[P-41]

Protective Effects of Naturally Occurring Antioxidants against beta-Amyloid-Induced Oxidative and Nitrosative Cell Death

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Introduction: beta-Amyloid peptide is considered to be responsible for the formation of senile plagues that accumulate in the brains of patients with Alzheimers disease. There has been a paucity of evidence to support the involvement of reactive oxygen and/or nitrogen species (ROS and/or RNS) in beta-amyloid-induced neuronal cell death. Recently, considerable atten- tion has been focused on identifying phytochemicals that are able to scavenge excess ROS and/or RNS, thereby ameliorating oxidative stress and cell death. In this study, we have investigated the effects of resveratrol, an antioxidant present in red wine, and ergothioneine (EGT), a naturally occurring thiol with peroxynitrite scavenging activity, on beta-amyloid-induced oxidative and/or nitrosative cell death in cultured rat pheochromocytoma (PC12) cells.

Methods: beta-Amyloid-induced oxidative and/or nitrative cell damage was assessed by measuring cytotoxicity, lipid peroxidation and the accumulation of ROS and/or RNS. The beta-amyloid- induced apoptosis was verified by characteristic morphological features, positive *in situ* terminal end-labeling, disruption of mitochondrial membrane potential and alterations in the expression of apoptotic signaling marker proteins. Activation of inducible nitric oxide synthase and production of nitric oxide were measured by Western blot analysis

and Griess assay, respectively. Gel electrophoretic mobility shift assay and immunocytochemistry were carried out to determine the activation of NF-κB, a redox sensitive transcription factor.

Results and Discussions: PC12 cells treated with beta-amyloid exhibited increased accumulation of intracellular ROS and/or RNS and underwent apoptotic death as determined by characteristic morphological alterations and positive *in situ* terminal end-labeling (TUNEL staining). beta-Amyloid treatment also led to the decreased mitochondrial membrane potential ($\triangle\Psi m$), the cleavage of poly(ADP-ribose)polymerase, an increase in the Bax/Bcl-XL ratio, and activation of c-Jun N-terminal kinase. Resveratrol attenuated beta-amyloid-induced cytotoxicity, apoptotic features, and intracellular ROI accumulation. Likewise, EGT pretreatment exhibited a protective effect against beta-amyloid-induced cytotoxicity, apoptosis and lipid peroxidation in PC12 cells, which appeared to be attributable to abrogation of peroxynitrite production. beta-Amyloid transiently induced activation of NF-κB in PC12 cells, which was suppressed by resveratrol or EGT pretreatment. These results suggest that resveratrol and EGT could modulate oxidative and/or nitrosative neuronal cell death caused by beta-amyloid, and may have preventive or therapeutic potential in the management of Alzheimer's disease.

References:

1) Jang J.-H. and Surh Y.-J. (2001) Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells. *Mutat. Res.* 496: 181-190.

2) Jang J.-H. and Surh Y.-J. (2002) beta-Amyloid induces oxidative DNA damage and cell death through activation of c-Jun N terminal kinase. *Ann. N. Y. Acad. Sci.* 973: 228-236.

3) Jang J.-H. and Surh Y.-J. (2003) Protective effect of resveratrol on beta-amyloid-induced oxidative PC12 cell death. *Free Radic. Biol. Med.* 34: 1100-1110.