

formation and remodeling are an important turning point in that they can act like estrogen by binding on estrogen receptors on target cell surface. We, therefore, believed that isoflavones may be applied in estrogen deficiency disease such as osteoporosis in terms of estrogen replacement therapy (ERT). As commonly known, osteoporosis is one of hormonal deficiency diseases, especially in menopausal women. When estrogen is no more produced in the body itself, a remarkable bone remodeling is occurred, and the events are regulated by growth factors in osteoblast lineage. In the present study, we investigated the effect of isoflavones (Isoval) extracted from *Sophorae Fructus* on growth factors, IGF-I and TGF- β related with bone formation in vitro. From the study, we found that the active control (PIII) effectively enhanced the level of nitric oxide, growth factors, and finally inhibited osteoclastogenesis. The most efficient concentration was observed at 10⁻⁸% for three to five days, whereas comparative control (soybean isoflavone) was not effective in lower concentration. In conclusion, the product which contained enriched glucosidic isoflavone and nutrient supplements such as shark cartilage and calcium can be used for treatment of osteoporosis by its role of enhancing the production of IGF-I and TGF- β , and nitric oxide produced through eNOS may play a role in inhibiting bone resorption.

[PC1-1] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Cooper ions and hypochlorite are mainly responsible for oxidative inactivation of paraoxon-hydrolyzing activity in human high density lipoprotein

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Paraoxonase, an antioxidant enzyme, exclusively located on HDL is well known for both hydrolysis of organophosphate and prevention against LDL oxidation. It have been reported that PON1 decrease its activity under oxidative stress and that PON1 activity is lower in subject with higher vulnerability to organophosphate poisoning. The aim of our study is to examine the effect of oxidative system on paraoxon-hydrolyzing activity and to elucidate the plausible mechanisms responsible for the decline of HDL-associated PON1 activity in vivo system. Of various oxidative systems, Ascorbate/Cu²⁺ was found to be the most potent in inactivating the paraoxon-hydrolyzing activity of purified PON1 as well as HDL-associated PON1. The inactivation of PON1 is protected by catalase but not other hydroxyl scavengers, supporting the important role of Cu²⁺ in catalyzing oxidative inactivation. In addition, several lipids including oleic acid and phosphatidyl dioleoyl glycerol also expressed partial protection. Noteworthy, Cu²⁺ cause HDL-associated PON1, but not purified PON, inactivation in a concentration- dependent manner, indicating that there may be an reducing component on HDL which facilitate the inactivation of Cu²⁺. Separately, PON1 both purified and HDL-associated form was also observed to be susceptible to HOCl. It is of interest that while susceptibility to hypochlorite (<1 mM) was similar between purified PON1 and HDL-associated PON1 the inactivation by hypochlorite at higher concentration seemed to be interfered by the membrane. Moreover, Ascorbate/Cu²⁺ in concert with HOCl exhibited the cooperative effect in inactivating HDL-associated PON1 (maximum 73%). On the basis of these result, it is suggested that metal-catalysed oxidation and HOCl-generating system is likely to be responsible for the reduction of HDL-associated PON1 activity under oxidative stress.

[PC1-2] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

ERGOPTHIONEINE RESCUES PC12 CELLS FROM BETA-AMYLOID-INDUCED APOPTOTIC DEATH

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beta-Amyloid (A β) peptide is the major component of senile plaques and considered to have a causal role in the development and progression of Alzheimer's disease. There has been compelling evidence supporting that A β -induced cytotoxicity is mediated through oxidative and/or nitrosative stress. Recently, considerable attention has been focused on dietary manipulation of oxidative and/or nitrosative damage. L-Ergothioneine (EGT) is a low-molecular weight naturally occurring thiol compound of dietary origin which exists in milimolar concentrations in

the brain, liver, kidney, erythrocytes, ocular tissues and in seminal fluids of mammals. This water soluble antioxidant has ability to scavenge hydroxyl and peroxynitrite radicals as well as activated oxygen species, such as singlet oxygen. In the present study, we investigated the effect of EGT on A β -induced oxidative and/or nitrosative cell death. Rat pheochromocytoma (PC12) cells treated with A β underwent apoptotic death as determined by positive in situ terminal end-labelling (TUNEL staining), decreased mitochondrial transmembrane potential ($\Delta\Psi_m$), an increased ratio of proapoptotic Bax to antiapoptotic Bcl-X_L, elevated caspase-3 activity and the cleavage of poly(ADP-ribose)polymerase. EGT pretreatment attenuated A β -induced apoptosis in PC12 cells. As compared to N-acetyl-L-cysteine that mainly scavenges reactive oxygen species, EGT effectively inhibited A β -induced cell death by suppressing peroxynitrite formation and subsequent nitration of protein tyrosine residues. The effects of EGT on the cytotoxicity induced by the nitric oxide donor sodium nitroprusside (SNP) and the peroxynitrite-generating 3-morpholinopyridone chlorhydrate (SIN-1) were compared. While EGT significantly protected against SIN-1-mediated cell death, it barely affected the cytotoxicity induced by SNP. These results suggest that EGT attenuates apoptosis caused by A β , preferentially by eliminating peroxynitrite derived from the neurotoxic peptide.

[PC1-3] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Counterion-dye staining method for DNA in agarose gels using indoine blue and methyl orange

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Sensitive and safe method for visualization of DNA in agarose gels using visible dye is described. To improve the sensitivity, we studied a counterion-dye staining method using methyl orange as a counterion-dye which contributes to reduce excessive background staining by indoine blue. Dye concentrations, pH of staining solution, mixing molar ratio of two dyes, and staining times were optimized for the counterion-dye staining. By the staining with a mixed solution of 0.005% indoine blue and 0.00165% methyl orange in 10% ethanol 0.2M sodium acetate, 8 ng of the 3 kb DNA in an agarose gel was detected within 1hr. The detection limit is 2 times lower than that of ethidium bromide.

[PC1-4] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Expression of osteopontin and this role in hepatic stellate cell motility and wound healing migration

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The activation of the hepatic stellate cell (HSC) is a key step in liver fibrogenesis. We investigated the changes of global gene expression during activation in hepatic stellate cells using a rat cDNA microarray with 5,000 sequence-verified clones. We identified osteopontin (OPN), a secreted matrix protein, as one of the upregulated factors. Northern analysis showed OPN mRNA was increasingly expressed during progressive activation of cultured rat HSCs and in models of experimental liver fibrosis. RT-PCR showed this mRNA was increased in human cirrhosis livers compared with normal liver. Incubation of primary HSC with recombinant OPN induced a significant migratory and proliferative effect. Anti-OPN antibody inhibited HSC migration induced by fetal bovine serum in wound healing assays. These findings provide the characterization of OPN expression and of this role in HSC migration, a key event in liver tissue wound healing and fibrogenesis.

[PC1-5] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Time-dependent Degradation of Polyphenol Oxidase in Perilla Frutescens Leaves

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