grown to confluence in 4 quadrant dishes. They were then placed in 0.1% FBS medium and 6 mM nicotine for two hours. Thereafter, a scratch wound assay was performed using a sterile 10 µl pipet tip on confluent cell monolayers. Pure AO compounds, ferulic acid (F), tetrahydrocurcuminoids CG (T), and resveratrol (R) in single, double or triple combinations (10^{-5}M) were tested. The migratory behavior of the wounded cells with or without nicotine in the presence of AO combinations was recorded every 15 min. for ten hours, using a Nikon Biostation. Immunohistochemical analysis of nicotine-treated cells had decreased signaling protein RacGTP at the leading edge of migrating cells. Treatment with single, double and triple combinations of antioxidants increased the level of Rac-GTP activation in HGF and HPDL cells at the leading edge. These results clearly demonstrated that combinations of AOs promote cell migration and counteract the effects of nicotine in cultured oral fibroblasts via the RacGTP signal transduction pathway.

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Modulation of 4-Hydroxy-2-Nonenal Induced Signaling by Glutathione S-Transferase A4-4

Rajendra Sharma[1], *Pankaj Chaudhary*[1], *Abha Sharma*[1], *Rit Vatsyayan*[1], *Sanjay Awasthi*[1], *and Yogesh C Awasthi*[1]
[1]*University of North Texas HLTH Sci. Center*

The lipid peroxidation product, 4-hydroxy-2-nonenal (4-HNE) has been recognized as an important second messenger in cell cycle signaling. Here we demonstrate that 4-HNE induces signaling for apoptosis via Fas mediated extrinsic and p53 mediated intrinsic pathway in HepG2 cells. 4-HNE induces apoptosis through p53 pathway by activating Bax, p21, JNK, and caspase-3. 4-HNE also induces Fas mediated DISC-independent apoptosis pathway by activating ASK1, JNK and caspase-3. 4-HNE exposure to HepG2 cells leads to the activation of both Fas and Daxx and promotes the export of Daxx from the nucleus to cytosol, where it binds to Fas and inhibits apoptosis. Depletion of Daxx by siRNA results in potentiation of apoptosis indicating that Daxx inhibits apoptosis by binding to Fas. 4-HNE-induced translocation of Daxx is also accompanied by the activation and nuclear translocation of HSF1 and up-regulation of heat shock protein Hsp70. All these effects of 4-HNE can be attenuated by transfection of cells with hGSTA4-4, the isozyme of glutathione S-transferase with high activity for 4-HNE. Through immunoprecipitation and liquid chromatography–tandem mass spectrometry, we have demonstrated covalent binding of 4-HNE to Daxx. We have also provided evidence that 4-HNE modification induces phosphorylation of Daxx at Ser668 and Ser671 in HepG2 cells to facilitate its cytoplasmic export. These results indicate that while 4-HNE contributes to oxidant toxicity through several mechanisms, in parallel it evokes signaling for defense mechanisms to self regulate its toxicity and can simultaneously affect multiple signaling pathways through its interactions with membrane receptors and transcription factors/ repressors.

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Thioredoxin 1 Down-regulates but Thioredoxin Reductase Up-regulates ROS Generation and Secretion/expression of MCP-1 in Endothelial-like cells

Xun Shen[1], *Beidong Chen*[1], *Zhen-Bo Liu*[1], *Dandan Guan*[1], *and Dandan Guan*[1]
[1]*Institute of Biophysics, Chinese Academy of Sciences*

To know if the high expression of thioredoxin 1（Trx1）and thioredoxin reductase 1 (TrxR1) in atherosclerotic plaques suggests a role of the enzymes in antioxidant defense mechanisms against atherosclerosis, the effect of Trx1 and TrxR1 on expression of MCP-1 and ROS generation was investigated in human endothelial cells. Opposite role of Trx1 and TrxR1 was observed: (1) Trx1 suppressed but TrxR1 enhanced ROS generation in the cells. The mechanism involves regulation of the expression of $p22^{phox}$, the subunit of NADPH oxidase. (2) Trx1 suppressed but TrxR1 enhanced the ox-LDL-stimulated MCP-1 release and expression. The former greatly promoted nuclear translocation of Ref-1 and subsequently reduced DNA-binding activity of AP-1, while the latter promoted nuclear translocation and DNA-binding activity of NF-κB. It seems that Trx1 inherently suppresses MCP-1 expression in vascular endothelium and may prevent atherosclerosis. However, TrxR1 appears a dualistic enzyme which catalyzes reduction of disulfide in oxidized thioredoxin, but enhances ROS generation and subsequent MCP-1 expression in endothelium, thus may promote rather than prevent vascular endothelium from forming atherosclerotic plaque.

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The Majority of Nox1 Resides at Internal Membranes and Traffics Differentially with Stimulation

Jennifer Streeter[1], *and Francis Miller*[1]
[1]*University of Iowa*

The Nox1 subunit of NADPH oxidase is a membrane protein that generates superoxide and activates redox-dependent signaling pathways in multiple cell types. Localization appears to be a regulatory mechanism for Nox1 activation. However, little is known regarding the relative membrane distribution of Nox1 and whether it traffics in response to agonist stimulation. Previous studies of Nox1 localization are limited by the use of heterologous over-expression systems. The goal of this study was to characterize endogenous Nox1 localization and trafficking under basal and stimulated conditions. Studies utilized cultured murine and rat aortic smooth muscle cells (SMCs). Biotinylation of plasma membrane (PM) proteins reveal that at basal conditions ~5% of total cellular Nox1 resides at the PM. Having previously shown that tumor necrosis factor (TNF)-α and thrombin activate Nox1 in SMCs, we found that treatment of SMCs with TNF-α protects biotinylated Nox1 from surface biotin cleavage, indicating internalization of PM Nox1 in response to TNF-α. In contrast, treatment of cells with thrombin does not protect Nox1 from surface biotin cleavage, signifying the absence of Nox1 internalization in response to thrombin. Stimulation of SMCs with TNF-α or thrombin in the presence of biotin increased the amount of biotinylated Nox1, suggesting possible recruitment of intracellular Nox1 to the PM. However, inhibiting endocytosis with a dominant-negative dynamin or 4°C prevents this increase in biotinylated Nox1 in response to TNF-α or thrombin. We interpret this finding to indicate that Nox1 is not recruited to the PM after agonist stimulation, but instead, agonist-induced endosomes fuse with intracellular Nox1-containing vesicles. These novel findings confirm differential activation of Nox1 in response to different