Beta-blocker dialyzability in chronic hemodialysis patients

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Supplemental Material

Beta-blocker Extraction and Liquid Chromatography

Blood samples were centrifuged at 2000g for 10 minutes within one hour of collection. Plasma was separated from blood cells and subsequently stored with dialysate samples at -80°C until analysis. We determined total plasma (free and protein-bound) and dialysate concentrations of atenolol, metoprolol, bisoprolol, and carvedilol using solid phase extraction (SPE) followed by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QToFMS). Beta-blockers were extracted from plasma and dialysate samples with SPE cartridges (C18, Strata-X Polymeric Reversed Phase 33 μm) obtained from Phenomenex (Torrance, CA) conditioned according to manufacturer's specifications. Deuterated beta-blockers (atenolol-d7, metoprolol-d7, bisoprolol-d7, and carvedilol-d3) at 50 ng/mL were used as internal standards for drug quantification. Plasma, dialysate, and internal standards were passed across the SPE cartridges and washed with 1 mL of nano-pure water followed by 1 mL of 20% methanol in water. Analytes were eluted into clean glass test tubes with 1 ml of methanol solution containing 0.1% trifluoroacetic acid. Eluents were dried in a 40°C water bath using an Organomation N-EVAP^{TM} nitrogen evaporator (Berlin, MA) and then reconstituted in mobile phase. To ensure adequate compound detection, plasma extractions containing carvedilol were concentrated by a factor of 10, while plasma samples containing other beta-blockers did not require concentration. Dried eluents from dialysate extractions were concentrated by a factor of 100. For analyte separation,

reconstituted samples were injected at a volume of 5 µL with a flow rate of 0.7 mL/min on a Phenomenex Kinetex C8 column (1.7 µm particle size, 50 x 2.1 mm). The Waters ACQUITY UPLC™ I-Class system (Waters, Milford, MA) autosampler maintained the column temperature at 40°C. Water (A) and acetonitrile (B), both containing 0.1% formic acid, were used as the mobile phase solutions for compound elution. The UPLC elution parameters were as follows: 0.00–0.20 min, 2% B; 0.20–1.50 min, 2–80% B; 1.50–2.50 min, 80% B; and 2.51–3.51 min, 2% B.

Beta-blocker Analysis with Mass Spectrometry

We conducted mass spectrometry using a Waters Xevo™ G2S-QTofMS, and measured beta-blockers in positive electrospray ionization. The capillary and cone voltages were set at 0.5 kV and 40 V, respectively, and a source temperature of 150°C was maintained. The desolvation gas flow was 1200 L/h at a temperature of 650°C, and the cone gas flow was 50 L/h. We acquired data in centroid mode using an MS^E method. Samples were acquired in positive polarity with extended dynamic range and the analyzer mode set to resolution. Both functions 1 (low energy collision) and 2 (high energy collision) of the centroid method acquired data within a mass range of 50- 1200 Da and a scan time of 0.05s. We set collision energy for function 1 of the MS^E method at 0V, while for function 2 it was ramped from 15–50 V. For function 3, lockspray was acquired to maintain accurate mass detection and reproducibility. The lockmass consisted of leucine-enkephalin (1ng/µL) set at a flow rate of 10 µL/min. Acquisition of data was controlled by Waters MassLynx v4.1 software and peak integration of sample chromatograms were conducted with QuanLynx software (Waters, MA, USA).