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A tea catechin, epigallocatechin-3-gallate, is a unique modulator of the farnesoid X receptor

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ABSTRACT

Farnesoid X receptor (FXR) is a ligand-activated nuclear receptor and serves as a key regulator to maintain health of the liver and intestine. Bile acids are endogenous ligands of FXR, and there are increasing efforts to identify FXR modulators to serve as biological probes and/or pharmaceutical agents. Natural FXR ligands isolated from plants may serve as models to synthesize novel FXR modulators. In this study, we demonstrated that epigallocatechin-3-gallate (EGCG), a major tea catechin, specifically and dose-dependently activates FXR. In addition, EGCG induced FXR target gene expression *in vitro*. Surprisingly, in a co-activator (SRC2) recruitment assay, we found that EGCG does not recruit SRC2 to FXR, but it dose-dependently inhibits recruitment of SRC2 to FXR (IC_{50}, 1 μM) by GW6064, which is a potent FXR synthetic ligand. In addition, EGCG suppressed FXR target gene expression induced by either GW4064 or chenodeoxycholic acid *in vitro*. Furthermore, wild-type and FXR knockout mice treated with an acute dose of EGCG had induced mRNA expression in a subset of FXR target genes in the intestine but not in the liver. In conclusion, EGCG is a unique modulator of FXR in the intestine and may serve as an important model for future development of FXR modulators.

Introduction

FXR (farnesoid X receptor, NR1H4) is a ligand-activated transcription factor and a member of the nuclear receptor superfamily. Bile acids are FXR's endogenous ligands. FXR regulates the biosynthesis and enterohepatic circulation of bile acids in addition to its regulation of triglyceride, cholesterol and glucose metabolism (Sinal et al., 2000; Cariou et al., 2005; Rizzo et al., 2005; Ma et al., 2006; Zhang et al., 2006). Recent evidence shows that FXR is also critical in maintaining innate immune responses in intestine (Vavassori et al., 2009). In addition, FXR deficiency in mice results in increased cholestasis, non-alcoholic fatty liver diseases, hepatocellular carcinoma and colon cancer (Gadaleta et al., 2010). This evidence suggests that FXR and FXR-mediated signaling pathways may be promising novel drug targets for the treatment of common metabolic diseases. However, use of synthetic FXR ligands in the clinic has not yet been approved.

The goal of the current study is to screen for FXR modulators in tea plant. Tea is a traditional medicinal plant and is also the most widely consumed beverage in the world, second only to water. The most commonly consumed teas are black, green, and oolong. These teas are all derived from the plant *Camellia sinensis*, which is a member of the Theaceae family. Epidemiologic and animal studies suggest that drinking green tea offers protection against cardiovascular diseases and a variety of cancers (oral cavity, esophagus, stomach, liver, small and large intestine, and mammary gland) (Setiawan et al., 2001; Zhang et al., 2002; Wu et al., 2003; Jian et al., 2004; Cabrera et al., 2006). Catechins are polyphenolic compounds and are one class of major chemicals found in tea. Examples of tea catechins include (−)-epigallocatechins-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC) (Fig. 1). EGCG is the major constituent and the most biologically active catechin of green tea. Numerous studies report that EGCG can lower oxidative stress, atherosclerosis, inflammation, and cholesterol (Dona et al., 2003; Frei and Higdon, 2003; Raederstorff et al., 2003; Chyu et al., 2004). However, no effect of tea compounds on FXR activity has been reported. Therefore, we aimed to determine the effects of tea catechins, particularly EGCG, in modulating FXR activity in this study.

Abbreviations: ALT, alanine aminotransferase; BSEP, bile salt efflux pump; CAR, constitutive androstane receptor; CDCA, chenodeoxycholic acid; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechins-3-gallate; Fgf15, fibroblast growth factor 15; FXR, farnesoid X receptor; Ibabp, ileum bile acid binding protein; I.P., intraperitoneally; OSTα, organic solute transporter α; P.O., orally; Q-PCR, quantitative real-time PCR; RXRα, retinoic acid receptor alpha; RXRβ, retinoic acid receptor beta; SHP, small heterodimer partner; TR-FRET, time-resolved fluorescence resonance transfer; PXR, pregnane X receptor; VDR, vitamin D receptor.

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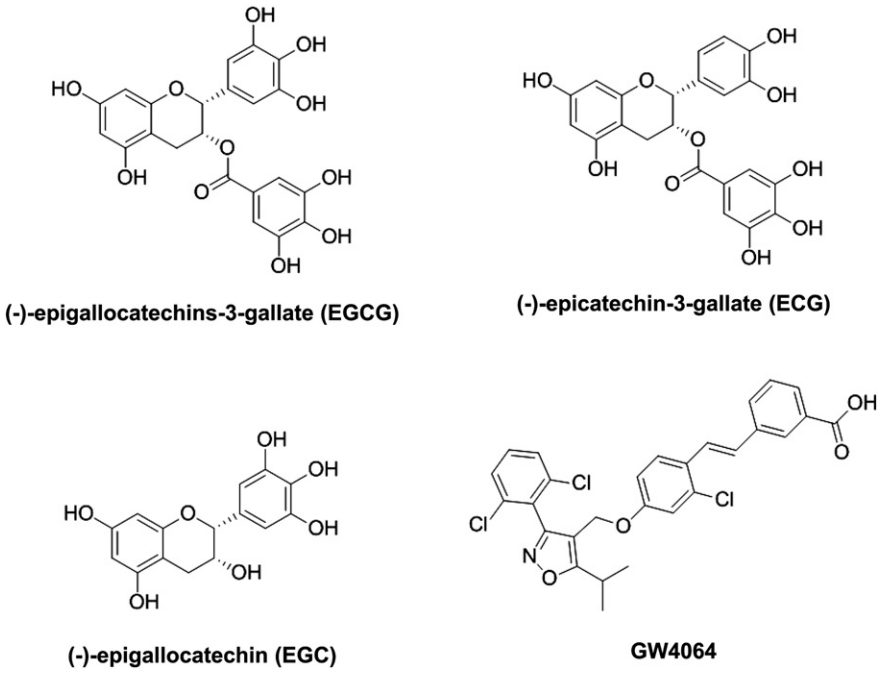

Fig. 1. Chemical structure of EGCG, EGC, and ECG, in comparison to a known FXR agonist, GW4064. Chemical structure of tea catechins, (−)-epigallocatechins-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and GW4064.

Materials and methods

Chemicals. Thirty-four commercially available samples of green tea (5 g) were extracted with 10 ml of H_2O (70 °C, for 10 min) to simulate conditions of regular infusion. The resulting aqueous extracts were concentrated *in vacuo* and dried overnight at 30 °C in a vacuum oven. All plant samples were prepared at the Department of Medicinal Chemistry, University of Kansas, Lawrence. GW4064 was synthesized by the Department of Medicinal Chemistry, University of Kansas. Chenodeoxycholic acid (CDCA), ECG, EGC, and EGCG were purchased from Sigma-Aldrich (St. Louis, MO). All compounds and catechins were dissolved in DMSO for *in vitro* studies. For the *in vivo* study, EGCG was dissolved in 0.9% saline and stored at −20 °C.

Cell culture. Human hepatocellular carcinoma cell lines HepG2 and Hep3B were purchased from American Type Culture Collection (Manassas, VA). The Huh7 cell line was purchased from RIKEN BioResource Center (Tsukuba, Japan). All cells were cultured in high-glucose DMEM supplemented with 1% penicillin/streptomycin, 1% L-glutamine, and 10% fetal bovine serum (Omega Scientific, Tarzana, CA). All cells were maintained in a 5% CO_2 humidified atmosphere at 37 °C.

Transient transfection and luciferase reporter gene assays. The generation of the pGL4-SHP-TK promoter luciferase reporter was described in detail previously (Li et al., 2010). Briefly, the downstream regulatory region of the *Nr0b2* gene, from +3639 to +4516 relative to the transcription start site, was amplified from mouse genomic DNA by PCR using pairs of primers containing XhoI and BglII restriction enzyme sites, respectively. The PCR product was subcloned upstream of the luciferase gene into the firefly luciferase pGL4-TK vector (Promega, Madison, WI) and was named pGL4-SHP-TK reporter vector. The sequence of the construct was confirmed by DNA sequencing. HepG2 cells were seeded in a 96-well plate and grown to 90% confluency prior to transient transfection with plasmids consisting of PGL4-SHP-TK reporter gene, pCMV-ICIS human FXR (Open Biosystems, Huntsville, AL), pSG5 human RXRα (Stratagene, La Jolla, CA), and pCMV-renilla luciferase vector (Promega, Madison, WI). Transient transfection was carried out according to the manufacturer's instructions using TurboFect *in vitro* Transfection Reagent (Fermentas, Glen Burnie, MD). To screen for plant compounds that activate FXR, cells were treated with 0.1% DMSO (negative control), 1 μM GW4064 (positive control), or various testing compounds at indicated concentrations 5 h after transfection. To test the effects of EGCG on GW4064 or CDCA activation of FXR, cells were treated 5 h after transfection with 500 nM GW4064 or 100 μM CDCA in the absence or presence of increasing concentrations of EGCG (0 to 100 μM) or 0.1% DMSO as a negative control. Thirty-six hours after treatment, firefly luciferase and renilla luciferase activities were quantified using the Dual-Glo Luciferase Kit (Promega, Madison, WI) in a Synergy-II HT plate reader (Bio-Tek Instruments, Inc., Winooski, VT). The firefly luciferase activity value was normalized as a ratio to that of renilla luciferase and expressed as fold over the pGL4-SHP-TK vector control. For plant compound screening, the data were presented as an average of three assays. For the dose response of ECG, EGC, and EGCG, the data were presented as an average of six wells. For the activation effects of EGCG combined with GW4064 or CDCA on FXR, the data were also presented as an average of six wells. All experiments were repeated at least twice.

FXR-mediated co-activator recruitment assay. Time-resolved fluorescence resonance transfer (TR-FRET) hFXR coactivator recruitment assays were performed according to the manufacturer's instructions (Invitrogen) with minor modifications. Briefly, the assays were performed in 384-well low volume (20 μl per well) solid black plates with 5 nM GST-FXR ligand-binding domain (LBD), 500 nM fluorescent-SRC2-2, 5 nM terbium-labeled anti-GST antibody and a test compound at various concentrations. A potent FXR agonist, GW4064, and DMSO were included in assays as positive and negative controls, respectively. The DMSO concentration was 1% in all assay wells.

In the reaction mixture containing GW4064, GST-FXR forms a complex with the terbium-labeled anti-GST antibody and co-activator peptide, fluorescent-SRC2-2. Excitation of terbium using a 340-nm excitation filter results in an energy transfer from terbium to the fluorophore of the labeled co-activator peptide. This energy transfer is detected by an increase in fluorescence emission of the labeled co-activator peptide at 520 nm and a decrease in fluorescence emission of terbium at 495 nm. The FRET ratio was calculated by dividing the emission signal at 520 nm by the emission signal at 495 nm. In the absence of a FXR agonist or addition of a FXR antagonist, GST-FXR fails