Supplementary materials_Methods

Methods include:

Method S1 The quantitative measurement bile acids in bile and gut content of hamsters

Method S2: Measurement of cholic acid

Method S3 mRNA and western blotting of CYP7A1 and CYP27A1

Method S1

The quantitative measurement bile acids in bile and gut content of hamsters

Standard references analysis and the calibration curves: The references standards cholic acid(CA), glycocholic acid (GCA), taurocholic acid (TCA), deoxycholic acid(DCA), ursodeoxycholic acid(UDCA), lithocholic acid(LCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and chenodeoxycholic acid (CDCA) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). For obtain the calibration curves, each reference standard was accurately weighed and dissolved in methanol as the stock solution. After dilution, a series concentrations at 0.01, 0.05, 0.1, 0.2 0.5, 1,2,5,10,20 μM of standard solutions were prepared with internal standards, dehydro-cholic acid(Tokyo Chemical Industry CO., LTD) and [$^{13}C_2$]-myristic acid (Cambridge Isotope Laboratories, Andover, MA, USA) in it. The samples were then analyzed using the Shimadzu LC/MS 2010, equipped with an Aglient ZORBAX SB-Aq column (2.1 × 150 mm, 3.5 μm).

An aliquot of 5 μl sample was injected into the column in the oven kept at 45 °C. The flow rate was 0.25 mL/min with mobile phase A (100 mg/L ammonium acetate and 50μL Ammonium Hydroxide) and mobile phase B (100% acetonitrile). The gradient is programmed as followed. Mobile phase B was initiated at 15% for 1 minute, increased to 25% from 1 to 4.5 min, and to 40% from 4.5 to 16 min, decreased to 15% within

0.5 min, where it was held for 3 min(The total run time was 19.5 min). The elution was introduced into mass spectrometry. Ionization of all bile acids and internal standard was achieved in an ESI source, which was detected in negative mode at -1.65 kV at an interval of 0.2 s. The gas (nitrogen) was set at a flow rate of 1.5 L/min, where the temperature was set at 200℃ and 250℃ for Block and CDL, respectively. The CDL voltage was 35.0 V. The Q-array RF was 150.

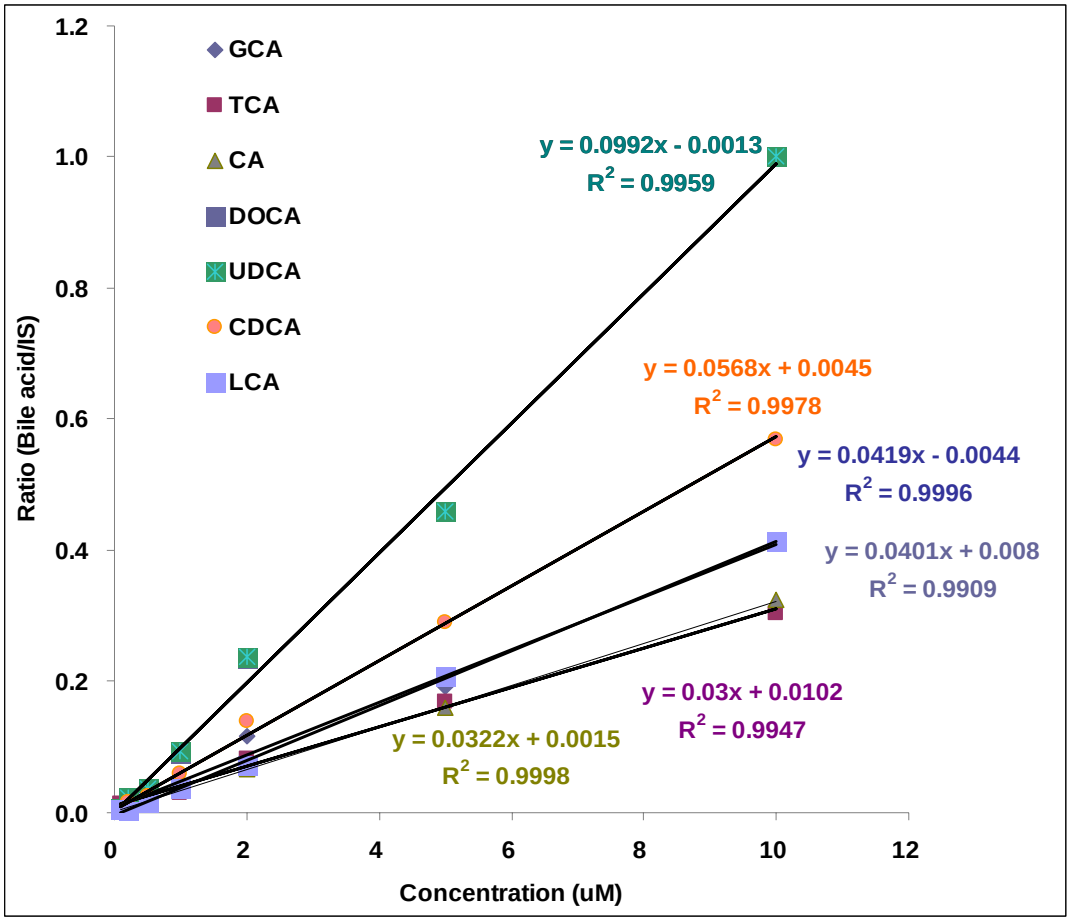
The peak areas of GCA, TCA, CA, DOCA, UDCA, LCA, dehydrocholic acid and [$^{13}C_2$]-myristic acid, were acquired with the quant masses at m/z 464.40, 514.45, 407.40, 391.25, 391.25, 375.25, 401.05, 229.25, and retention times at 8.11, 8.21, 8.91, 10.83, 9.84, 14.80, 7.58, 15.69 min, respectively. Peak areas were normalized by internal standard, and the calibration curves and the equations were obtained to calculate the concentrations of bile acids in bile and gut content (Method S1-Table 1 and Figure 1).

Analysis of bile acids in bile and gut content of hamsters: The collected samples, i.e., the hamster liver, bile, gut content and feces were pretreated before extraction of the bile acids. In detail, livers were homogenized at 9000 rpm in ultra-pure water (1:3, w/v) for 2 min twice; bile, gut content and feces were mixed with ultrapure water at 2:3, 1:2, 1:5 (w/v), respectively, and vigorously vortexed for 3 min, followed by ultrasonic extraction for 5 min. All the homogenous mixtures were added into methanol (1:3, v.v), vortexed for 3 min and centrifuged at 18,000 rpm for 10 min. One hundred microliter supernatant was transferred into a LC vial, and 5μl supernatant was injected and analyzed as above. The concentration and amount of

bile acids were calculated based on the calibration equations listed in the table below.

Method S1-Table 1 Standard curve equations of bile acids

Bile acids	Correlation Coefficient (r)	Calibration Curve Equation
Glycocholic acid	0.9954	y = 0.0401x + 0.008
Taurocholic acid	0.9973	y = 0.03x + 0.0102
Cholic acid	0.9999	y = 0.0322x + 0.0015
Deoxycholic acid + Chenodeoxycholic acid	0.9989	y = 0.0568x + 0.0045
Ursodeoxycholic acid	0.9959	y = 0.0992x - 0.0013
Lithocholic acid	0.9996	y = 0.0419x - 0.0044



Method S1-Figure 1 Standard curve of bile acids

Method S2:

Measurement of cholic acid

Ceca collected from each group of the hamsters fed with HFD or treated with berberine were combined, and 0.2 ml of the mixture was added in 1 ml oxygen free cultured solution. The incubation was carried out with berberine or without it at 37 °C for 12 h in nitrogen. Then the stable isotope-labelled cholic acid, $^{13}C_2$ was added in the system for incubation for another 6 h. the percent of metabolized cholic acid-$^{13}C_2$ were calculated in each group.

A series of working solutions were prepared by diluting stable isotope-labeled cholic acid with ethanol to yield the concentrations of 1.0,2.0,4.0,10,20,40,100,200 μg/mL. The calibration curve was prepared by adding 20 μL working solution into 180 μL inactivated gut content (boiled after incubation in yeast medium 1:10), and the final concentrations in the germ liquid were 0.1, 0.2, 0.4, 1, 2, 4, 10, 20 μg/mL. Linearity was weighed ($1/x^2$) and assessed using least squares regression analysis. Precision was evaluated by measuring replicate samples (0.4, 2.0 and 16 μg/mL), and it was calculated by intra- and inter-day RSD.

For each Ceca sample (100 μl), 500 μl of acetylacetate containing internal standard [$^{13}C_2$]-myristic acid (12.5 μg/ml) was added. Then the mixture was vigorously extracted for 3 min and was centrifuged at 10000 *g* for 10 min. Two hundred microliters of supernatant was transferred to a GC vial, then evaporated to dryness under vacuum. Fifty micro-liters MSTFA was added in the vial for trimethylsilylation for 1 h. At last, 50 μl heptane was added and vortexed for GC/MS analysis.

Cholic acid was analyzed in a gas chromatography mass spectrometry system

SHIMADZU QP2010Ultra/SE (Kyoto, JAPAN). In detail, 2 μL of the sample was splitlessly injected into inlet (250 °C) and was chromatographically separated by an RTx-5MS column (Restek Corporation, PA, USA) with helium as the carrier gas at 1.5 ml/min and a purge flow of 12.0 ml/min. The column temperature was programmed as follows: 100 °C for 5 min, increasing at 10 °C/ min to 300 °C, and holding at 300 °C for another 5 min. The ion source temperature was set at 200 °C, and the interface temperature was 220 °C. The ions were generated by an electron beam of 70 eV, and the detector voltage was set at -950 V. The masses between 50 and 800 m/z were acquired from 7 to 30 min, with a scan speed of 2500 Hz and event time of 0.30 seconds. The peak areas of cholic acid and the IS were acquired at m/z 520.3 and 287.1, respectively, and retention times of 28.9 and 10.2 min, respectively. Cholic acid amount in hamster gut content was calculated based on the calibration equation ($y = 0.1631x - 0.0162$, $R = 0.9998$).

Method S3:

mRNA and western blotting of CYP7A1 and CYP27A1

mRNA Expression: Hepatocellular carcinoma cell line HepG2, normal hepatic cell line L-02, and colorectal adenocarcinoma cell line Caco2 (the American Type Culture Collection, Manassas, VA) were maintained in DMEM (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% nonessential amino acids, 110 mg/L pyruvic acid sodium, and 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA). Both the cell lines were cultured at 37°C in a humidified atmosphere with 5% CO_2 and and passages 10-20 were used for experimentation. To investigate the effect of berberine on FXR pathway, cells were incubated with different concentration of berberine (0.25, 1.0, 2.5, 10.0 μg/ml) (dissolved in dimethyl sulfoxide, DMSO) for fixed time periods (24 hours).

Total RNA was isolated from hamster livers or in vitro cell lines using Trizol reagent (Invitrogen). RNA (1ug) was used for first-strand complementary DNA synthesis with a reverse transcription polymerase chain reaction (RT-PCR) kit (TaKaRa Bio Inc.). RT-qPCR was performed in a CFX96 real-time RT-PCR detection system combined with a C1000 thermal cycler (Bio-Rad, USA). Reactions were performed in a 15 μl volume containing 7.5 μl of 2 × SYBR Premix Ex Taq (TaKaRa Bio Inc.), 2 μl of properly diluted cDNA (corresponding to 1 ng total RNA input), and 1μM primers. Specific primers used in the study were listed in Method S3-Table 2. The negative controls (with no DNA template, only primer pair, water and 2 × SYBR Green PCR for each primer set were included in each run. The thermal cycling conditions included initial denaturation at 95°C for 5 min., followed by 40 cycles each of denaturation (10 s at 95°C), annealing (15 s at 50-60°C) and extension (15 s at 72°C with a single fluorescence measurement), melt curve program (60 to 95°C with a heating rate of 0.11°C/s and a continuous fluorescence measurement) and finally a cooling step to 40°C. The relative mRNA levels were calculated by the comparative threshold cycle method using *β-actin* as the internal control.

Western Blot Analysis: After treatment, total cell lysate, cytosolic proteins were prepared and used for western blot analysis as described previously (Chen et al., 2009; Zhang et al., 2010). In brief, Cells were gently scraped with cell scrapers and subjected to total protein extraction. Protein concentrations were measured using a BCA protein assay kit (Pierce Chemical, Rockford, IL) according to the manufacturer's instructions. Samples reconstituted in SDS-polyacrylamide gel electrophoresis sample loading buffer were boiled for 5 min for protein denaturation. Equal amount of protein samples were separated on a 10% SDS-polyacrylamide gel and transferred onto a PVDF membrane (Millipore Corporation). After blotting, the membrane was blocked with 5% fat-free milk in Tris-buffered saline-Tween 20 (TBST) for 1 h at 37°C. Immunoblots were reacted with human anti CYP7A1 (1:200; Bioworld Technology, Inc., MA, USA), CYP27A1 (1:200; Santa Cruz Biotechnology, Inc., MA, USA), or β-actin (1:500 dilution; Boster Biological Technology, Wuhan, China), and then probed with secondary antibody tagged with horse radish peroxidase. After washing with TBST, the membrane was incubated with HRP-conjugated secondary antibody (KeyGen, Nanjing, China) for 1 h. The signal was visualized by enhanced chemiluminescence (ECL, Millipore, MA, USA) and the density of the immunoreactive bands was analyzed using Image J software (NIH, MD, USA).

Method S3-Table 2 Primer sequences of target and reference genes

Name	Forward Primer(5'-3')	Reverse Primer (5'-3')
Human-CYP7A1	TGGTGGTAGAAATGATTCA	GAGTCTGTGATAGCATCTG
Human-CYP27A1	AATGAGGTGATTGATGAC	AAGTAGTAGAAGAGTTGAG
Hamster-CYP7A1	AGCAAATAGTCTCCCAGGGC	TGCAATCTACCCAGACCCTC
Hamster-CYP27A1	TTGGAAAGGTGATACAGGGC	ATCTGGCTACCTGCACTTCC
Hamster-β-actin	CCTTCCTTCCTGGGTATG	TGTTGGCATAGAGGTCTT
Human-β-actin	GCGTGACATTAAGGAGAAG	GAAGGAAGGCTGGAAGAG
Rat-β-actin	TCAGGTCATCACTATCGGCAAT	AAAGAAAGGGTGTAAAACGCA

References

1. Chen, L., Jarujaron, S., Wu, X., Sun, L., Zha, W., Liang, G., Wang, X., Gurley, E.C., Studer, E.J., Hylemon, P.B.*, et al.* (2009). HIV protease inhibitor lopinavir-induced TNF-alpha and IL-6 expression is coupled to the unfolded protein response and ERK signaling pathways in macrophages. Biochem Pharmacol *78*, 70-77.
2. Zhang, J., Zhou, F., Wu, X., Gu, Y., Ai, H., Zheng, Y., Li, Y., Zhang, X., Hao, G., Sun, J.*, et al.* (2010). 20(S)-ginsenoside Rh2 noncompetitively inhibits P-glycoprotein in vitro and in vivo: a case for herb-drug interactions. Drug Metab Dispos *38*, 2179-2187.