Investigation of Arachidonic Acid and Its Metabolites as Biomarkers for Potential Efficacy Endpoints for Monoacylglycerol Lipase Inhibition

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To further develop monoacylglycerol lipase (MAGL) inhibition as a therapeutic strategy for diseases with a neuroinflammatory component, endpoints are needed to monitor treatment efficacy. Brain inflammation can occur with infection, traumatic brain injury, or disease. Typically, this inflammation is helpful in restoring tissue structure and physiological function, but if it becomes chronic or uncontrolled, it can cause neurological damage. MAGL is an enzyme that controls brain levels of arachidonic acid (AA) and pro-inflammatory downstream metabolites, which are significantly increased in inflamed tissue and are key players in the generation of the inflammatory response. MAGL inhibitor treated animals or MAGL-deficient mice show neuroprotection and attenuated neuroinflammation in models of brain inflammation, Parkinson's and Alzheimer diseases. To investigate AA and its metabolites as potential biomarkers of neuroinflammation, we evaluated levels of these metabolites in cerebral spinal fluid in patients with brain inflammation.

A quantitative assay was developed using reverse phase ultra-high pressure liquid chromatography coupled with a triple quadrupole mass spectrometer (UPLC/MS/MS) to measure arachidonic acid and several metabolites in human cerebral spinal fluid (CSF). Samples were prepared for analysis by first adding an internal standard followed by protein precipitation with an organic solvent. The analytes measured included 6-keto prostaglandin F1α (6-keto PGF1 α), prostaglandin E2 and D2 (PGE2, PGD2), (±)15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-HETE), (±)12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (12-HETE), (±)5-hydroxy-6E, 8Z,11Z,14Z-eicosatetraenoic acid (5-HETE), leukotriene E_4 (LTE_4) and thromboxane (TXB). Detection limits ranged from 0.5 to 1000 ng/ml for PGE2, PGD2 and TXM, from 1 to 500 ng/ml for 12-HETE and 5-HETE, from 1 to 50 ng/ml for 15-HETE, from 0.5 to 50 ng/ml for LTE4, and from 15 to 30,000 ng/ml for AA. The assay was used to measure AA and metabolites from

control patients and patients with brain inflammation resulting from intra-cerebral hemorrhage, ischemic stroke, and traumatic brain injury.

The most dramatic increase in AA and metabolites compared to control was observed in patients who had an intra-cerebral hemorrhage; AA and 6-keto PGF1 α was increased nearly 10 fold, 12-HETE 8 fold, 15-HETE, 5-HETE and LTE4 nearly 4-fold. The results obtained from this sample set have enabled us to identify potential biomarkers to further investigate for the development of MAGL inhibitors.