NITROARACHIDONIC ACID MODULATES INFLAMMATION THROUGH THE NRF2/ARE PATHWAY.

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Nitrated lipids have been recently detected in cell membranes and human plasma. In particular, nitration of arachidonic acid (AA) occurs under oxidative/nitrative conditions, redirecting AA-dependent cell signaling pathways. Herein, we synthesized and characterized the major isomers of nitroarachidonate ($AANO_2$) and demonstrated its ability to modulate inflammation. $AANO_2$ was synthesized by AA incubation with sodium nitrite at acidic pH, and purified by TLC and HPLC. Mass spectrometry, IR and NMR spectra analysis showed the presence of mononitrated nitroalkenes. The position of the NO_2 group was determined by MS fragmentation, where four major isomers (9-, 12-, 14- and 15-$AANO_2$) were identified. All isomers exert anti-inflammatory activities, including inhibition of inducible nitric oxide synthase (NOS2) expression in LPS/IFNγ activated macrophages. The involvement of the Nrf2/Antioxidant Responsive Elements (ARE) pathway was evaluated: $AANO_2$ as well as the Nrf2 agonist t-butylhidroquinone, induced heme oxygenase 1 (HO-1) expression in macrophages as well as in astrocytes. Moreover, HO-1 expression was inhibited when cells where transfected with a negative dominant plasmid for Nrf2, further confirming the presence of Nrf2 activation. These data support that $AANO_2$ represents a novel anti-inflammatory signaling molecule by activation of the Nrf2/ARE pathway.
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