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