Determination of a broad range of polyunsaturated fatty acid-derived bioactive lipid mediators by a new UPLC-MS/MS method

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Here, we present a new ultra-performance liquidchromatography tandem mass spectrometry (UPLC-MS/MS) method, for the detection of a broad range of polyunsaturated fatty acid (PUFA)-derived bioactive metabolites. Lipid mediators (LM) are small molecules produced for instances by immune cells that can act as pro- and non-resolving molecules during the inflammatory process. They are oxygenated derivatives of PUFA present in cellular membranes such as arachidonic acid (AA, 20:4 ω-6), dihomo-γ-linolenic acid (DGLA, 20:3 ω-6), eicosapentaenoic acid (EPA, 22:5 ω-3), or docosahexaenoic acid (DHA, 22:6 ω-3) through the activity of 5-, 12-, or 15-lipoxygenases (LO) and cyclooxygenases (COXs). The LM production was verified in primary human polymorphonuclear leukocytes (PMNL), in HL-60 cells, and in PMA-differentiated THP-1 immortalized cells. The stimulation was performed in the presence or after pre-treatment of cells with different exogenous PUFA in order to evaluate their effect on LM profile produced by the various cells. Upon ionophore or lipopolysaccharide (LPS) stimulation, primary PMNL synthesized higher amounts of eicosanoids compared to the immortalized cells (HL-60; THP-1 derived macrophages): around 50 eicosanoids were simultaneously separated in a single analytical run, with major interest on pro-resolving LM production. The new method was applied also on samples from mouse peritoneal exudates obtained from *in vivo* experiments. In conclusion, the new highly sensitive UPLC-MS/MS method provides a faster qualitative and quantitative method to study LM production in immune cells during inflammation and may help to understand their regulation in health and disease.