Bile acid signaling through FXR induces intracellular adhesion molecule-1 expression in mouse liver and human hepatocytes

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Qin, Pu, Lisa A. Borges-Marcucci, Mark J. Evans, and Douglas C. Harnish. Bile acid signaling through FXR induces intracellular adhesion molecule-1 expression in mouse liver and human hepatocytes. Am J Physiol Gastrointest Liver Physiol 289: G267–G273, 2005. First published April 7, 2005; doi:10.1152/ajpgi.00043.2005.—Previous studies have demonstrated a dramatic induction of inflammatory gene expression in livers from mice fed a high-fat, high-cholesterol diet containing cholate after 3–5 wk. To determine the contribution of cholate in mediating these inductions, C57BL/6 mice were fed a chow diet supplemented with increasing concentrations of cholic acid (CA) for 5 days. A dose-dependent induction in the hepatic levels of TNF-α, VCAM-1, ICAM-1, and SAA-2 mRNA were observed. As positive controls, a dose-dependent repression of cholesterol 7α-hydroxylase and a dose-dependent induction of small heterodimer partner (SHP) expression were also observed, suggesting that farnesoid X receptor (FXR) was activated. In addition, ICAM-1 and SHP mRNA levels were also induced in primary human hepatocytes when treated with chenodeoxycholic acid or GW4064, a FXR-selective agonist. The involvement of FXR in CA-induced inflammatory gene expression was further investigated in the human hepatic cell line HepG2. Both ICAM-1 and SHP expression were induced in a dose- and time-dependent manner by treatment with the FXR-selective agonist GW4064. Moreover, the induction of ICAM-1 by GW4064 was inhibited by the FXR antagonist guggulsterol or with transfection of FXR siRNA. Finally, the activity of FXR was mapped to a retinoic acid response element (RARE) site containing an imbedded farnesoid X response element (FXRE) on the human ICAM-1 promoter and FXR and retinoid X receptor were demonstrated to bind to this site. Finally, FXR-mediated activation of ICAM-1 could be further enhanced by TNF-α cotreatment in hepatocytes, suggesting a potential cooperation between cytokine and bile acid-signaling pathways during hepatic inflammatory events.

Inflammation

Bile acids are amphipathic detergents necessary for the digestion and absorption of fat-soluble nutrients. Bile acids are synthesized in the liver by either a neutral or classic pathway resulting in formation of cholic acid (CA) or by an acidic or alternative pathway leading to the synthesis of chenodeoxycholic acid (CDCA). In humans, the classic biosynthetic pathway predominates via the rate-limiting enzyme cholesterol-7α-hydroxylase (CYP7A1). After their synthesis, bile acids are excreted to the intestine where they facilitate the absorption of dietary lipid and lipid-soluble nutrients. In addition, bile acids are also important in the excretion of cholesterol and toxic metabolites. The biosynthesis of bile acids from cholesterol is the most significant pathway for the elimination of cholesterol from the body.

It has been recently demonstrated that bile acids are the physiological ligands for the farnesoid X receptor (FXR) (24, 38). FXR is expressed mainly in tissues that are exposed to high concentrations of bile acids including the liver and intestine (24, 38). Activated FXR binds to the promoters of its target genes typically via an IR-1 element and enhances the transcription of these genes, including small heterodimer partner (SHP) (11, 33), bile salt export pump (BSEP), and ileal bile acid binding protein (1, 3, 12, 22). The induction of SHP by FXR results in the negative regulation of bile acid synthesis through the regulation of CYP7A1 and sterol 12α-hydroxylase (CYP8B1) (11, 22, 42). Thus FXR is an important regulator for bile acid homeostasis, and inactivation of FXR in mice results in elevated serum bile acids and cholesterol, increased triglyceride levels, increased hepatic cholesterol, and a proatherogenic serum lipoprotein profile (17).

Although the role of bile acids in regulation of bile acid and lipid homeostasis has been well established, its potential role in inflammation has not been defined. There has been a number of reports in the literature suggesting a link between bile acids and inflammation. When accumulated in high concentrations, hydrophobic bile acids can result in an inflammatory phenotype as evidenced during cholestasis (13). The induction of proinflammatory genes such as EGF, transforming growth factor-β1 (TGF-β1), TNF-α, IL-1, and ICAM-1 have been demonstrated in experimental models of cholestasis after bile duct ligation resulting in neutrophil-induced liver injury (14, 30). In addition, before the development of genetically modified mice that are prone to atherosclerosis, cholate supplementation to a high-fat, high-cholesterol diet was necessary to induce consistent aortic lesion formation in C57BL/6 mice (28). This diet has also been shown to induce the expression of a number of inflammatory genes in the liver including serum amyloid A2 (SAA-2), monocyte chemotactic protein, VCAM-1, TNF-α, and regulated on activation normal T cell expressed and secreted (RANTES) (9, 18) with the induction of VCAM-1 specifically being attributed to the presence of cholate (36).

The mechanism by which bile acids could be inducing hepatic and vascular wall inflammation is unclear. The inflammation induced by prolonged exposure to high levels of bile acids could simply be due to its associated cytotoxicity (7). Alternatively, bile acids could elicit their proinflammatory phenotype through activation of kinase pathways including protein kinase C (34), extracellular signal-regulated kinase (31), or c-Jun NH2-terminal kinase (15). In

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addition, bile acids can stimulate cytokine production in macrophages that can then modulate adjacent hepatocytes (26). In this report, we demonstrate a novel pathway in which bile acids can induce inflammatory gene expression through direct FXR signaling.

In this study, we demonstrate that treatment of C57BL/6 mice with CA results in the induction of ICAM-1, VCAM-1, TNF-α, and SAA-2. Moreover, in HepG2 cells, ICAM-1 expression was induced by the synthetic FXR agonist GW4064 (25) in a dose- and time-dependent manner. The induction of ICAM-1 by GW4064 could be antagonized by treatment with the FXR antagonist guggulsterone or through inhibition of FXR expression by siRNA. FXR was demonstrated to directly interact with a RARE/FXRE site within the human ICAM-1 promoter. Finally, we demonstrate that the activation of ICAM-1 by liganded FXR could be further enhanced by cotreatment with cytokines suggesting a potential cooperation between the two signaling pathways during inflammatory events.

MATERIALS AND METHODS

Mice. Male C57BL/6 mice (16–20 g) (Taconic) were separated into groups of eight. The mice were fed a chow diet or high-fat diet (20% fat, 0.15% cholesterol) supplemented with CA (0.1–1%) as indicated for up to 5 wk. At the end of the experimental period, the liver was dissected and immediately frozen in liquid nitrogen. Liver total RNA was prepared by using Trizol reagent (GIBCO-BRL) and further purified using RNeasy kit (Qiagen). Gene expression analysis was performed using real-time RT-PCR as described previously (9).

Cell culture. Primary human hepatocytes cultured in a 24-well plate (Cambrex) were treated immediately on arrival. HepG2 cells were maintained in growth medium (DMEM; GIBCO-BRL) supplemented with heat-inactivated 10% FBS, 1% Glutamax, 1% MEM nonessential amino acids, 100 U/ml penicillin, and 100 μg/ml streptomycin. All cells were maintained in 37°C in a 5% CO2 incubator. The cells were seeded in at 3.0 × 105 cells/well in a 12-well dish. The cells were treated with 1–100 μM CDCA (Sigma), 1–1,000 nM GW4064, 5 μM guggulsterone (Steraloids), or 1 μM GW4064 for 24 h. Total RNA was isolated, and the levels of SHP, FXR, and ICAM-1 were assayed by real-time RT-PCR and normalized to GAPDH. Values are reported as means ± SE of three replicates from at least two experiments.

RESULTS

CA induces inflammatory gene expression. Previous studies (9, 18) have demonstrated a dramatic induction of inflammatory gene expression in livers from mice fed a high-fat diet containing CA. Studies were undertaken to determine the involvement of cholate in mediating these inflammatory gene inductions. C57BL/6 mice were fed a Chow, high-fat diet supplemented with 0.5% cholate or high-fat diet alone for 5 wk. Induction of the inflammatory genes ICAM-1, VCAM-1, SAA-2, and TNF-α mRNA occurred in mice fed a high-fat diet containing CA but not in mice fed a high-fat diet without CA

![Fig. 1. Hepatic inflammatory gene expression is induced by cholate. C57BL/6 mice were fed with a chow diet, high-fat diet (HF) with 0.5% cholate (CA) or high-fat diet without CA for 5 wk. The hepatic mRNA levels of ICAM-1, VCAM-1, TNF-α, and serum amyloid A2 (SAA-2) were examined by real-time RT-PCR and normalized to GAPDH. Values are reported as means ± SE with the mean expression level in mice fed the chow diet defined as 1. *P < 0.05 vs. mice on a chow diet.](http://ajpgi.physiology.org/)

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This suggests that the induction of these genes is dependent on CA.

To further prove the role of CA in the induction of these inflammatory genes and to eliminate the potential confounding toxic metabolic effects associated with 5-wk exposure to CA (7), C57BL/6 mice were fed a chow diet supplemented with increasing concentrations of CA (0.01–1%) for 5 days. Real-time RT-PCR analysis demonstrated a dose-dependent induction in the hepatic mRNA levels of ICAM-1, VCAM-1, TNF-α, and SAA-2 with maximal induction observed at the 0.3% dose for TNF-α (2-fold) and at 1.0% for ICAM-1 (3.1-fold), VCAM-1 (2.2-fold), and SAA-2 (28-fold; Fig. 2). As positive controls, the induction of SHP and repression of CYP7A1 by CA were observed as previously reported (19). These data support the premise that bile acids such as CA can induce inflammatory gene expression in the liver.

FXR is required for the induction of ICAM-1 in HepG2 cells. To further elucidate the role of bile acids in the induction of hepatic inflammatory genes, studies were performed in primary human hepatocytes and the human hepatic cell line HepG2. Primary human hepatocytes were treated with 100 μM CDCA or the synthetic FXR agonist GW4064 (1 μM) for 6 and 24 h, and the mRNA levels were determined by real-time RT-PCR. Both SHP and ICAM-1 were induced by CDCA and GW4064 treatment at both time points (Fig. 3). In contrast, SAA-2 mRNA levels were only slightly induced (1.4-fold) by GW4064, whereas VCAM-1 mRNA levels were not regulated at all (data not shown). To further study the regulation of ICAM-1 by FXR, HepG2 cells were treated with increasing concentrations of CDCA or GW4064 for 24 h. ICAM-1 mRNA levels were dose dependently increased by both CDCA and GW4064 treatment with the maximal fold induction of 2.7 and 2.5 at 100 μM CDCA and 100 nM GW4064, respectively (Fig. 4A). Similar results were also obtained in the human hepatocyte cell line, Hep3B (data not shown). As expected, SHP mRNA was upregulated, whereas CYP7A1 mRNA was downregulated by CDCA and GW4064 in a dose-dependent manner consistent with previous observations (16). Time-course studies demonstrated a rapid induction of ICAM-1 and SHP mRNA within 2 h by GW4064 treatment and persisting for at least 24 h (Fig. 4B), which resulted in the induction of ICAM-1 protein levels (Fig. 4C). The ability of the selective FXR agonist GW4064 to induce ICAM-1 expression supports the involvement of FXR signaling in mediating this effect.

To further confirm the role of FXR in the induction of ICAM-1, HepG2 cells were treated with 100 nM GW4064 alone or cotreated with 5 μM of the FXR-selective antagonist guggulsterone (Fig. 5A) (5, 35). The GW4064-mediated elevation of SHP and ICAM-1 mRNA was effectively inhibited by guggulsterone coadministration. In addition, transfection of FXR siRNA resulted in a 50% suppression of endogenous FXR mRNA levels, which led to an ~50% decrease in the GW4064-mediated induction of SHP mRNA levels (from a 60% increase to a 30% increase over vehicle-treated cells, Fig. 5B). A similar decrease in the GW4064-mediated induction in ICAM-1 expression was also observed following FXR siRNA transfection (from a 130% increase to a 60% increase over vehicle-treated cells, Fig. 5B). These data further support a role for FXR in mediating ICAM-1 expression in HepG2 cells.
Mapping the FXR-responsive element in the human ICAM-1 promoter. To determine the mechanism of FXR regulation on ICAM-1, a series of promoter deletion constructs was created and tested in HepG2 cotransfection assays. As shown in Fig. 6, GW4064 treatment resulted in a threefold induction in luciferase activity with the ICAM-1 promoter construct containing sequences from /H11002 to /H11001. Deletion of promoter sequences from /H11002 to /H11002 did not affect the GW4064-mediated induction in luciferase activity. However, deletion from /H11002 to /H11002 resulted in a complete loss of GW4064-mediated induction. The sequence between /H11002 and /H11002 contains a RARE (reviewed in Ref. 37) and mutation of this site within the context of the /H11002 to /H11001 promoter region (see Fig. 7A) also resulted in the loss of the FXR-mediated induction. This RARE site appears to contain an imbedded FXRE site (Fig. 7A). To determine whether FXR directly binds to this element, EMSA assays were performed. In vitro transcribed/translated FXR and RXR-α proteins were incubated with labeled WT RARE probe from the ICAM-1 promoter, consensus FXRE probe (IR-1 element from BSEP), or the mutated RARE probe (MUT; Fig. 7B and C). A shifted band is observed when both FXR and RXR-α proteins are present and only with the WT and IR-1 probe but not with the MUT probe. The shifted band can be competed off by excess amounts of unlabeled WT or IR-1 probe (Fig. 7C),
whereas excess amounts of unlabeled MUT probe or a control nucleotide probe (unrelated sequences) does not compete off the shifted band. To further confirm that FXR and RXR-α proteins bind to this element, FXR and RXR-α proteins were incubated with labeled WT probe and antibodies against the NH2-terminal of FXR or RXR-α (Fig. 7D). Both antibodies greatly reduced the intensity of the shifted band, suggesting that the FXR-RXR heterodimer can bind to this RARE/FXRE site to mediate the induction of ICAM-1 transcription directly.

Coordinative induction of ICAM-1 expression by FXR agonist and proinflammatory cytokine. Because cytokine signaling can regulate genes involved in bile acid synthesis (6, 10) and is linked with inflammatory gene expression, we wanted to determine whether there was any cooperation between bile acids and cytokines in influencing ICAM-1 expression. From previous experiments (4), TNF-α was shown to modulate ICAM-1 expression through the NF-κB element just downstream from the newly identified FXRE, and it is likely that these two signaling pathways may coordinately impact the ICAM-1 promoter. HepG2 cells were treated with TNF-α and GW4064 for 24 h, and the mRNA levels of ICAM-1 and FXR were examined by real-time RT-PCR (Fig. 8). TNF-α and GW4064 treatment elevated ICAM-1 mRNA levels to a similar extent (2- to 3-fold) in HepG2 cells without affecting FXR mRNA levels. When given in combination, an additive effect on ICAM-1 induction was observed. These data suggest that the FXR-dependent induction of ICAM-1 in hepatocytes can be further enhanced by additional proinflammatory signals such as TNF-α.

DISCUSSION

It has been well established that high doses of bile acids can induce inflammation in the liver and cause liver damage in disease conditions such as primary biliary cirrhosis, cholestasis, and primary sclerosing cholangitis (8). In this study, we determined that bile acids alone, in the absence of high fat and high cholesterol, could mediate the induction of a number of inflammatory genes in the mouse liver. In addition, we identified a novel role for FXR being involved in this activity, specifically in mediating the induction of ICAM-1 in human hepatocytes. This induction of inflammatory gene expression is likely to initiate or contribute to bile acid-induced inflammation.

Our study initiated from the observation of the increased expression of proinflammatory genes in the liver of C57BL/6 mice fed an atherogenic diet (20% fat, 0.15% cholesterol, and 0.5% cholate) for 5 wk (9). We demonstrate here that CA alone can mediate the induction of at least a subset of these proinflammatory genes such as VCAM-1, ICAM-1, TNF-α, and SAA-2. This is supported by a previous study (36) in which the induction of VCAM-1 as well as other proinflammatory genes such as the chemokines scya6 and scya9 were attributed solely to the cholate component of the atherogenic diet. In that study, SAA-2 expression was also demonstrated to be induced by either the cholesterol or cholate component of the diet. Therefore, it appears that bile acids themselves are inducers of proinflammatory gene expression in the liver.

Through a series of in vitro experiments including agonist, antagonist, and siRNA, we provide strong evidence that bile acids induce the expression of ICAM-1 through an FXR-dependent mechanism. Using promoter mapping and EMSA assays, we showed that FXR and RXR bind to a RARE/FXRE site within the promoter of ICAM-1. Mutations in both the upstream half-site (Fig. 6) as well as the downstream half-site (data not shown) of this RARE/FXRE site in ICAM-1 promoter abolished the induction in luciferase activity by GW4064. This suggested that FXR likely functions as a heterodimer with RXR and was supported by the EMSA experiments. Interestingly, the EMSA experiments showed that the amount of binding of FXR-RXR to the ICAM-1 promoter did not change following GW4064 treatment. Thus the induction in the transcription of ICAM-1 may be a result of ligand-dependent displacement of corepressors with coactivators associated with the FXR-RXR heterodimer but not due to the amount of FXR-RXR associated with the response element.

ICAM-1 can be induced by proinflammatory cytokines such as TNF-α, INF-γ, and IL-1β in endothelial cells as well as hepatocytes (21, 27, 32, 41). When HepG2 cells were cotreated with TNF-α and GW4064, an additive effect in the induction of ICAM-1 was observed, suggesting that the FXR- and cytokine-signaling pathways can cooperate and may impact inflammatory events associated with elevated levels of bile acids. This may occur during cholestasis where both elevated levels of cytokines and bile acids are
observed. The neutrophil-induced liver injury in the bile duct-ligated mouse model of cholestasis is reduced in both ICAM-1- and CD18-deficient mice (13, 14). Therefore, the potential arises that the neutrophil-induced liver injury resulting from elevated bile acid levels is mediated in part due to the activation of ICAM-1 expression by FXR. On the other hand, FXR activation can also have beneficial effects in this model through induction of bile salt exporters that facilitate the clearance of bile acids during cholestasis, thereby reducing the toxic and potentially proinflammatory effect of bile acids (20).

The FXR-dependent induction of ICAM-1 may also translate into the vasculature where FXR expression has recently been demonstrated (2). The induction of ICAM-1 is known to contribute to the leukocyte-induced inflammation in vascular tissue that can eventually lead to the formation of atherosclerotic lesions. The contribution of FXR in atherosclerotic lesion development has not yet been studied. However, studies by Paigen’s laboratory (28) demonstrating the requirement of cholate for the development of consistent aortic lesions in the C57BL/6 strain of mice support a potential link between bile acid signaling and vascular wall inflammation. This may be relevant in settings of a high-cholesterol diet because cholesterol is converted to bile acids in the liver. Mice on a high-cholesterol diet do have increased bile acid secretion and serum bile acid levels (20, 29, 39, 40), which may directly impact the vasculature.

In summary, we demonstrate that bile acid signaling can result in hepatic inflammatory gene expression in vivo and have identified a novel role for FXR in mediating ICAM-1 gene expression. Moreover, bile acid and cytokine signaling can result in an additive enhancement in ICAM-1 expression. The consequence of this proinflammatory mechanism in vivo is currently being investigated but may play a role in diseases associated with elevated levels of bile acids such as cholestatic diseases or atherosclerosis.
REFERENCES


