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ORIGINAL RESEARCH

Intestinal Farnesoid X Receptor Activation by Pharmacologic Inhibition of the Organic Solute Transporter α-β

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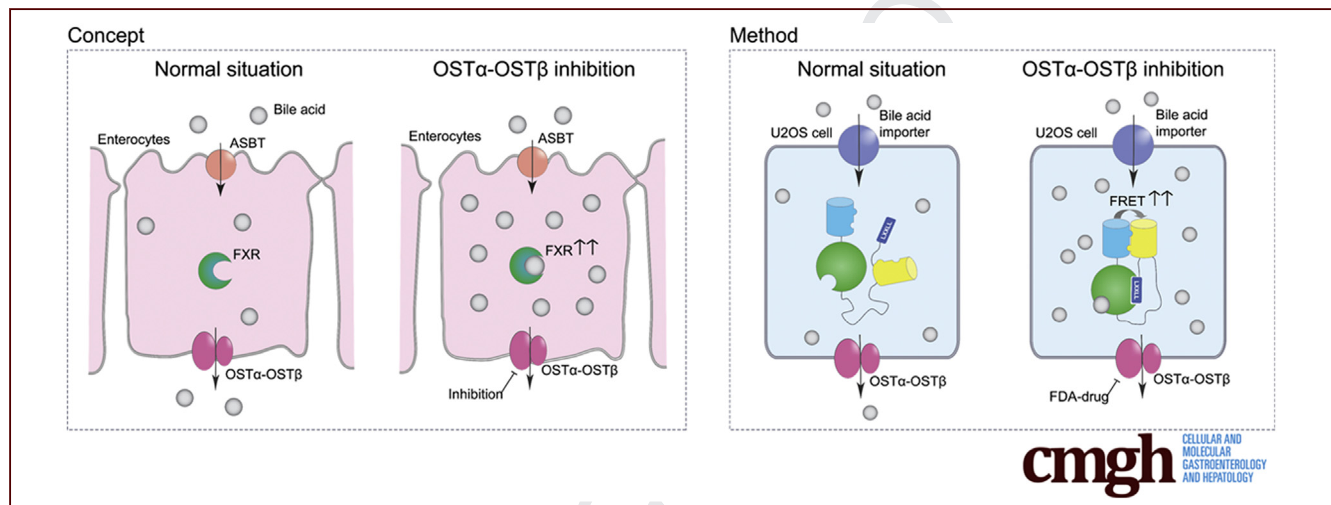

SUMMARY

A fluorescent resonance energy transfer–based, high-throughput screen was established to identify inhibitors of organic solute transporter (OST)α-OSTβ–mediated bile acid efflux. We identified clofazimine as an inhibitor of OSTα-OSTβ and showed that such inhibition enhanced intestinal farnesoid X receptor activation.

BACKGROUND & AIMS: The organic solute transporter α-β (OSTα-OSTβ) mainly facilitates transport of bile acids across the basolateral membrane of ileal enterocytes. Therefore, inhibition of OSTα-OSTβ might have similar beneficial metabolic effects as intestine-specific agonists of major nuclear receptor for bile acids, the farnesoid X receptor (FXR). However, no OSTα-OSTβ inhibitors have yet been identified.

METHODS: Here, we developed a screen to identify specific inhibitors of OSTα-OSTβ using a genetically encoded Förster Resonance Energy Transfer (FRET)–bile acid sensor that enables rapid visualization of bile acid efflux in living cells.

RESULTS: As proof of concept, we screened 1280 Food and Drug Administration–approved drugs of the Prestwick chemical library. Clofazimine was the most specific hit for OSTα-OSTβ and reduced transcellular transport of taurocholate across Madin–Darby canine kidney epithelial cell monolayers expressing apical sodium bile acid transporter and OSTα-OSTβ in a dose-dependent manner. Moreover, pharmacologic inhibition of OSTα-OSTβ also moderately increased intracellular taurocholate levels and increased activation of intestinal FXR target genes. Oral administration of clofazimine in mice (transiently) increased intestinal FXR target gene expression, confirming OSTα-OSTβ inhibition in vivo.

CONCLUSIONS: This study identifies clofazimine as an inhibitor of OSTα-OSTβ in vitro and in vivo, validates OSTα-OSTβ as a drug target to enhance intestinal bile acid signaling, and confirmed the applicability of the Förster Resonance Energy Transfer–bile acid sensor to screen for inhibitors of bile acid efflux pathways. *(Cell Mol Gastroenterol Hepatol 2017;■:■–■; https://doi.org/10.1016/j.jcmgh.2017.11.011)*

Keywords: Fluorescence Resonance Energy Transfer (FRET); FXR; OSTα-OSTβ; Bile Acids.

Abbreviations used in this paper: ASBT, apical sodium-dependent bile acid transporter; BAS, bile acid sensor; FACS, fluorescence-activated cell sorting; FDA, Food and Drug Administration; FGF15/19, fibroblast growth factor 15/19; FRET, fluorescent resonance energy transfer; FXR, farnesoid X receptor; MDCKII, Madin–Darby canine kidney epithelial cells; mRNA, messenger RNA; nucleoBAS, nucleus-localized bile acid sensor; OSTα-OSTβ, organic solute transporter α-β; TCDCA, taurochenodeoxycholic acid; TICE, transintestinal cholesterol excretion; U2OS, human bone osteosarcoma epithelial cells.

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Bile acids are released in the duodenum during a meal, where they act as digestive detergents crucial for intestinal absorption of lipids, fat-soluble vitamins, and other lipophilic nutrients.[1] After the meal, most bile acids are reabsorbed from the ileum and return to the liver via the hepatic portal vein. In the hepatocyte, the bile acid pool is replenished via de novo bile acid synthesis from cholesterol.[2] Subsequently, bile acids are conjugated and stored in the gallbladder until the next meal. The meal-regulated dynamics renders bile acids as potent signaling molecules that modulate triglyceride, lipid, glucose, and energy homeostasis, making bile acid signaling an interesting target for metabolic diseases.[3] Activation of the farnesoid X receptor (FXR), a major nuclear receptor involved in bile acid signaling, has been suggested to be beneficial in many metabolic disorders. In particular, selective intestinal activation of FXR led to metabolic improvements such as enhanced glucose tolerance, reduced diet-induced weight gain, and reduced inflammation,[4] but also enhanced transintestinal cholesterol excretion (TICE).[5]

The organic solute transporter α-β (OSTα-OSTβ) mainly facilitates transport of bile acids across the basolateral membrane of ileal enterocytes.[6,7] It consists of 2 proteins forming a heterodimer, OSTα (encoded by *SLC51A*) and its subunit OSTβ (encoded by *SLC51B*), which are both required for normal trafficking and function of OSTα-OSTβ.

OSTα knockout mice show a reduction in the bile acid pool and serum levels combined with increased FXR activation in ileal enterocytes.[8] In addition, deficiency of OSTα is protective for liver injury during obstructive cholestasis, and leads to decreased body fat and lipid accumulation and improved insulin sensitivity.[9–11] Furthermore, OSTα knockout mice show increased elimination of cholesterol in the feces and decreased levels of cholesterol and triglyceride in serum.[8,12] These data point to OSTα-OSTβ as a novel target to treat diabetes and obesity, but also lipid and cholesterol disorders, for instance, by inducing the TICE pathway via intestinal FXR activation.[13] However, no inhibitors for OSTα-OSTβ have yet been identified and techniques available for measuring bile acid efflux are limited. Therefore, we developed a novel assay for cell-based high-throughput screening to identify specific inhibitors for OSTα-OSTβ, making use of a fluorescent resonance energy transfer (FRET)-based bile acid sensor that enables rapid visualization of bile acid efflux in living cells.[14] This screen specifically measures bile acid efflux, which simultaneously provides a readout based on increased FXR activation as a consequence of OSTα-OSTβ inhibition. We screened 1280 Food and Drug Administration (FDA)-approved drugs of the Prestwick chemical library and confirmed several positive hits. Here, we show that inhibition of OSTα-OSTβ leads to intestinal FXR activation.

Materials and Methods

Reagents

The Prestwick chemical library (1280 FDA approved compounds in 96-well plates) was purchased from Prestwick Chemical. Bifonazole, bromhexine HCl, clofazimine, lovastatin, meclozine 2HCL, and simvastatin were purchased from Sigma-Aldrich. The fibroblast growth factor (FGF)19 human enzyme-linked immunosorbent assay kit was purchased from BioVendor R&D. [^{3}H]-taurocholate and [^{14}C]-inulin were purchased from Perkin Elmer.

Cell Culture

Human bone osteosarcoma epithelial cells (U2OS) wild-type cells (HTB-96; American Type Culture Collection) were cultured in Dulbecco's modified Eagle medium (high glucose) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine. NucleoBAS (bile acid sensor localized in the nucleus),[14] NA$^+$-taurocholate co-transporting polypeptide, and OSTα-OSTβ–expressing U2OS cells were engineered by transfecting cells using polyethylenimine. Stable cell lines were generated by colony picking using cloning rings over well-separated colonies. Apical sodium-dependent bile acid transporter (ASBT) and mouse OSTα-OSTβ co-expressing Madin–Darby canine kidney epithelial cells (MDCKII) cells were a gift from Paul Dawson (Emory University School of Medicine, Atlanta, GA).[15] All cells were cultured at 5% CO_2 at 37°C.

Fluorescence-Activated Cell Sorting

Two days before the experiments, wild-type and transfected U2OS cells were cultured in 5% charcoal-treated fetal bovine serum to prevent bile acid overload of the sensor. The adherent cell layer was trypsinized by 5 mmol/L EDTA to create a suspension of single cells for fluorescence-activated cell sorting (FACS) analysis. Cells were harvested by centrifugation and the resulting pellet was suspended in FACS uptake buffer (0.3 mmol/L EDTA, 0.5% bovine serum albumin, 0.01% NaN_3, and 10 mmol/L D-glucose), plated in 96-wells and subsequently incubated with 10 μmol/L of 1 of 1280 compounds of the Prestwick Chemical library. After 5 minutes, 3 μmol/L TCDCA was added and incubated for 30 minutes at room temperature while shaking. Citrine and cerulean intensity was measured by a violet 405-nm laser. Spectral range for emission detections were as follows: cerulean, 450/40 nm; and citrine, 525/20 nm.

Taurocholate Uptake Assay in MDCKII–ASBT Cells

Cells were cultured in a 24-well plate format at 50%–60% confluency. The next day, cells were washed with uptake buffer and incubated with compounds at 37°C for 30 minutes. Uptake buffer was aspirated and a mix of trace amounts of [^{3}H]-taurocholate and 20 μmol/L nonradiolabeled trichloroacetic acid was administered. After 2 minutes of incubation at 37°C, the cells were washed using ice-cold phosphate-buffered saline and lysed in 0.05% sodium dodecyl sulfate in distilled water. Tritium signal of each sample was measured in 3 mL of scintillation liquid.

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