Hydrogen Peroxide-Induced Superoxide Production in Endothelial Cells: Role of Nitric Oxide Synthase

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Background: Hydrogen peroxide causes oxidant injury to vascular cells and may contribute to the pathogenesis of vascular disease and endothelial dysfunction. In smooth muscle cells (SMC), hydrogen peroxide causes oxidant injury via NAD(P)H oxidase dependent production of superoxide and superoxide scavengers protect against hydrogen peroxide-induced SMC cytotoxicity. We investigated the potential enzymatic sources and consequences of hydrogen peroxide-induced superoxide production in quiescent endothelial cells and compared expression and activity to physiological shear.

Methods: Superoxide production was examined by dihydroethidium fluorescence. Nitric oxide production was quantified via Greiss assay and intracellular staining with DAF-FM diacetate. Cell viability was determined by propidium iodide staining, and cell detachment with lower activity of the endothelium and shear stress.

Results: Under static conditions, 60 μM hydrogen peroxide induced superoxide production in porcine aortic endothelial cells (PAEC). In the presence of L-NAME (1 mM), a specific inhibitor of nitric oxide synthase (NOS), there was a sixteen percent reduction in cellular superoxide production (n=9, p<0.001). Apocynin (0.5 mM), an inhibitor of NAD(P)H oxidase, combined with L-NAME (1 mM) was also found to reduce superoxide production. Manganese superoxide dismutase (SOD) overexpression by adenoviral transduction decreased. In contrast, the level of PRMTI and III was increased in tissue from patients with cardiovascular disease and is extensively produced by adipose tissue. Fibrates are lipid lowering agents activating peroxisome proliferator activated receptor-α (PPAR-α). Recent experimental as well as clinical data attributed additional antiatherosclerotic properties to these substances. The mechanisms, however, remain to be fully understood. The current study investigates a possible modulation of PAI-1 expression in adipose tissue by fibrates.

Methods: Human preadipocytes in primary culture were exposed to selected concentrations of gemfibrozil and fenofibrate (100, 350, 700μM) in the presence or absence of 40pM transforming growth factor-beta (TGF-β). An established stimulus for PAI-1 expression, PAI-1 protein was measured by ELISA, PAI-1 mRNA by light cycle RT-PCR. Parallel experiments were performed in cultured differentiated human adipocytes. Results: In both cell fractions of human adipose tissue, in preadipocytes and adipocytes, gemfibrozil and fenofibrate reduced PAI-1 protein expression by up to 39±10% (n=6, P=0.01) and 27±8% (n=6, P=0.01) under basal conditions and up to 35±6% (n=6, P=0.01) and 18±5% (n=6, P=0.04) when stimulated with 40pM TGF-β. Coincubation with 350μM gemfibrozil and 5μM troglitazone, a PPAR-γ agonist known to downregulate PAI-1 expression in adipose tissue, did not produce an additional effect compared to either of the substances alone suggesting a common final pathway (n=4, P=ns). Time course experiments showed a maximal effect after 24 hours (n=6, P=0.05 each). Total quantity quantification indicated persistent viability of cells. Quantification of mRNA proved that regulation was at least in part at the transcriptional level (n=3, P<0.01 each). Incubation with nonfibrate PPAR-α agonists showed similar reductions in PAI-1 expression suggesting that effects were mediated by PPAR-α gene regulation. The finding is consistent with previous investigations indicating that the potential significance of PPAR-α activation is an antiatherosclerotic phenomenon that may diminish cardiovascular risk, possibly by downregulation of PPAR-α expression in adipose tissue.