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

results, we show that these flavanoids with other antioxidant substrates are incresed antioxidant activity level.

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

Antioxidant effect of chitosan in the renal failure

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Oxidative stress has been implicated in a range of disease states, including end-stage renal failure treated with hemodialysis [Westhuyzen J. et al, 2003]. Free radicals react with biological molecules and destroy the structure of cells, which eventually causes free-radical induced disease such as cancer, renal failure, aging, etc. Exogenous or endogenously produced nitric oxide (NO) inhibits superoxide-stimulated urea permeability. In the inner medulla, superoxide generation by local oxidases may stimulate urea transport, and the role of endogenous NO may be to dampen this effect by decreasing superoxide levels [Zimpelmann J. et al, 2003 (Epub ahead of print)]. Xie W. et al (2001) reported that water-soluble chitosan derivatives had antioxidant activity. In the present series of experiments, we studied the amount of NO (nmol/mg protein) and the activity of superoxide dismutase (SOD, units/mg protein) in mouse kidney (in vivo) and porcine proximal tubules (in vitro) from normal and $HgCl_2$-induced renal failure models. In vitro, the amount of NO and the activity of SOD were increased in a concentration dependent manner (0.025, 0.05, 0.075, and 0.1%) of chitosan only or with $HgCl_2$ (20μM). The production of NO by $HgCl_2$ (0.715±0.343) increased more than normal (0.525±0.192). But, $HgCl_2$ did not affect on the activity of SOD significantly. In vivo, the amount of NO and the activity of SOD were changed each time (24, 48, and 72 hour) after injection of $HgCl_2$ (1㎎/㎏); normal (NO: 0.104±0.069, 0.083±0.049, and 0.093±0.067; SOD: 6.366±2.011, 5.421±1.925, and 6.232±1.593), only $HgCl_2$ (NO: 0.145±0.048, 0.183±0.023, and 0.176±0.053; SOD: 7.017±1.203, 6.525±0.990, and 6.211±1.698), chitosan with $HgCl_2$ (NO: 0.209±0.074, 0.154±0.052, and 0.113±0.059; SOD: 9.964±0.824, 8.611±1.224, and 6.835±1.431). These data suggest that (a) $HgCl_2$ does not affect the activity of SOD and (b) chitosan may help to overcome oxidative stress caused by superoxide in the renal failure.

[PC1-11] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Dye-silver double staining method for proteins in SDS-polyacrylamide gels using a dye as a silver sensitizer

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We have developed a silver staining method using a dye as a silver sensitizer. Dye staining is performed in combination with silver nitrate staining. Dye-silver staining shortens the time of silver staining (~1 hr) and improves the sensitivity better than that of silver diamine stain (1-10 ng) or comparable to that of silver nitrate stain with glutaraldehyde as a silver sensitizer. In dye staining (silver sensitizing step), it has been proven that the sensitivity is at least 4 times comparing with that of CBBR stain and the staining time is about 45 min. It is convenient to succeed in silver staining following dye stain. The dye, an azo compound having polycyclic aromatic sulfonic acid, binds to both protein and silver ion. Sulfonic acid group of dye and chelation with silver produces binding sites for silver ion and so enhances silver nucleation. In addition, azo group of dye may contribute to silver ion reduction as a silver sensitizer when azo group breaks down to N2 evolution in alkaline solution. These can enhance the sensitivity of the dye-silver staining up to 0.1-1 ng. This staining method can be applied to detect for the trace amount of protein in 1D and 2D-PAGE and compatible to MALDI-TOF MS (silver nitrate with glutaraldehyde is not MALDI-TOF MS compatible) for proteom research.

[PC1-12] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Agrobacterium-mediated transformation of Eleutherococcus senticosus with the