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## THE ANTIOXIDANT TEMPOL ATTENUATES ANG II INDUCED OXIDATIVE STRESS, HYPERTENSION AND HYPERTROPHY IN MICE OVEREXPRESSING NOX1 IN SMOOTH MUSCLE CELLS

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Reactive oxygen species (ROS) play a significant role in the development of cardiovascular pathologies. NADPH oxidases are one of the main sources of ROS in the vessel wall. To assess the role of ROS in Ang II–mediated hypertension and hypertrophy in vivo we used smooth muscle-specific nox1-overexpressing mice ($Tg^{SMnox1}$). We tested the hypothesis that overexpression of VSMC nox1 exacerbates Ang II-induced superoxide production, vascular hypertrophy and hypertension, and that it can be reversed by antioxidant treatment with Tempol. Male $Tg^{SMnox1}$ mice and their negative littermate controls were infused with Ang II (0.7 mg/kg per day) for 14 days. Superoxide formation in mouse aortas was quantified by HPLC analysis of the DHE-superoxide product, oxyethidium, and ESR using spin probe CMH. Overexpression of nox1 led to 2-fold increase in superoxide in the aorta after Ang II infusion. Blood pressure was significantly higher in $Tg^{SMnox1}$ mice treated with Ang II than in their wild type littermate controls. In addition, Ang II–induced aortic hypertrophy was greater in $Tg^{SMnox1}$ mice. To confirm that this potentiation of vascular hypertrophy and hypertension was due to increased ROS formation, additional groups of mice were coinfused with Tempol. Antioxidant treatment decreased the level of Ang II-induced superoxide production in mouse aortas and partially prevented the development of aortic hypertrophy and hypertension. These data indicate that upregulation of Nox1 does indeed affect both the pressor and hypertrophic responses to Ang II in a ROS-dependent manner, supporting the concept that nox1 may be involved in the development of cardiovascular pathologies.

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## INDUCTION OF PHASE II DETOXIFYING ENZYMES AND INHIBITION OF AP-1-MAPKS PATHWAY BY NATURAL ANTIOXIDANT CHLOROGENIC ACID

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Chlorogenic acid, the ester of caffeic acid with quinic acid, is one of the most abundant polyphenols in the human diet. The antioxidant and anticarcinogenic properties of chlorogenic acid have been established in animal studies. However, little is known about the molecular mechanisms through which chlorogenic acid inhibits carcinogenesis. In this study, we report that chlorogenic acid inhibited the proliferation of A549 human cancer cells in vitro. Results of soft agar assays indicated that chlorogenic acid suppressed TPA-induced neoplastic transformation of JB6 P+ cells in a dose-dependent manner. Pretreatment of JB6 cells with chlorogenic acid blocked UVB- or TPA-induced transactivation of AP-1 and NF-ƒÛB over the same dose range. At low concentrations, chlorogenic acid decreased the phosphorylation of c-Jun NH2-terminal kinases as well as MAPK kinase 4 (MKK4) induced by UVB/TPA, while higher doses are required to inhibit p38 kinase and extracellular signal-regulated kinases. Chlorogenic acid also increases the enzymatic activity of glutathione S-transferases (GST) as well as that of antioxidant response element (ARE)-mediated induction of a luciferase reporter. Further study indicated that chlorogenic acid stimulates the nucleus translocation of NF-E2-related factor (Nrf2). These results provide the first evidence that chlorogenic acid could protect against environmental carcinogen-induced carcinogenesis and suggest that the chemopreventive effects of chlorogenic acid may be through its up-regulation of cellular antioxidant enzymes and suppression of ROS-mediated NF-ƒÛB and AP-1-MAPKs activation.

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## PEROXIREDOXIN 6 (PRDX6) IS A NOVEL AND IMPORTANT LUNG ANTI-OXIDANT ENZYME

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GSH is known to play an important role in lung defense against hyperoxic stress but mice with knock-out of cytosolic GSH peroxidase (GPx 1) do not show altered sensitivity to oxygen exposure. Prdx6 is a recently described peroxidase that uses GSH as the physiological reductant for phospholipid and other hydroperoxides. This enzyme is a member of the peroxiredoxin family that has only a single conserved cysteine moiety and does not utilize thioredoxin as a reductant. In this study, we evaluated whether genetic inactivation of Prdx6 increases sensitivity of mice to oxygen toxicity. These mice develop and reproduce normally and have no obvious phenotypic alteration under ambient conditions. GSH peroxidase activity in lung homogenate with phosphatidylcholine hydroperoxide substrate was 70.5 „b 3 nmol/min/mg in wild type and 4.5 „b 1 nmol/min/mg in Prdx6 -/- mice. The mean duration of survival (LT50) for Prdx6 -/- mice (42 „b 1 h) was significantly shorter than that observed in wild-type mice (88.5 „b 1.5 h) on exposure to 100% O2; survival of Prdx6 +/- mice was intermediate (59.4 „b 2.2 h). After 72 h O2 exposure, lungs of Prdx6 -/- mice showed more severe injury compared to wild-type with increased wet/dry weight, epithelial cell necrosis and alveolar edema on microscopic examination, increased protein and nucleated cells in bronchoalveolar lavage fluid, and higher content of TBARS and protein carbonyls in lung homogenate. These findings show that Prdx6 -/- mice have increased sensitivity to hyperoxia and provide in vivo evidence that Prdx6 is an important lung antioxidant enzyme. We speculate that the ability to reduce phospholipid hydroperoxides differentiates Prdx6 from GPx1 and accounts for its role in protection from oxidant stress.

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## OXIDATIVE STATUS OF TYPE 2 DIABETICS CONSUMING MILLED FLAXSEED AND FLAXSEED OIL.

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The global prevalence of Type 2 diabetes (T2D) mellitus has increased exponentially. T2Ds may be prone to oxidative stress because hyperglycemia depletes natural antioxidants and facilitates the production of free radicals. Study of antioxidant therapy in persons with T2D is limited. Flaxseed is a key source of phytochemicals and is rich source of α-linolenic acid, fiber and lignans and consumption may reduce oxidative stress in T2D. A 12 week study was conducted where participants with T2D managed by diet were provided with baked products containing: 1) milled flax (n=12), 2) flax oil (n=13) and 3) placebo (n=10). We measured FRAP (Ferric Reducing Ability of Plasma) using a spectrophotometric procedure. FRAP values at different intervals for each group were: baseline 1) 977 ± 25 um 2) 1057 ± 26 um 3) 1037 ± 29 um; week 4, 1) 964 ± 25 um 2) 1056 ± 26 um 3) 981 ± 29 um; week 8, 1) 958 ± 25 um 2) 1047 ± 26 um 3) 1080 ± 29 um; week 12, 1) 953 ± 25 um 2) 1064 ± 28 um 3) 1046 ± 29 um,


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