SUPPLEMENTARY INFORMATION

This Supplemental Material section describe the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of pravastatin, TDA, HDA, CP-I and CP-III.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of pravastatin

LC-MS/MS analyses of pravastatin and atorvastatin (internal standard (IS)) was performed on a Shimadzu LC-20AD (Kyoto, Japan) HPLC system coupled to an Sciex API (Framingham, MA) 4000 triple quadrupole mass spectrometer outfitted with a Turbo V ion source using negative ionization mode. HPLC analysis were performed on a reverse phase column (XBridge 3.5 µm, 2.1 x 100 mm) utilizing a gradient system of aqueous ammonium formate (5 mM) and acetonitrile (ACN), 5% ACN (v/v) to 95% over 9 min at a flow rate of 0.3 ml/min. Samples were prepared using solid phase extraction through reverse phase hydrophilic-lipophilic balance extraction cartridges (Agela Technologies). The cartridges were conditioned with methanol prior to addition of samples, and were washed with water and 5% methanol after sample addition. Samples were eluted with methanol and then separated on the mass spectrometer with a gradient and flow rate mentioned above. Analysis of pravastatin and atorvastatin (IS) via LC-MS/MS was performed by multiple reaction monitoring (MRM) transitions $[M-H]^-$ m/z 423 to m/z 321 and $[M-H]^-$ m/z 557 to m/z 397, respectively. The acquisition of mass spectral data was acquired with Analyst software (version 1.5.2, AB Sciex Framingham, MA) and concentrations of pravastatin were calculated by plotting peak area ratios of pravastatin to IS versus the concentration of pravastatin. This method allows detection of pravastatin and 3'α-hydroxy-pravastatin, which is the major isomer catalyzed by nonenzymatic isomerization[1]. Unless mentioned otherwise, we focused the analysis of pravastatin level to include both pravastatin and 3'α-hydroxy-pravastatin as total pravastatin, which we referred to as pravastatin in the article.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses of dicarboxylic acids

This method has been previously described by Shen et al. 2017[2]. In brief, HDA and TDA calibration standards were prepared in human plasma stripped four times with charcoal: 2.5, 5.0, 10.0, 25.0, 50, 100, 250, and 500 nM. Quality control (QC) samples were also prepared in a similar fashion at the concentrations of 2.5, 7.5, 400.0, and 800.0 nM. An aliquot of 100 µl plasma sample was transferred to a 96-well polypropylene plate, spiked with 400 µl of chilled methanol containing internal standard (100 nM each of HDA-d4 and TDA-d4). The plate was vortexed at room temperature for 5 min and then centrifuged at 3000 rpm using a Sorvall Legend RT centrifuge at 4°C for 15 min. One hundred microliters of the supernatant was

transferred into a 96-well plate containing 50 µl of deionized water using a Janus mini liquid handler, and subjected to LC-MS/MS analysis in a Shimadzu Nexera UHPLC system (Shimadzu Scientific Instruments, Inc., MD, USA) coupled to an API 4000 mass spectrometer (Sciex, CA, USA). Chromatography was carried out with a Cortecs C18+ (2.1 × 100 mm, 1.6 µm) from Waters (MA, USA) employing mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in 90/10 acetonitrile/methanol) at a flow rate of 0.5 ml/min. The gradient program was set as follows: mobile phase B set at 52% (0–1.5 min), increase mobile phase B linearly from 52% to 75% (1.5–4.0 min), increase mobile phase B linearly from 75% to 95% (4.0–4.2 min), maintain 95% mobile phase B (4.2–4.8 min), then decrease mobile phase B from 95% to 52%. The mass spectrometer was operated with negative electrospray ionization and multiple reaction monitoring using the transitions of the protonated forms of HDA (m/z 285.1 → 223.1), HDA-d4 (m/z 289.1 → 225.1), TDA (m/z 257.1 → 239.2), and TDA-d4 (m/z 261.1 → 198.2 Declustering potentials were 95, 85, 60, and 80 V for HDA, HDA-d_{28}, TDA, and TDA-d_{24}, respectively. Declustering potential (DP) for TDA/TDA-d4 and HDA/HDA-d4 was -80 and -70, respectively. Collision energy (CE) was -28 for TDA, -30 for TDA-d4, -34 for HDA and -30 for HDA-d4. Collision cell exit potential (CXP) was -7 for TDA, -13 for TDA-d4, -7 for HDA and -15 for HDA-d4. The dwell time was set to 100 ms for all four compounds.

Quantification of coproporphyrin by HPLC-MS/MS

This method has been previously described by Kandoussi et al[3]. All samples were kept in the dark and sample processes were carried out by protecting them from light as much as possible. A 100 µL sample aliquot was mixed with a 100 µL volume of 10.0 nM CP-I $^{15}N_4$ and 25.0 nM CP-III d_8 internal standard solution prepared in 6M formic acid. After vortexing the samples for 5 minutes, a 200 µL of the mixture was loaded onto a Biotage Isolute SLE+, 200-µL, 96-well plate and allowed to equilibrate for at least 5 minutes. Then a 500 µL aliquot of ethyl acetate was added to each well for three times, and allowed to elute under gravity and a positive pressure of 3 psi for 30 seconds to ensure completion of elution. Samples were dried under a nitrogen stream and reconstituted with 100 µL of 0.1:30:70 formic acid / acetonitrile / water, v/v/v. A UHPLC system consisting of an Acquity™ HSS T3 UPLC column (2.1 x 50 mm, 1.7 µm) analytical column with a VanGuard BEH C18, 2.1 mm x 10 mm, 1.7 µm guard column was used to separate of CP-I and CP-III isomers. Mobile phase B was 0.1:2:98 formic acid / water / acetonitrile, and the mobile phase A was 0.1:100 formic acid / water, v/v). The UHPLC program starting conditions are 35% B. At 0.9 min, %B is increased to 40% over 3.6 min, and then further increased to 95% in 0.15 min, maintaining 95% B for 1.35 min and then decreasing to starting conditions (35%B) in 0.2 min and maintaining the condition until 7.5 min. The UHPLC

eluent was introduced into a Triple Quad™ 6500 mass spectrometer. The mass spectrometer was operated in positive electrospray ionization mode with the following settings: curtain gas, 30 psi; CAD gas, 8 psi; gas 1, 75 units; gas 2, 60 units; turbo ion spray voltage 4000 V and turbo probe temperature at 650°C. The mass spectrometer was operated in MRM mode with the transitions of m/z 655.4 → 596.3 for both CP-I and CP-III, m/z 659.3 → 600.3 for CP-I $^{15}N_4$ and m/z 663.3 → 602.3 for CP-III d_8. The chromatographic peak integration were performed using Analyst® software (version 1.6.2, AB SCIEX) and the peak areas were exported to Assist LIMS™ (version 6.06 a validated LIMS system).

References

1. van Haandel, L., Gibson, K.T., Leeder, J.S. & Wagner, J.B. Quantification of pravastatin acid, lactone and isomers in human plasma by UHPLC-MS/MS and its application to a pediatric pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* **1012-1013**, 169-77 (2016).
2. Shen, H. et al. Comparative Evaluation of Plasma Bile Acids, Dehydroepiandrosterone Sulfate, Hexadecanedioate, and Tetradecanedioate with Coproporphyrins I and III as Markers of OATP Inhibition in Healthy Subjects. *Drug Metab Dispos* **45**, 908-919 (2017).
3. Kandoussi, H. et al. UHPLC-MS/MS bioanalysis of human plasma coproporphyrins as potential biomarkers for organic anion-transporting polypeptide-mediated drug interactions. *Bioanalysis* **10**, 633-644 (2018).