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 Aβ-induced oxidative and/or nitrosative cell death. Rat pheochromocytoma (PC12) cells treated with Aβ 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 Aβ-induced apoptosis in PC12 cells. As compared to N-aceryl-L-cysteine that mainly scavenges reactive oxygen species, EGT effectively inhibited Aβ–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-morpholinosydnonimine 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 Aβ, 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 agarosegels using indoine blue and methyl orange

Hwang Sun-Young , Jin Li-Tai , Yoo Gyurng-Soo, Choi Jung-Kap
College of Pharmacy, Chonnam National University, Kwangju 500-757, South Korea, .

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

Lee Sung Hee°, Zhao Yu-zhe, Park Eun-jeon, Song Hye Min, Sohn Dong Hwan
Department of Pharmacy, Wonkwang University

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 Fructescens Leaves

An Keun Kim, Yoo Kyung Kim°

Sookmyung Women's University, *Seoul Metropolitan Government Research Institute of Public Health & Environment and Sookmyung Women's University*

Time-dependent PPO activity was determined at 4℃ and 30℃. The result of activity determination, PPO extracted by phosphate buffer containing triton x-114(tPPO) was more stable than PPO by phosphate buffer(bPPO). The result of electrophoresis, at first a band was appeared at 48kd. After 1-3days a partial degrade band was appeared in bPPO and three partial degrade bands in tPPO. No activity band was appeared in PPOs at 30℃ and bPPO at 4℃ after 4 days. Two degrade bands (39kd and 37kd) in tPPO were remained after 30 days at 4℃. The result of activity and electrophoresis, detergent like triton x-114 was important for stability of PPO.

[PC1-6] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Proteomic analysis of nitrated and HNE-adducted proteins in the aging process

Kim Chul Hong, Zou Yani, Jung An Sung, Chung Hae Young
Pusan Bioinformatics & Biocomplexity Research Center, Pusan National Univsersity, Korea, College of Pharmacy, Pusan National Univsersity, Korea

Peroxynitrite and 4-hydroxynonenal (4-HNE) are highly reactive molecules which are generated under oxidative stress condition and during aging. Many proteins in living organism are modified by them and consequently associated with various diseases including cardiovascular and neurodegenerative diseases. We hypothesize that peroxynitrite and 4-HNE modified serum proteins are also associated with aging process. To establish information on peroxynitrite and 4-HNE adducted proteins for aging study, we used proteomic methods, 2D-PAGE and MALDI-TOF MS, to identify modified proteins from young (7-month) and old (25-month) rat serum. As a result of immunodetection, levels of nitrotyrosine, HNE-histidine, and free HNE were increased in old rat serum. Also, we identified 16 immunopositive proteins like alpha-1-macroglobulin, apolipoprotein H, albumin, prothrombin, transferrin, T-kininogen I, and haptoglobin from young and old 2D-gels. Among of nitrated proteins, Alpha-1-inhibitor III and inter-alpha-inhibitor H4 heavy were shown in young rat serum, but T-kininogen I and alpha-1-antiproteinase were observed in old rat serum. In HNE-adducted proteins, T-kininogen I, apolipoprotein E, and haptoglobin were shown in old rat serum. Moreover, some proteins were double modified by both 4-HNE and peroxynitrite. These modified proteins are involved in homeostasis, transport, regulation of proteolysis and peptidolysis, and acute-phase responses. Our data indicate dysfunction of serum proteins through 4-HNE adduction and nitration, which may be associated with aging-related vascular diseases via endothelial cell damage and contribute to vascular aging and aging process.

[PC1-7] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Celecoxib Attenuates Nitric Oxide-Induced Apoptosis in PC12 Cells by Inhibiting AP-1 Activation and COX-2 Expression.

Li Meihua, Jang Junghee, Surh Youngjoon
College of pharmacy, Seoul National University

Recent studies suggest that inflammatory events are implicated in a variaty of ailments such as cancer and neurodegenerative diseases, and certain non-steroidal anti-inflammatory drugs have beneficial effects for the treatment or prevention of these disorders. Cyclooxygenase-2 (COX-2), the rate-limiting enzyme in the prostaglandin (PG) synthesis, is induced by various pro-inflammatory stimuli including nitric oxide (NO) and has been reported to cause and/or aggravate neuronal cell death. In this study, we have investigated the possible protective effect of celecoxib, a selective COX-2 inhibitor, against inflammatory cell death induced by the NO releasing compound sodium nitroprusside (SNP) in cultured rat pheochromocytoma (PC12) cells. PC12 cells treated with SNP underwent apoptotic cell death as revealed by cleavage of poly(ADP-ribose)polymerase, decreased mitochondrial membrane potential (△Ym), an increased Bax/Bcl-XL ratio and internucleosomal DNA