role in the pathogenesis or hepatic fibrosis. In this study, we investigated apoptosis stimulation by baicalein in activated rat hepatic stellate cells (T-HSC/Cl-6). Transformed rat hepatic stellate cells (T-HSC/Cl-6) were treated with baicalein(100uM, 70uM, 40uM). Apoptosis was determined by detection of DNA fragmentation in gel electrophoresis, morphological alternations by flow cytometry and quantification of phosphatidylserine externalization by Annexin V labeling. Activation of caspase-3, caspase-9 and cytochrome c release and the proteolytic cleavage of poly(ADP-ribose) polymerase in a concentration-dependent manner. In conclusion, results above demonstrated that baicalein stimulates apoptosis via Caspase-3, caspase-9 activation and release of cytochrome C in T-HSC/Cl-6.

**[PA3-1] [ 2003-10-11 09:00 - 12:30 / Grand Ballroom Pre-function ]**

## Identification of a novel $Ca^{2+}$-independent Phospholipase $A_2$ in Bovine Brain

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Phospholipase $A_2$($PLA_2$) catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate arachidonic acid(AA), a precursor of eicosanoids including prostaglandins(PGs) and leukotrienes (LTs). The same reaction also produces lyso-phospholipids. So far, at least 19 enzymes that possess PLA2 activity have been identified, consists of low-molecular-weight, $Ca^{2+}$-requiring, secretory enzymes that have been implicated in a number of biological processes, such as modification of eicosanoid generation, inflammation, host defense, and atherosclerosis. The cytosolic $PLA_2$ ($cPLA_2$) family(Group IV) consists of 3 enzymes, among which $cPLA_2\alpha$ plays an essential role in the initiation of AA metabolism. Intracellular activation of $cPLA_2\alpha$ is tightly regulated by $Ca^{2+}$ and phospholylation. The $Ca^{2+}$-independent $PLA_2$($iPLA_2$) family(Group VI) contains 2 enzymes and may play a major role in membrane phospholipid remodeling and apoptosis. Recently, we detected an $iPLA_2$ activity in 10,000g supernatant in bovine brain homogenates. This brain form of $iPLA_2$ was purified by sequential use of pH 5.0-extraction, and DEAE-Cellulose anion exchange, Phenyl-5PW hydrophobic, Heparin-Sepharose affinity, Sephacryl S-300 gel filtration, Mono S cation exchange, Mono Q anion exchange, Superose 12 gel filtration column chromatographies. The enzyme activity eluted as the highest peak at an apparent molecular mass of 150~200kDa on a superose 12 gel filtration column. The active fraction from Superose 12 gel filtration column as a final step migrated as a single spot of a molecular mass of 156kDa and isoelectric point of 5.3 on two dimensional electrophoresis. And the 156kDa protein was proved as a novel protein through MALDI-TOF analysis and data base search of peptide profiles. Our purified $iPLA_2$ was insensitive to boromoenol lactone(BEL) and ATP but inhibited trifluoromethyl-arachidonyl ketone(AACOCF3), Triton X-100 , iron, and $Ca^{2+}$.

**[PA3-2] [ 2003-10-11 09:00 - 12:30 / Grand Ballroom Pre-function ]**

## Comparative Study of the Inhibitory Effect of Luteolin and Luteolin-7-Glucoside on Rat Aortic Vascular Smooth Muscle Cell Proliferation

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It has been previously reported that luteolin and luteolin-7-glucoside displayed the potent anti-oxidant and anti-inflammatory effects, which have also been successful in reducing vascular smooth muscle cells(VSMCs) proliferation. In this study, a possible anti-proliferative effect and its mechanism on rat aortic VSMCs by luteolin and luteolin-7-glucoside were investigated. Luteolin significantly inhibited the platelet-derived growth factor(PDGF)-BB-induced proliferation of rat aortic VSMCs. While luteolin-7-glucoside weakly inhibited the proliferation. In order to elucidate the anti-proliferative mechanism, we examined the effects of luteolin and luteolin-7-glucoside on the PDGF-BB-induced activation of PDGF-Rβ by western blot in cultured VSMCs. Pre-treatment of VSMCs with luteolin resulted in a significant inhibition of the PDGF-BB-induced phosphorylation of PDGF-Rβ. Downstream of PDGF-Rβ such as extracellular signal-regulated kinase 1/2 (ERK1/2), phospholipase

C-γ1 (PLC-γ1) and Akt cascade were also inhibited by luteolin. Luteolin-7-glucoside showed weak inhibition of PDGF-Rβ, ERK1/2, PLC-γ1 and Akt cascade. The anti-proliferative effect of luteolin was reduced by the presence of a glucose. Taken together, these results suggest that the inhibition of vascular smooth muscle cell proliferation by luteolin and luteolin-7-glucoside may be mediated mainly by inhibition of PDGF-β receptor, which leads to the inhibition of downstream such as ERK1/2, PLC-γ1 and Akt cascade.

**[PA3-3] [ 2003-10-11 09:00 - 12:30 / Grand Ballroom Pre-function ]**

## Determination of Sulfur Dioxide in Herb Drugs

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This study was carried out to investigate sulfur dioxide in herb drugs.(Puerariae Radix, Lycii Fructus, Platycodi Radix, Dioscoreae Rhizoma, Mori Radicis Cortex, Nelumbo Seed, Paeoniae Radix, Remotiflori Radix, Astragali Radix, Polygonatum Rhizome) Experimental subjects included 105 cases of 10 species of herb drugs collected in Kyung-dong market from December in 2001 to February in 2002. Sulfur dioxide was determined by Optimized Monier-Williams method. In 65 cases(61.9%) of the 105 cases, we detected $SO_2$ over 10mg/kg. Sulfites were detected in 10 cases(100.0%) of Dioscoreae Rhizoma, 10 cases(100.0%) of Paeoniae Radix, 8 cases(72.7%) of Mori Radicis Cortex, 7 cases(70.0%) of Platycodi Radix, and 7 cases(70.0%) of Polygonatum Rhizome. In domestic 29 cases of 60 cases(48.3) were detected over 10mg/kg. Otherwise, 36 cases(80.0%) were in imported samples.

**[PA3-4] [ 2003-10-11 09:00 - 12:30 / Grand Ballroom Pre-function ]**

## Heat Shock Protein 60 Is a $Mg^{2+}$-dependent, Membrane-associated and Neutral Sphingomyelinase That Mediates TNF-alpha Signaling

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The hydrolysis of sphingomyelin (SM), known as the SM pathway, is induced by the activation of sphingomyelinase (SMase) to generate the second messenger ceramide, which plays a key role in cellular responses such as apoptosis, differentiation, senescence, and inflammation. Here, we identified a 60 kDa membrane-associated, neutral and $Mg^{2+}$-dependent SMase, termed N-SMase ε, from mammalian brains, which was revealed as the heat shock protein 60 (HSP60) through cDNA cloning and mass spectrometrical analysis. This finding was further confirmed by using anti-HSP60 antibodies. Hsp60 gene transfection of human neuroblastoma cells produced a significant increase in N-SMase activity as well as a increase in protein levels. And both Hsp60-antisense and siRNA reduced significantly SMase activity in HEK293 cells. Also Hsp60-siRNA block ceramide generation by serum deprivation in HEK293 cells. Immunoblotting analysis showed that N-SMase ε exists as a majority in brain and localizes in cortical neurons. TNF-α stably enhanced the enzyme activity up to ~2-fold with parallel up-regulation of N-SMase ε, biphasic increase in ceramide and concomitant apoptosis in cortical neuron cells. Thus, HSP60 as N-SMase ε mediates TNF-α-induced neuronal apoptosis through the production of ceramide.

**[PA3-5] [ 2003-10-11 09:00 - 12:30 / Grand Ballroom Pre-function ]**

## Saponin isolated from Platycodon grandiflorum induces cell cycle arrest in hepatic stellate cells

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