Application of liquid chromatography/mass spectrometry for evaluation of plasma ATP concentration and turnover rate

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Nucleotides and its derivatives in the extracellular fluid, especially in plasma play a prominent role in many physiological processes. In particular, its role in vascular pathology is well proven. Nucleotides released from cells to extracellular space can trigger thrombosis, inflammation and ectopic calcification. However, quantitative analysis represents a challenge due to relatively low concentration of these compounds. Reduction of flow in chromatographic separations linked to mass detectors offers massive gain in sensitivity but at the expense of complexity of work flows. Nanoflow LC/MS is extensively used in proteomic while its application in small molecule separation is not common. This study aimed to test several options and to develop procedure to quantitate ATP concentration in plasma using liquid chromatography/mass spectrometry capable to resolve ATP isotopomers to facilitate turnover rate studies.

Several chromatographic strategies were tested that include porous carbon, ion-pairing reversed phase and HILIC. Both standard HPLC and nano HPLC were used in connection with triple quadrupole (TSQ Vantage, HESI II source) and ion trap (LCQ Deca XP, ESI or nano ESI sources) mass detectors operating in negative ion mode. Stable isotope labeled ATP (15N) or normal ATP were added to heparinised human blood for up to 5 min at 0, 300nM, 1 µM, 3 µM concentrations followed by plasma isolation, extraction with acetonitrile and analysis.

HILIC method provided best robustness and reproducibility. Triple quadrupole mass detector offered sensitivity below 10 nM but suffered from considerable in source fragmentation of ATP ion (20-40%). Ion trap detector had much less in source fragmentation (<5%) and equivalent sensitivity. Application of nano HPLC resulted in 50 fold gain in sensitivity but

added technical problems such as ATP retention in the system. Human plasma ATP concentration estimated with HILIC method and ion trap of was 0.43(±0.21) µM. Half-life of 15N ATP in human blood within concentrations studied was estimated at 2 to 5 min.

We have developed several procedures for liquid chromatography/mass spectrometry analysis of ATP concentration and isotope pattern for turnover studies and highlighted benefits and problems of nanoHPLC for such analyses. Our results demonstrated ATP concentration equivalent to previous studies and relatively long half-life of extracellular ATP in isolated human blood.