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An LPS-inducible anti-inflammatory epoxygenase pathway in the endothelial cell line EA.Hy926

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Oxylipids are produced through three major metabolic pathways, the cyclooxygenase, lipoxygenase and cytochrome P450 (CYP) monooxygenase systems (1). Here we investigated the expression of the epoxygenase CYP2J2 and the enzyme responsible for the removal of its epoxy-products, soluble epoxide hydrolase (sEH) to dihydroxy- metabolites, in the stable human endothelial cell line EA.Hy926.

EA.Hy926 were grown in M199 media supplemented with 10% FCS and Pen-Strep. Released oxy-lipids were measured by LC-MS/MS (2). CYP2J2 and sEH mRNA was assessed by Taqman qRT-PCR. TNFalpha release was measured by ELISA (eBioscience) at 7h. In some experiments cells were treated with LPS (1ug/ml), IL-1beta (10ng/ml) or PMA (5nM) alone or in the presence of the sEH inhibitor AUDA (3; 10uM) or vehicle (0.01% DMSO).

EA.Hy926 produced active epoxygenase/ sEH metabolites of arachidonic acid (5,6-DHET, and 14,15,DHET), linoleic acid (9,10-EPOME and 12,13-EPOME and their sEH products 9,10-DHOME and 12,13-DHOME), docosahexaenoic acid (sEH product 19,20-DiHDPA) and eicosapentaenoic acid (sEH product 17,18-DHET); Fig 1. * $p<0.05$ by paired t-test; n=3; 24h. LPS further induced 5,6-DHET and the DHOMEs, and induced CYP2J2 mRNA (2.1±0.4fold ; $p<0.05$; n=4) but not sEH mRNA (0.85±0.1 fold). The sEH inhibitor AUDA inhibited basal, LPS, IL-1β and PMA induced TNFα release (Fig 2; * $p<0.05$; 1-sample t-test; n=8).

Fig. 1

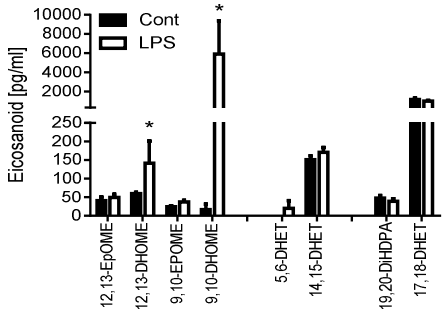
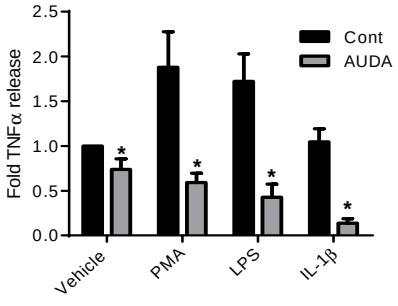


Fig. 2



In summary, the human endothelial cell line EA.Hy926 contains a LPS-regulated epoxygenase CYP2J2 and metabolises linoleic acid> eicosapentaenoic acid > arachidonic acid > docosahexaenoic acid to active products. AUDA, a sEH inhibitor, greatly attenuated inflammatory stimuli-induced TNFalpha release. sEH inhibitors may therefore be useful therapeutic avenues for human vascular inflammatory disorders.

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