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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

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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.

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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

fragmentation. SNP treatment also led to the depletion of intracellular GSH and lipid peroxidation. In addition, SNP caused elevated COX-2 expression and PGE2 production, which was accompanied by AP-1 activation. Pretreatment with celecoxib rescued PC12 cells from apoptotic death, nitrosative stress and repressed COX-2 expression and subsequent PGE2 production. Interestingly, both DNA binding and transcriptional activities of AP-1 induced by SNP were blocked by celecoxib. These results suggest that activated AP-1 mediates SNP- induced COX-2 expression, subsequent PGE2 production, and apoptosis. Additional studies are in progress to determine whether attenuation of SNP-induced nitrosative PC12 cell death by celecoxib is associated with its inhibition of AP-1 activation and COX-2 expression.

[PC1-8] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Requirement of PI3K-PKCε Signaling Pathway for Apicidin Induction of $p21^{WAF1/Cip1}$

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We previously reported that the activation of $p21^{WAF1/Cip1}$ transcription by histone deacetylase inhibitor apicidin was mediated through Sp1 sites and pointed to the possible participation of protein kinase C (PKC). In this study, we investigated the role and identity of the specific isoforms of PKC involved and identified phosphatidylinositol 3-kinase (PI 3-kinase) as an upstream effector in HeLa cells. Using an isoform-specific pharmacological inhibitor of PKC, a PKCε dominant-negative mutant, and antisense oligonucleotide to inhibit PKCε specifically, we found that among PKC isoforms, PKCε was required for the $p21^{WAF1/Cip1}$ expression by apicidin. In addition to PKCε, PI 3-kinase appeared to participate in the activation of $p21^{WAF1/Cip1}$ promoter by apicidin, since inactivation of PI 3-kinase either by transient expression of dominant negative mutant of PI 3-kinase or its specific inhibitors, LY294002 and wortmannin, attenuated the activation of $p21^{WAF1/Cip1}$ promoter and $p21^{WAF1/Cip1}$ protein expression by apicidin. Furthermore, membrane translocation of PKCε in response to apicidin was blocked by the PI 3-kinase inhibitor, indicating the role of PI 3-kinase as an upstream molecule of PKCε in the $p21^{WAF1/Cip1}$ promoter activation by apicidin. However, the $p21^{WAF1/Cip1}$ expression by apicidin appeared to be independent of the histone hyperacetylation, since apicidin-induced histone hyperacetylation of $p21^{WAF1/Cip1}$ promoter region was not affected by inhibition of PI 3-kinase and PKC, suggesting that the chromatin remodeling through the histone hyperacetylation alone might not be sufficient for the expression of $p21^{WAF1/Cip1}$ by apicidin. Taken together, these results suggest that the PI 3-kinase–PKCε signaling pathway plays a pivotal role in the expression of the $p21^{WAF1/Cip1}$ by apicidin.

[PC1-9] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Antioxidant effect of flavonoid, myricetin with GSH, vitamin E, vitamin C on B16F10, murine melanoma cell

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Reactive Oxygen Species (ROS) are produced during normal cellular function. ROS are very transient species due to their high chemical reactivity that leads to lipid peroxidation and oxidation of some enzyme, massive protein oxidation and degradation. Under normal conditions, antioxidant are substances that either directly or indirectly protect cells against adverse effects of ROS. Several biologically important compounds have been reported to have antioxidant functions. These incluce vitamin C, vitamin E, GSH, flavonoids. superoxidee dismutase(SOD), glutathione peroxidase(GPX) and catalase(CAT). The various antioxidant either scavange superoxide and free radicals or stimulate the detoxification mechanisms within cells resulting in increased detoxification of free radicals formation and thus in prevention of many pathophysiologic processes. This study carried out to investigate the antioxidant activity of flavonoids, myricetin and (+)-catechin with other antioxidants, GSH, vitamin E and vitamin C on B16F10. In order to investigate the efficacy of antioxidant activity, we mesured antioxidant enzyme activity(SOD, GPX, CAT), RT-PCR and intracellular reactive oxygen intermediate. In this