Hydroxyoctadecadienoic acids (HODEs) increase apoptosis in human THP1 monocytes and macrophages

V. Vangaveti, U. H. Malabu, V. Shashidhar, B. T. Baune, R. L. Kennedy
Department of Medicine, James Cook University, Townsville, QLD, Australia

Certain fatty acids function as signaling molecules. HODEs are stable oxidation products of linoleic acid (LA; C18:2), are abundant in atherosclerotic plaque, and known to signal through GPR132 (9-HODE only) or PPARgamma (9-HODE and 13-HODE). Macrophage apoptosis is an important process, contributing to atherosclerosis progression. Both GPR132 and PPARgamma were expressed in THP1 (RT-PCR and immunohistochemistry) with expression of both increased when cells were differentiated into macrophages (PMA). In 24-hour cultures, 9-HODE but not 13-HODE or LA decreased cell number (68%, $p<0.001$). We aimed to determine whether this was due to apoptosis and how it was mediated. Using a caspase 3/7 assay, 9-HODE and 13-HODE (30-100mM) but not LA increased caspase activity in monocytes and macrophages, with 9-HODE being more potent ($p<0.001$). This was accompanied by decreased cell viability (ATP generation assay, both $p<0.001$). FACS was used to quantify cells that were either viable or apoptotic (7AAD and annexin V positive). There was a time-dependent (over 24 hours) increase in apoptotic cells with 9-HODE and 13-HODE (both $p<0.001$), with 9-HODE being more potent ($p<0.001$). The effect of HODEs was replicated with campothecin (10mM) but not with the PPARgamma agonist rosiglitazone (1mM). The pro-apoptotic effects of HODEs were abolished by addition of the caspase inhibitor DEVA-CHO but not affected by the PPARgamma antagonist T0070907. In a gel-based assay, DNA fragmentation was apparent with campothecin and 9-HODE but not with LA or 13-HODE. GPR132 expression was silenced using siRNA oligonucleotides. There was no evidence of decreased effect of either 9-HODE or 13-HODE with GPR132 silencing. In conclusion, HODEs, and particularly 9-HODE, are potent regulators of macrophage apoptosis. They do not appear to be signaling through GPR132 or PPARgamma, both of which have regulatory roles in atherosclerosis. Further study of their mode of action may lead to identification of novel therapeutic targets for atherosclerosis.