

**S011** Analysis of HOCl- and free radical-induced damage to biomolecules by LCMS

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In inflammatory diseases, release of oxidants leads to oxidative damage to biomolecules. Free radical damage results in peroxidation of lipids and various post-translational oxidative modifications of proteins. HOCl, released by the myeloperoxidase-H<sub>2</sub>O<sub>2</sub>-Cl<sup>-</sup> system, can cause formation of phospholipid chlorohydrins and alpha-chloro-fatty aldehydes from plasmalogens. HOCl can attack several amino acid residues in proteins, but the formation of 3-chlorotyrosine is one of the most stable markers of HOCl-induced damage. Similarly, reactive nitrogen species such as peroxynitrite result in formation of 3-nitrotyrosine. We have used LCMS to detect the formation of phospholipid hydroperoxides and chlorohydrins in oxidized LDL and cultured cells subjected to oxidative stress. To allow analysis of oxidative modifications of proteins in complex mixtures, we are currently developing LCMS<sup>2</sup> and LCMS<sup>3</sup> methods using precursor ion scanning to detect mass changes in oxidized residues on fragmentation of peptides from tryptic digests. We have identified indicative fragment ions for chlorotyrosine, nitrotyrosine, hydroxytyrosine and hydroxytryptophan, but nano-LC/MS<sup>3</sup> is required to overcome the problem of false positives from isobaric ions in unmodified peptides. The use of LCMS to detect specific oxidative modifications in both lipids and proteins is important for understanding disease mechanisms and offers potential for development of novel biomarkers of inflammatory diseases.