Biphasic Regulation of STAT1α Expression in Vascular Smooth Muscle Cells by Oxidized Low-Density Lipoprotein

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Atherosclerosis is a pro-inflammatory disease involving the effects of many cytokines and growth factors, which signal via distinct pathways, such as the janus kinases (JAKs) and signal transducers and activators of transcription (STAT). Oxidized LDL (OxLDL) is critical for atherosclerotic plaque generation and progression. To examine potential regulation of STAT1α by OxLDL in vascular smooth muscle cells (VSMC), we incubated VSMC with 0-100 μm/mL OxLDL or native LDL (nLDL) and measured STAT1α expression by real-time PCR and Western blotting. OxLDL, but not nLDL, dose-dependently increased STAT1α expression at 3h (51±1% mRNA increase; 23±1% protein increase n=3, p<0.01), but markedly reduced STAT1α expression at later time points (83±10%, p<0.01). To determine intracellular signaling pathways mediating OxLDL effects on STAT1α expression, we studied the role of candidate proteins implicated in OxLDL signaling. OxLDL components are high-affinity ligands for PPAR-γ (PPAR-γ..), but the effect of OxLDL was not blocked by the PPAR-γ inhibitor liprostat. The synthetic PPAR-γ ligand ciglitazone did not regulate STAT1α expression. Furthermore, inhibition of the p38 MAPK pathway (SB203580) or p42/44 MAPK pathway (PD98059) did not modify OxLDL effects, indicating that the ability of OxLDL to regulate STAT1α was not mediated via a p38 or p42/44 MAPK dependent pathway. Likewise, inhibition of the ubiquitin/proteasome pathway (MG132) did not blunt OxLDL regulation of STAT1α. However, 40nM Fucoidan (a scavenger receptor A inhibitor) and Anti-CD36 anti-body (a scavenger receptor B inhibitor) markedly blocked OxLDL regulation of STAT1α expression (64%, 71% inhibition, respectively). Furthermore, the water-soluble Vitamin E derivative, Trolox, completely inhibited OxLDL regulation of STAT1α. In conclusion, OxLDL causes biphasic regulation of VSMC STAT1α expression via a PPAR-γ, p38 MAPK, p42/44 MAPK independent and redox-dependent pathway that is mediated via scavenger receptors A and B. These findings have important implications for understanding cellular signaling events regulated by OxLDL, and hence mechanism of atherogenesis.

Large Oligomeric Grape Skin Polyphenolics Are Most Effective as Antioxidants and Antiplatelet Agents

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Background: Grape skin polyphenolics (PP) exhibit antioxidant and antiplatelet effects, which may reduce the risk of CVD in individuals consuming them. As these PP are chemically diverse, it is unknown which classes of PP are most effective. We hypothesized that different classes of PP will be differently effective as antioxidants and antiplatelet agents. Methods: We fractionated a grape skin extract into 6 distinct PP fractions (F1-6; normal-ized to 5μm gallic acid equivalents of redox potential) using multiple solvent elutions through a Sephadex LH-20 column. F1-6 were characterized using HPLC and MALDI-TOF mass spectrometry. Antioxidant effect was determined by measuring the ability of F1-6 and ascorbate (control) to extend the lag time to Cu2+-induced oxidation of LDL (n=5): 1) by direct incubation (DI) with the LDL, and 2) by incubating LDL with the compounds and then washing the LDL to remove unbound compounds (TW). Antiplatelet effect was measured using collagen induced whole blood platelet aggregation (PA).

Results: F1-3 contained oligosaccharides, hydroxycinnamic acids, anthocyanins, flavonoids and low-MW polygalloyl polyflavan-3-ols (PGPF). F4-6 contained PGPF with 4-8, 5-10, and 6-16 degrees of polymerization, respectively. In DI study, F1-3 extended lag time to LDL oxidation by 64±1%, 156±2%, 147±4%, but failed to retain this effect in the TW study. F1-2 did not have an effect on PA, however F3 significantly stimulated PA by 31%, F4-6 extended DI lag time by 18±1%, 113±7%, and 144±2% and retained 80%, 100%, and 100% of this effect in TW, respectively. F4-6 significantly inhibited PA by 55±14%, 98±9%, and 98±9%, respectively. Conclusions: F5-6, containing PGPF with 5-16 degrees of polymerization, were most effective as antioxidants and antiplatelet agents. They were also most effective at binding LDL. This suggests that large MW PGPF may be primarily responsible for the beneficial effects of grape skin PP. Conversely, certain PP such as F3, which stimulated oxidation, may have undesirable effects on CVD risk factors. Careful study of various classes of PP in a diet may be crucial in determining the overall effect of the PP on CVD.