile acid-containing atherogenic diet (Fig. 1C). In C57BL/6 mice, the bile acid-containing atherogenic diet increased the hepatic expression of mRNAs encoding IL-1α (7-fold, p < 0.01), IL-1β (4-fold, p < 0.01), TNFα (3-fold, p < 0.01), IFNγ (6-fold, p < 0.01), and TGF-β1 (7-fold, p < 0.01) (Fig. 1C). In marked contrast, the expression of hepatic cytokines by C3H/HeJ mice was unaffected by the bile acid-containing atherogenic diet (Fig. 1C). The concordance between the ability of the bile acid-containing atherogenic diet to induce the expression of mRNAs encoding cytokines while repressing CYP7A1 mRNA expression suggested the possibility that cytokines might mediate the repression of CYP7A1 caused by the bile acid-containing atherogenic diet. Indeed, recent studies have shown that administering lipopolysaccharide as well as the cytokines TNFα or IL-1 to hamsters resulted in a marked suppression of CYP7A1 (28).

Based on these combined findings, we formulated the following experimentally testable model (Fig. 1D). Following their active absorption in the distal intestine, bile acids return to the liver via the portal vein entering the hepatic parenchymal cell by crossing through the sinusoids. As bile acids move across the sinusoids, they may interact with resident macrophages (i.e.
Kupffer cells) which reside along the sinusoidal surface. At sufficient concentration, bile acids may cause the activation of cytokines by Kupffer cells. These regulatory cytokines may subsequently act on hepatic parenchymal cells, leading to the repression of CYP7A1 (28).

We attempted to reconstruct this model using the cultured rat hepatoma cell line (L35 cells) and human monocyte/macrophages (THP-1 cells). To approximate the intercellular relationships that may exist between hepatic macrophages and parenchymal cells (Fig. 1D), THP-1 cells were exposed to bile acids and the effects of the conditioned medium was examined on the expression of CYP7A1 by L35 cells. CYP7A1 expression by L35 cells was unaffected by changing the culture medium to serum-free DMEM, without dexamethasone but containing either 0.1% BSA (lanes 1 and 3) or 0.1% BSA containing 100 μM UDCA (lanes 2 and 6), 0.1% BSA containing 100 μM CDCA (lanes 3 and 7) or 0.1% BSA containing 100 μM CDCA and 500 nM rosiglitazone (BRL) (lanes 4 and 8). RNA was isolated and subjected to RNase protection assay for the indicated cytokines. L32 and GAPDH are RNA loading controls. C, the PPARγ agonist rosiglitazone blocks the CDCA-induced repression of CYP7A1 in L35 cells by conditioned medium from THP-1 cells. Rat hepatoma L35 cells were cultured in serum-free medium containing 100 μM dexamethasone for 24 h. The cultured medium was then changed to serum-free DMEM containing the following additions (50% by volume): conditioned medium obtained from THP-1 cells incubated with 0.1% BSA (lanes 1 and 5), 0.1% BSA containing 100 μM UDCA (lanes 2 and 6), 0.1% BSA containing 100 μM CDCA (lanes 3 and 7) or 0.1% BSA containing 100 μM CDCA and 500 nM rosiglitazone (BRL). After 24 h, cells were harvested, and the relative level of CYP7A mRNA to β-actin mRNA was quantitated. Each value represents the mean of duplicate plates of cells. D, TNF-α represses the expression of CYP7A1 mRNA by L35 cells. L35 cells cultured in serum-free DMEM medium containing 100 μM dexamethasone were treated with the indicated concentrations of human TNF-α for 24 h. Each value represents the level of CYP7A mRNA to β-actin mRNA as the mean ± S.D of three replicate plates of cells.

According to our hypothesis (Fig. 1D) cytokines mediate the repression of CYP7A1 caused by dietary bile acids. Thus, our model predicts that blocking the activation of cytokines by bile acids should block the repression of CYP7A1. PPARγ agonism inhibits the production of inflammatory cytokines by monocyte/macrophages (29). Therefore, if our model is valid, the PPARγ agonist rosiglitazone should prevent repression of CYP7A1. Treating THP-1 cells with rosiglitazone completely blocked the ability of CDCA to induce the expression of CYP7A1 (Fig. 2B). Rosiglitazone also blocked the ability of THP-1 cells exposed to CDCA to produce conditioned medium that could repress CYP7A1 expression by L35 cells (Fig. 2C). The additional finding that TNFα caused a dose-dependent decrease in the expression of CYP7A1 mRNA by L35 cells (Fig. 2D) further indicates that cytokines produced by THP-1 cells in response to CDCA are responsible for repression of CYP7A1.

Further analysis showed that L35 cells did not express detectable levels of mRNAs encoding the regulatory cytokines TNFα, TGF-β1, or IL-1β (data not shown). Treatment of L35
cells with culture medium containing CDCA or rosiglitazone did not induce the expression of these cytokines to detectable levels (data not shown). In marked contrast to L35 cells, HepG2 cells display the ability to express most inflammatory cytokines (30, 31). The inability of L35 cells to express TNF-α did not induce the expression of these cytokines to detectable levels with culture medium containing CDCA or rosiglitazone.

Therefore, it is likely that the composition and concentration of the bile acid pool within the enterohepatic circulation determines which of several possible mechanisms will be invoked in regard to regulating the expression of CYP7A1.

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Fig. 3. The PPARγ agonist rosiglitazone blocks the repression of CYP7A1 mRNA caused by feeding C57BL/6 mice the bile acid-containing atherogenic diet. Female C57BL/6 mice (n = 24) were fed either chow or the bile acid-containing atherogenic diet. Half the mice in each feeding group were given either vehicle (0.25% Tween 80, 1% carboxymethylcellulose) alone or vehicle containing 1 mg/ml of rosiglitazone daily by oral gavage. After 3 weeks, mice were sacrificed, and the relative content of rat CYP7A1 mRNA compared with GAPDH was determined. Each value represents the mean ± S.D of six separate mice. The asterisk denotes a significant difference between the values for the rosiglitazone-treated chow-fed mice and the rosiglitazone-fed mice fed the bile acid-containing atherogenic diet, p < 0.01.