SUPPLEMENTARY METHODS

In vitro conversion of atorvastatin to atorvastatin lactone in simulated gastric fluid

Atorvastatin acid standards were prepared in simulated gastric fluid (SGF), adjusted to pH 9.5 to minimize conversion of acid to lactone. Atorvastatin lactone standards were prepared in 0.2% formic acid to minimize conversion of lactone to acid. All samples were prepared in glass vials. Samples of SGF were warmed to 37°C for 5 minutes prior to the addition of atorvastatin stock solution for a final nominal starting concentration of 100 nM atorvastatin acid (prepared in duplicate). The samples were then injected onto the LC/MS-MS system at 37°C and resampled every 7.5 minutes up to 2 hours. The LC/MS-MS system consisted of a Leap autosampler, Shimadzu pumps and controller and Sciex API 6500+ mass spectrometer. Mobile phases were 10mM ammonium formate at pH 4.5 and acetonitrile and a Thermo Fisher Betasil C18 5um 2.1x20 mm column was used. Mass transitions monitored were 559.3 → 440.2 m/z (atorvastatin) and 541.2 → 448.2 m/z (atorvastatin lactone). An Eli Lilly proprietary internal standard was used.

In vitro hepatocyte uptake

Uptake of atorvastatin and o-hydroxyatorvastatin was evaluated human hepatocytes in suspension using the oil-spin method. Female human cryopreserved hepatocytes (lot JYS) was purchased from Bioreclamation IVT (Baltimore, MD). Hepatocytes were thawed at 37°C and then placed in InVitroGRO HT medium. Cells were spun down and reconstituted in 4% BSA in KHB to a density of 1.5×10^6 viable cells/ml. Cell viability was >85%, as determined by Trypan blue staining. Uptake was evaluated from 0.25 to 15 minutes at 1 µM at 37°C in the absence and

presence of 250 or 500 µM rifamycin SV. All time points were evaluated in triplicate. Uptake of rosuvastatin, a probe OATP substrate, was concurrently assessed as a positive control. Cells were prewarmed for 5 minutes prior to the initiation of uptake. To initiate uptake, an equal volume of 4% BSA in KHB containing 2× the concentration of test article was added. 100 µl aliquots were taken at specified time points and added to microcentrifuge tubes containing 100 µl of an 8:1 silicone (1.05 g/ml) to mineral oil (1.015 g/ml) mixture (final density= 1.027 g/ml) and 100 µl of 5 M ammonium acetate and then spun down using table-top centrifuges at 12,500 rpm for 15 seconds to stop transport. Tubes were immediately placed on dry ice and stored at −80°C until atorvastatin and o-hydroxyatorvastatin concentrations were determined by LC-MS/MS. Conditions and equipment were as above. Mass transitions monitored were 559.3 → 440.2 m/z (atorvastatin) and 575.2 → 440.2 m/z (o-hydroxyatorvastatin). Atorvastatin-d5 was used as the internal standard, the mass transition monitored was 564.3→ 445.2 m/z. Total uptake rates (pmol/10^6 cells/min) were determined from the linear portion of uptake over time and uptake clearances (µL/10^6 cells/min) calculated by dividing the rates by measured medium concentrations. The free uptake clearances were determined by dividing the total uptake clearances by the fraction unbound in 4% BSA, which was determined separately using equilibrium dialysis for atorvastatin and assumed similar for both compounds. Passive clearance was determined as the clearance in the presence of rifamycin. Active clearance was calculated by subtracting this from uptake in the absence of rifamycin.