

# **Prevention of atherosclerosis by blocking lipid binding to cysteines in human aortic endothelial cells in vitro**

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## **Abstract**

Atherosclerosis is the buildup of fatty materials along the arterial wall. It is the underlying condition that can lead to heart attacks and strokes. Recent research has shown that oxidized phospholipids (Ox-PL) will bind to specific proteins in human aortic endothelial cells (HAEC) inside the arterial wall. It has also been found that Ox-PL regulates over one thousand genes in HAECs. Some of the genes that are up regulated in the process cause monocytes to migrate and enter the arterial wall; eventually this leads to a buildup of plaque which is known as atherosclerosis. Because PEIPC has been determined to be the most reactive phospholipid in this process we are centering our studies on it. Currently our group believes that PEIPC binds to cysteines in HAECs via an electrophilic  $\alpha,\beta$  unsaturated enone group. A major goal of our group is to reduce this binding and to reduce the pro-inflammatory effects of PEIPC in HAECs. While testing the mechanism by which PEIPC binds to proteins, it was found that the free fatty acid in the sn-2 position of PEIPC, EI, may also be important in the regulation of inflammation. EI is produced when PEIPC is cleaved in the sn-2 position by PLA2 (phospholipase A2). Because PEIPC has an inflammatory effect it was speculated that EI would probably also have an inflammatory effect, but surprisingly, evidence suggests that EI may have an anti-inflammatory effect. This raises many interesting questions. Because lipoproteins associated PLA2 (Lp-PLA2) is mainly found in HDL, which has been said to have an anti-atherosclerotic effect, and Lp-PLA2 is also reported to be anti-atherogenic, the cleavage of PEIPC to EI by PLA2 may constitute a mechanism of HDL activity. If this is the case, EI, or an analog of EI, may have the potential to be used as a therapeutic with limited side effects because it is a naturally occurring compound that inhibits atherosclerosis. To progress toward answering these questions, we need to identify key proteins that PEIPC is binding to. Our current objective is to identify if OxPAPC binds to any of 3 specific proteins; VEGFR2, EP2 and GRP78. We can synthesize a biotin-tagged analog of PEIPC or OxPAPC, treat HAECs with the lipid, then use gel imaging and gel electrophoresis to create a western blot to estimate the molecular weight of lipid-bound proteins. We may also use affinity chromatography to pull down lipid-bound proteins and identify these lipid protein targets with LC-MS/MS. This will give further insight into the mechanism by which oxidized lipids affect the inflammatory pathway in HAECs. We can also use this information to model the binding of PEIPC to proteins in order to develop compounds and methods to inhibit this binding.