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Development and Application of a Mass Spectrometry-Based Quantitative Assay for Apolipoprotein M in Human and Mouse Serum

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Abstract:

Apolipoprotein M (apoM) is necessary for the formation of lipid-poor preβ-HDL particles, the initial precursor of HDL and acceptors of cholesterol efflux from peripheral cells. An assay to quantify apoM in serum is not widely-available, hampering the efforts to further understand apoM and to develop therapeutic methods to increase circulating levels of apoM. An antibody-free, high throughput mass spectrometry (MS)-based assay was developed to quantitatively measure apoM from a variety of species including human, mouse, and rat. Apolipoproteins were enriched by selectively binding to Liposorb, an affinity resin, followed by enzymatic digestion. This peptide mixture was separated by HPLC coupled in-line with tandem MS/ MS. Signal intensities from the MS/ MS fragmentation of apoM-specific peptides were measured simultaneously in a targeted method spanning many commonly used species. The same amount of purified human apolipoprotein A-IV uniformly labeled with 15N was spiked into all samples and was used as an internal standard to correct for any variation in sample handling and recovery. Assay variability and accuracy was statistically validated in a three-day spike recovery experiment to determine the working range of the assay. The concentration range for quantification of apoM using this assay was 11.2-500 nM, whereas average concentration of human apoM measured from a large sampling (n>100) was 370 nM. This assay was used to measure changes in apoM in mouse serum from a pre-clinical study that was designed to evaluate the effects of a microsomal triglyceride transfer protein (MTTP) inhibitor. All measured lipoproteins and apolipoproteins showed a dose-dependent decrease in concentration and the response of apoM closely followed the response of HDL. In a clinical application of the assay, apoM was measured in human serum to evaluate the effects of two cholesterol-lowering compounds, a statin drug and an experimental PPAR-α agonist. ApoM levels did not change with PPAR-α agonist or combination treatments, but significantly decreased with atorvastatin. The measurement of apoM provided additional information on the effects of these drug treatments that previously could not be measured. The availability of a quantitative assay for apoM provides a valuable tool in the development of cardio-protective therapeutics and understanding the mechanisms of these drugs.

Description:

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