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Guggulsterone antagonizes farnesoid X receptor induction of bile salt export pump but activates pregnane X receptor to inhibit cholesterol 7α-hydroxylase gene☆

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Abstract

Bile acids activate a nuclear receptor, farnesoid X receptor (FXR), that induces bile salt export pump (BSEP) but inhibits cholesterol 7α-hydroxylase (CYP7A1) gene transcription in the liver. Guggulsterone, a plant sterol that lowers serum cholesterol, has been shown to antagonize FXR activated genes. Transient transfection assay of a human BSEP/luciferase reporter in HepG2 cells transfected with FXR reveals that guggulsterone strongly antagonizes bile acid induction of the BSEP gene. On the other hand, guggulsterone has no effect on FXR inhibition of the *CYP7A1* gene, but strongly inhibits the human *CYP7A1* gene by activation of pregnane X receptor (PXR). These results suggest that guggulsterone inhibits bile acid secretion from hepatocytes into bile and activates PXR to inhibit bile acid synthesis in the liver. Reduced conversion of cholesterol and bile acid excretion may lead to an increase of hepatic cholesterol and decrease of intestinal cholesterol absorption, and results in lowering serum cholesterol.

Keywords: Bile acid synthesis; Nuclear receptor; Cholesterol metabolism; Bile acid transport; Pregnane X receptor

Conversion of cholesterol to bile acids is the predominant pathway for cholesterol catabolism and plays an important role in maintaining cholesterol homeostasis in humans [1]. CYP7A1 catalyzes the first and rate-limiting reaction of bile acid biosynthesis and is feedback inhibited by bile acids returning to the liver via enterohepatic circulation. Bile acids are not just physiological detergents for absorption, transport, and distribution of lipid soluble vitamins, sterols, and xenobiotics, but also are signaling molecules that activate nuclear receptors, FXR, PXR, and vitamin D receptor (VDR) [2]. It is thought that bile acid-activated FXR induces a negative receptor, small heterodimer partner (SHP), which subsequently inhibits *CYP7A1* gene transcription [3,4]. On the other hand, FXR induces BSEP in the liver to excrete bile acids into bile [5] and induces ileum bile acid-binding protein (IBABP) in the intestine to facilitate bile acid absorption [6]. Bile acids regulate xenobiotic metabolism by activating the PXR to induce the CYP3A family of cytochrome P450 drug metabolizing enzymes that metabolizes about 60% of clinical drugs, steroids, and bile acids in the liver and intestine [7]. We reported previously that pregnenolone 16α-carbonitrile (PCN), a PXR agonist, inhibits CYP7A1 activity and mRNA and protein expression in the liver [8,9]. In *Pxr* knockout mice, PCN does not inhibit CYP7A1 mRNA expression, indicating that PXR plays a role in bile acid inhibition of the *CYP7A1* gene [7].

FXR antagonists may have the potential to be developed as therapeutic drugs for lowering serum cholesterol by stimulating the conversion of cholesterol to bile acids. It was reported recently that guggulsterone, a plant sterol that has been used to reduce serum cholesterol and triglyceride levels in humans, is an FXR antagonist that inhibits FXR induction of IBABP and

☆ *Abbreviations:* BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; CYP 3A4, cytochrome P450 family 3A4; CYP7A1, cholesterol 7α-hydroxylase; FXR, farnesoid X receptor; IBABP, ileum bile acid-binding protein; PXR, pregnane X receptor; RXR, retinoid X receptor; SHP, small heterodimer partner; VDR, vitamin D receptor.

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SHP expression [10,11]. It is predicted that guggulsterone may induce the *CYP7A1* gene by antagonizing the inhibitory effect of FXR and SHP. However, the effect of guggulsterone on *CYP7A1* and *BSEP* gene expression has not been studied. In this communication we studied the effect of guggulsterone on human *CYP7A1* and *BSEP* gene transcription. We found that guggulsterone antagonizes FXR induction of the *BSEP* gene but strongly inhibits the *CYP7A1* gene.

Materials and methods

Reporter gene and receptor expression plasmids. The mouse *IBABP* (*p-496*/Luc) reporter plasmid containing nucleotides −496 to +40 of the gene encoding mouse ileum bile acid-binding protein (IBABP) was kindly provided by Dr. David Moore (Baylor College of Medicine, Houston, TX). The human *BSEP* reporter plasmid (p-145/Luc) was kindly provided by Dr. M. Ananthanarayanan (Mt. Sinai Medical Center, NY). The human CYP7A1 reporter plasmid (ph-1887/Luc) containing nucleotides −1887 to +24 was constructed as described previously [12]. Rat FXR (pRSVFXR) and human RXR (pCMX-hRXRα) were kindly provided by C. Weinberger (National Institute of Environmental Health Sciences, Research Triangle Park, NC) and R. Evans (Salk Institute, La Jolla, CA), respectively. FXR and RXR cDNA were moved to the pcDNA3 vector (Invitrogen, Carlsbad, CA). Human PXR expression plasmid (pSG5-hPXR) was kindly provided by Dr. Steven Kliewer (University of Texas Southwestern Medical Center, Dallas, TX).

Cell Culture and transient transfection assay. HepG2 cells (ATCC HB80565, American Type Culture Collection, Rockville, MD) were cultured in DMEM/F-12 (50:50) (Sigma, St. Louis, MO) and supplemented with 10% heat-inactivated fetal bovine serum (Irvine Scientific, Santa Ana, CA) and penicillin–streptomycin solution (10,000 IU/10,000 μg/ml). Cells were grown in 12-well plates for 24 h and transfected with 2.5 μg reporter plasmid by the calcium phosphate DNA coprecipitation method. Cells were grown in serum-free medium for 40 h after transfection. For cotransfection assays, 2.5 μg of reporter plasmid and 0.5 μg of FXR or PXR and RXR expression plasmids were transfected into HepG2 cells. The total amount of DNA used in each assay was kept constant by adding pcDNA3 empty vector. Luciferase activity was assayed using a luciferase assay kit (Promega, Madison, WI). pCMV-β-galactosidase was transfected as an internal standard for normalization of transfection efficiency. Chenodeoxycholic acid (Sigma) and Z-guggulsterone (Steraloids, Newport, RI) were dissolved in 70% ethanol and added in cultures.

Results

Guggulsterone antagonizes CDCA/FXR induction of the human BSEP gene

Transient transfection assay in HepG2 cells was performed to study the effect of guggulsterone on bile acid induction of the human *BSEP* gene. BSEP is the principal bile acid transporter located in the canalicular membrane for excretion of bile acids into bile. BSEP is expressed at a very low level in the liver and is markedly induced by bile acids [5]. When assayed in HepG2 cells

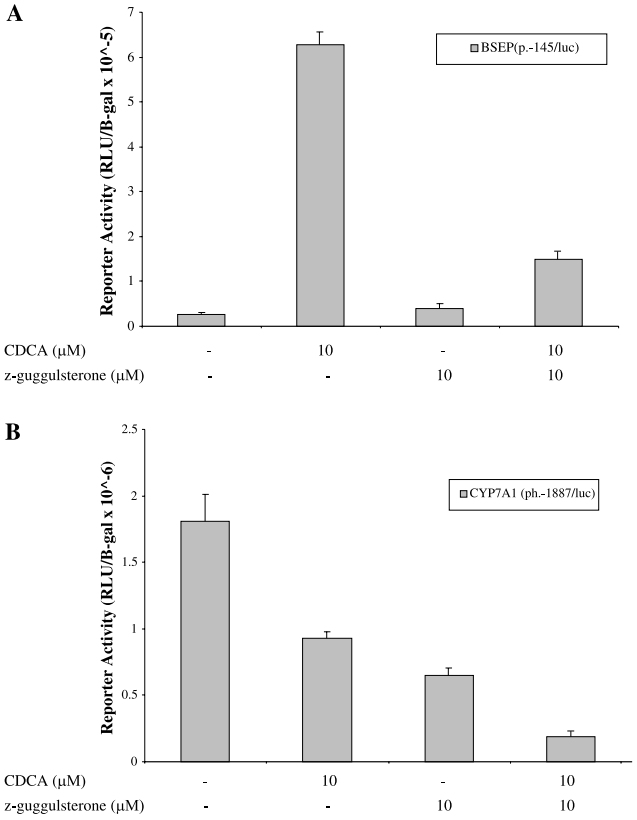

Fig. 1. Effects of guggulsterone on FXR regulation of the *BSEP* and *CYP7A1* genes. HepG2 cells were transfected with FXR and RXR expression plasmids for transfection assay of BSEP reporter (p-145/Luc) (A) or human CYP7A1 reporter (ph-1887/Luc) (B) as described under Materials and methods. CDCA and/or guggulsterone were added as indicated. Results shown are an average of triplicate assays. Error bars are standard errors of the means.

transfected with FXR/RXR, CDCA (10 μM) markedly stimulated BSEP reporter activity by about 23-fold (Fig. 1A). Without CDCA, guggulsterone (10 μM) did not have any effect on BSEP reporter activity. Guggulsterone strongly reduced BSEP reporter activity stimulated by CDCA. These results suggest that guggulsterone antagonizes bile acid-activated FXR induction of the human *BSEP* gene.

Guggulsterone does not antagonize, but enhances, FXR inhibition of the human CYP7A1 gene

We reported previously that FXR enhanced bile acid inhibition of human *CYP7A1* [13]. As shown in Fig. 1B, CDCA (10 μM) inhibited human *CYP7A1* reporter activity by 50% in HepG2 cells transfected with FXR/RXR, and guggulsterone (10 μM) strongly inhibited CYP7A1 reporter activity by 64%. Addition of both CDCA and guggulsterone inhibited CYP7A1 by 90%. These results suggest that guggulsterone does not antagonize, but enhances, the inhibitory effect of FXR on the human *CYP7A1* gene.

Guggulsterone dose-dependently inhibits CYP7A1 and BSEP gene transcription

We then studied the guggulsterone dose–response effect on CYP7A1 and BSEP reporter activity. As shown in Fig. 2A, CDCA slightly inhibited CYP7A1 reporter activity when added up to 10 μM in HepG2 cells without cotransfection with FXR. Addition of guggulsterone strongly inhibited CYP7A1 reporter activity in a dose-dependent manner. In contrast, CDCA did not induce BSEP reporter activity without cotransfection with FXR, and guggulsterone had no effect on the *BSEP* gene under this condition. This result also indicates that the endogenous FXR, if any, must be too low to induce BSEP. When HepG2 cells were transfected with FXR/RXR, the BSEP reporter activity was markedly stimulated by CDCA in a dose-dependent manner, and guggulsterone dose-dependently inhibited BSEP reporter activity (Fig. 2B). On the other hand, CDCA dose-dependently inhibited CYP7A1 reporter activity by about 50% at 10 μM when cotransfected with FXR, and addition of guggulsterone further inhibited CYP7A1 reporter activity in a dose-dependent manner. As a positive control, CDCA strongly stimulated IBABP reporter activity by 9-fold in HepG2 cells transfected with FXR/RXR (Fig. 3). Guggulsterone strongly antagonized bile acid induction of IBABP as expected. Without FXR/RXR, IBABP had extremely low reporter activity and guggulsterone had no effect on the gene.

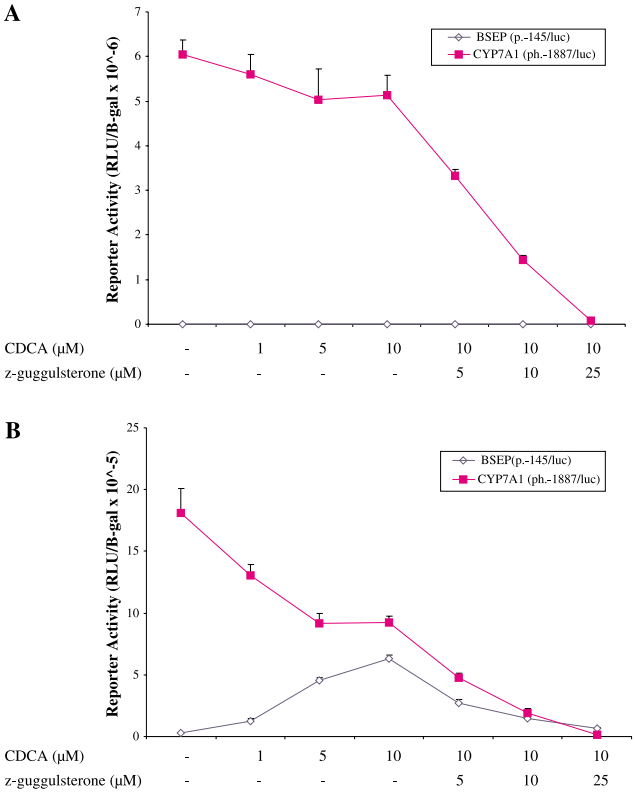

Fig. 2. Dose effects of CDCA and guggulsterone on the *BSEP* and *CYP7A1* genes. Transfection assays were done in HepG2 cells without (A) or with (B) cotransfection of FXR and RXR expression plasmids. Human *CYP7A1* (ph-1887/Luc) and human *BSEP* (p-145/Luc) reporters were assayed in the presence of CDCA and guggulsterone in the amounts indicated.

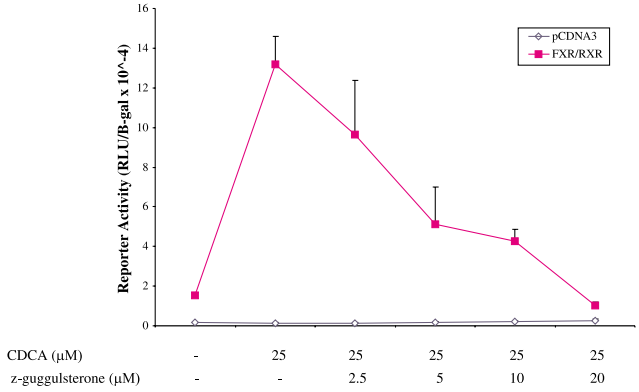

Fig. 3. Effect of CDCA and guggulsterone on IBABP reporter activity. HepG2 cells transfected with FXR and RXR expression plasmids were used for transfection assay. CDCA and guggulsterone are added in the amount indicated. pcDNA empty vector was used in place of FXR/RXR as a negative control.

Effect of PXR and guggulsterone on CYP7A1 gene transcription

Since guggulsterone is a pregnane and has been shown to be a strong PXR agonist [11], we studied the effect of guggulsterone on the *CYP7A1* gene in HepG2 cells cotransfected with PXR and RXR. CDCA is known to activate PXR and inhibits *CYP7A1* gene expression [14]. Fig. 4 shows that CDCA (10 μM) inhibits CYP7A1 reporter activity by about 44%. Interestingly, guggulsterone (10 μM) strongly inhibited the *CYP7A1* gene by about 93%. Addition of both CDCA and guggulsterone had the same effect as guggulsterone alone. These results suggest that guggulsterone indeed is a

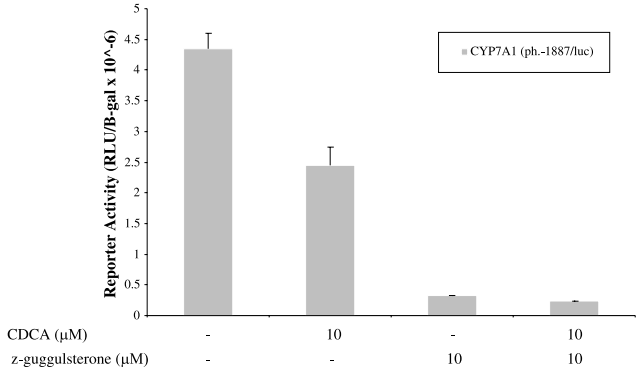

Fig. 4. Effect of CDCA and guggulsterone on PXR inhibition of the *CYP7A1* gene. HepG2 cells were transfected with PXR and RXR and used for transfection assay of a human CYP7A1 reporter (ph-1887/Luc). CDCA and guggulsterone are added in the amounts indicated.

PXR agonist that is much more potent than CDCA in inhibition of the *CYP7A1* gene. Guggulsterone does not have any effect on FXR inhibition of *CYP7A1* gene transcription.

Discussion

It seems clear that guggulsterone antagonizes the FXR induced genes in both the liver and intestine. This plant sterol may reduce serum cholesterol by antagonizing FXR induction of IBABP in the intestine to reduce bile acid reabsorption. It is thus expected that guggulsterone may reduce bile acid feedback, decrease FXR induction of SHP, and subsequently stimulate CYP7A1 to convert cholesterol to bile acids in the liver. Our results show that guggulsterone strongly antagonizes the FXR induction of BSEP in the liver. This is consistent with guggulsterone being an FXR antagonist. However, guggulsterone inhibition of the human *CYP7A1* gene seems to be unexpected because guggulsterone should antagonize the inhibitory effect of FXR and SHP and stimulate the *CYP7A1* gene. It should be noted that guggulsterone is a very potent PXR agonist that strongly inhibits the *CYP7A1* gene [8,14]. The agonistic effect of guggulsterone on PXR might be dominant over its antagonistic effect on FXR and SHP, thus inhibiting *CYP7A1*. This is consistent with the idea that the SHP-dependent mechanism is just one of the several mechanisms for bile acid inhibition of *CYP7A1* gene transcription [2]. Genetic knockout of the *Shp* gene does not abolish bile acid inhibition of Cyp7a1 expression, indicating that redundant pathways must exist for bile acid feedback inhibition in *Shp* null mice [15]. The mechanism by which PXR inhibits CYP7A1 is not known at present. Results from this study suggest that PXR inhibition of the *CYP7A1* gene is independent of FXR and SHP.

It is recently reported that a retinoid-like compound, AGN34, is a gene-selective bile acid receptor modulator that acts as an agonist on CYP7A1, an antagonist on IBABP, and had no effect on SHP [16]. The action of guggulsterone may be different from AGN34; guggulsterone is an FXR antagonist and a PXR agonist, whereas AGN34 is both agonist and antagonist of FXR but not a PXR agonist [11]. In contrast to our results, Cui et al. [17] show that guggulsterone is a BSEP agonist, but has no effect on IBABP, SHP, and CYP7A1. These results also are in complete contrast to the antagonistic effect of guggulsterone on IBABP and SHP reported by two other laboratories [10,11].

Our results may explain the serum cholesterol lowering effect of guggulsterone. This plant sterol may inhibit bile acid reabsorption in the intestine by antagonizing FXR induction of IBABP. However, guggulsterone antagonizes FXR induction of BSEP, which regulates the rate-limiting step of the enterohepatic recirculation of bile. Increased bile acids in the liver activate PXR, which inhibits the *CYP7A1* gene to reduce bile acid synthesis, and induces the *CYP3A* genes to detoxify bile acids, thus protecting the liver from bile acid induced cholestasis. The consequence of reduced bile acid synthesis is the increased hepatic cholesterol content, which is observed by Cui et al. [17]. In conclusion, guggulsterone may reduce bile acid synthesis in the liver and bile acid secretion into bile. A decrease of circulating bile acid pool may lead to a decrease of cholesterol absorption in the intestine and results in lowering serum cholesterol.

Acknowledgment

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