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Original Article

Transgenic overexpression of steroid sulfatase alleviates cholestasis☆

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Background and Aim: Sulfotransferase (SULT)-mediated sulfation and steroid sulfatase (STS)-mediated desulfation represent two critical mechanisms that regulate the chemical and functional homeostasis of endogenous and exogenous molecules. STS catalyzes the hydrolysis of steroid sulfates to form hydroxysteroids. Oxygenated cholesterol derivative oxysterols are known to be endogenous ligands of the liver X receptor (LXR), a nuclear receptor with anti-cholestasis activity, whereas the sulfated oxysterols antagonize LXR signaling. The conversion of sulfated oxysterols to their non-sulfated counterparts is catalyzed by STS. The aim of this study is to determine whether STS can alleviate cholestasis by increasing the activity of LXR.
Methods: Liver-specific *STS* transgenic mice were created and subject to the lithocholic acid (LCA)-induced model of cholestasis.
Results: Transgenic overexpression of *STS* in the liver promoted bile acid elimination and alleviated LCA-induced cholestasis. The protective effect of the *STS* transgene was associated with the activation of LXR and induction of LXR target genes, likely because of the increased conversion of the antagonistic oxysterol sulfates to the agonistic oxysterols.
Conclusions: *STS* has a novel function in controlling the homeostasis of bile acids by regulating endogenous LXR ligands.
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1. Introduction

Sulfotransferase (SULT)-mediated sulfation and steroid sulfatase (STS)-mediated desulfation represent two opposing mechanisms involved in regulating the chemical and functional homeostasis of endogenous and exogenous chemicals. STS catalyzes the hydrolysis of steroid sulfates to form hydroxysteroids and is expressed in many tissues including the liver, where the circulating steroids are extensively metabolized. By altering the levels of sulfated versus non-sulfated steroids, STS is important in the regulation of the cellular actions of steroid hormones.[1]

Bile acids are the major catabolic end products of cholesterol in the liver. As a major constituent in the bile, bile acids play an important physiological role in the solubilization and absorption of lipids and other lipophilic nutrients. Bile acids also function in signaling by activating receptors such as the farnesoid X receptor (FXR) and G protein-coupled receptor 5 (TGR5, also known as G protein-coupled bile acid receptor 1, or GPBAR1).[2,3] In addition to their beneficial functions, excessive bile acids are potentially toxic when they accumulate in the body. For example, LCA, a secondary bile acid, is a potent cholestatic agent and can induce liver injury and other pathological changes when it is not efficiently eliminated.[4]

The nuclear receptor liver X receptor (LXR) represents a promising therapeutic target for bile acid detoxification by enhancing the metabolism and elimination of bile acids as well as by reducing inflammation.[5] Both pharmacological stimulation and genetic activation of LXR prevented LCA-induced hepatotoxicity. In contrast, LXRα and β double knockout mice were more sensitive to bile acid toxicity.[5] However, several existing synthetic LXR agonists not only regulate bile acid homeostasis, but also promote lipogenesis and are pro-atherogenic.[6] By contrast, oxysterols, natural LXR agonists, showed selective modulation of LXR target genes that are responsible for cholesterol elimination, while not over-activating lipogenic genes.[7]

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STS plays a key role in regulating the homeostasis of endogenous ligands for LXR. Oxysterols, oxygenated cholesterol derivatives, are endogenous ligands of LXR. Like many other cholesterol derivatives, oxysterols can be sulfated by sulfotransferases such as the cholesterol sulfotransferase SULT2B1b, and the sulfonated forms of oxysterols can antagonize LXR signaling.[8–11] The conversion of sulfated oxysterols to their non-sulfated counterparts is catalyzed by STS. Knowing that LXR has anti-cholestasis activity, we hypothesize that STS may attenuate cholestasis by activating LXR through increased availability of endogenous LXR agonists and reducing the level of endogenous LXR antagonists.

In this report, we show that transgenic over-expression of *STS* in the liver and small intestine enhanced bile acid elimination and attenuated LCA-induced liver damage in mice. The protective effect of *STS* was associated with the induction of LXR target genes. Our results suggest a novel function of *STS* in controlling the homeostasis of bile acids by regulating endogenous LXR ligands.

2. Methods

2.1. Generation of STS transgenic mice

The generation of the STS transgenic mice was previously described.[12] In brief, the cDNA encoding human *STS* was cloned into the *TRE-SV40* transgene cassette to construct the *TRE-STS* transgene. The *TRE-STS* transgenic mice were then bred with the liver and intestinal specific FABP-tetracycline-controlled transactivator (tTA) transgenic mice to generate *TRE-STS/FABP-tTA* double transgenic mice.[12] The mice were maintained in a C57BL/6J background strain. When necessary, doxycycline (DOX, 2 mg/ml) was given in drinking water to silence the transgene expression. All results presented are derived from male mice. The use of mice in this study complied with relevant federal guidelines and institutional policies.

2.2. Drug treatment, histology, and serum biochemistry

For LCA treatment, mice were gavaged daily with LCA (8 mg/day) or vehicle for 4 days and sacrificed 24 h after the last treatment as previously described.[13] Fecal samples were collected by using mouse metabolic cages. For histology evaluation, the liver tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E). The histological damage was defined by the appearance of necrotic foci and was evaluated in a blinded fashion. Serum levels of ALT, AST (Stanbio Laboratory, Boerne, TX) and total bile acids (Bio-Quant, San Diego, CA) were measured by using commercial assay kits according to the manufacturer's instructions.

2.3. Northern blot, real-time PCR and immunohistochemistry

For Northern blot analysis, total RNA was isolated using the TRIZOL reagent from Invitrogen. Northern blot analysis was performed using ^{32}P-labeled full-length STS cDNA to probe the transgene expression. For real-time PCR analysis, cDNA was synthesized by reverse transcription with random hexamer primers and Superscript RT III enzyme from Invitrogen. SYBR Green-based real-time PCR was performed with the ABI 7300 real-time PCR system. Data were normalized against the housekeeping gene cyclophilin. The immunohistochemical staining of paraffin sections was performed using a monoclonal anti-STS antibody (dilution 1:50) purchased from Abcam (Cambridge, MA) following heat-induced antigen retrieval procedures.

2.4. Transient transfection and luciferase reporter gene assay

COS-7 cells were seeded onto a 48-well plate and transfected using polyethyleneimine polymer transfection reagents. When necessary, cells were treated with vehicle or 22-hydroxycholesterol (10 μmol) in medium containing 10% charcoal-stripped FBS for 24 h before the luciferase assay. The luciferase activity was normalized using the β-gal activity from a co-transfected pCMX-β-gal vector to determine the transfection efficiency. The relative reporter activity was calculated by comparing with cells transfected with empty vector and vehicle-treated cells. All transfections were performed in triplicate.

2.5. Statistical analysis

Results are expressed as the mean ± SD. Statistical significance between groups was determined by an unpaired two-tailed Student *t*-test, one-way ANOVA or two-way ANOVA. A *P*-value less than 0.05 is considered to be statistically significant.

3. Results

3.1. Generation of transgenic mice expressing STS in the liver and small intestine

To investigate the *in vivo* function of STS in cholestasis, we created tetracycline-responsive *STS* transgenic mice (STS mice) by crossing transgenic mice expressing human STS under the control of the tetracycline response element (TRE) with transgenic mice expressing tTA in the liver and small intestine under the control of the fatty acid binding protein (L-FABP) gene promoter. In the double transgenic mice and as outlined in Fig. 1A, binding of the tTA protein to TRE induces the expression of the *STS* transgene, whereas administration of doxycycline (DOX) prevents the binding of tTA to TRE and thus silences the *STS* transgene expression. The two transgenes were independently genotyped by PCR (Fig. 1B). The expression of the *STS* transgene at the mRNA level in the liver and small intestine was confirmed by Northern blot analysis using a transgene-specific probe (Fig. 1C, top panel). As expected, the expression of the *STS* transgene was completely reversed upon DOX treatment (Fig. 1C, top panel). The transgene was not detected in a panel of non-targeting tissues including the brain, kidney, skeletal muscle, brown (BAT), and white (WAT) adipose tissues (Fig. 1C, bottom panel). To localize cells that expressed the transgene, immunohistochemistry analysis was performed on liver and small intestine sections. STS was detected both in the liver and in the epithelial cell lining of the villi of the jejunum of the STS transgenic mice (Fig. 1D), which was consistent with the tissue specificity of the *FABP* gene promoter.[14] The expression of the *STS* transgene in the liver and small intestine was also confirmed by measuring the enzymatic activity of STS. When tritium-labeled estrone sulfate was used as the substrate, the STS mice exhibited higher enzymatic activity compared with the WT mice in both the liver and small intestine, which was normalized by DOX treatment (Fig. 1E). These results demonstrated that the STS transgene was expressed and functional in the STS transgenic mice.

3.2. Over-expression of STS attenuates lithocholic acid (LCA)-induced cholestasis

Gastric feeding of the secondary bile acid LCA to mice is an established model to study bile acid-induced cholestasis and hepatotoxicity *in vivo*.[5,13] To examine the effects of STS over-expression on LCA-induced hepatotoxicity, STS transgenic mice and their WT littermates were given a daily treatment of vehicle or LCA by oral

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