

To satisfy the increasing medical demanding especially for sever burn patients to regenerate full thickness wound cure, this study developed dermis with gelatin based scaffold and perform the biocompatibility tests. To prepare scaffold 30% of gelatin was mixed with sieved salt and dried in the mold to shape then, cross linked with a water-soluble cross-linker, EDAC. Preparing the cell for seeding from a rabbit skin, the fibroblast and keratinocyte were successfully isolated and cultured in vitro. After cell and scaffold were ready, the fibroblast was seeded to the scaffold ($\sim 10^6$ cell/cm²) for preparing dermis and keratinocyte was cultured until forming the sheet. As a result for identity test for cells the morphology in the inverted microscope and histochemical staining were used and typical shape of each cell were shown. As the characteristics of scaffold the water uptake rate was $18,900 \pm 800\%$ and the SEM image showed the porosity $70.09 \pm 0.9\%$ and the bio-safety test include sterility showed no contamination. The growth rate of fibroblast was checked from passage 0 to 3 and the result showed very similar pattern of growth at each passage. The histochemical result of seeded cell's attachment, proliferation and distribution was confirmed from day 1 through 15. As the biocompatibility tests for the scaffold and cell seeded one, in vitro cell cytotoxicity with various cells include L929, skin irritation test, intracutaneous reactivity test and sensitization test were performed and no significant reaction was demonstrated. With the in vitro cultured keratinocyte sheet and dermis we developed, the attachment agent fibrin will be adjusted and full thickness artificial skin will tested with transplantation. As far the salt leaching method is very useful in developing artificial skin and gelatin scaffold is very promising material for the skin cell attachment and growth and both scaffold and artificial dermis is biocompatible and bio-safe.

[PA2-5] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Honokiol induces apoptosis in activated rat hepatic stellate cells via cytochrome c release and caspase activation

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The therapeutic goal in liver fibrosis is to reverse fibrosis and selective clearance of activated hepatic stellate cells (HSCs), which play a central role in liver fibrogenesis, by apoptosis might be essential during resolution of fibrosis. Past several years we screened for natural products which mediate apoptosis in activated HSCs, and among the candidates honokiol, isolated from Magnoliae Cortex, was found to induce apoptotic death in activated rat HSCs in a dose- and time-dependent manner at the concentration between 12.5 microM and 50 microM. 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 and -9, and the proteolytic cleavage of poly(ADP-ribose) polymerase were found during apoptosis induced by honokiol. In addition, pan-caspase, selective caspase-3 and selective caspase-9 inhibitors but not selective caspase-8 inhibitor blocked honokiol-induced apoptosis. Honokiol induced the reduction of mitochondrial transmembrane potential and the release of cytochrome c into cytoplasm. And honokiol also down-regulated bcl-2 protein. Take together, our findings indicate that honokiol mediates apoptosis in activated rat HSCs through mitochondria alternations to caspase-9 and that then the downstream effector caspases are activated sequentially.

[PA2-6] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Baicalein induced Apoptosis of Rat Hepatic Stellate Cells

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Baicalein (5,6,7-trihydroxyflavone), a flavonoid originated from the root of Chinese medicinal herb *Scutellaria baicalensis*, has been shown to exert anti-inflammatory and antioxidant effects and hepatic stellate cells play an important

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 Ca²⁺-independent Phospholipase A₂ in Bovine Brain

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Phospholipase A₂(PLA₂) 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, Ca²⁺-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 PLA₂ (cPLA₂) family(Group IV) consists of 3 enzymes, among which cPLA₂ α plays an essential role in the initiation of AA metabolism. Intracellular activation of cPLA₂ α is tightly regulated by Ca²⁺ and phosphorylation. The Ca²⁺-independent PLA₂(iPLA₂) family(Group VI) contains 2 enzymes and may play a major role in membrane phospholipid remodeling and apoptosis. Recently, we detected an iPLA₂ activity in 10,000g supernatant in bovine brain homogenates. This brain form of iPLA₂ 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 iPLA₂ was insensitive to boromoenol lactone(BEL) and ATP but inhibited trifluoromethyl-arachidonyl ketone(AACOCF₃), Triton X-100, iron, and Ca²⁺.

[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-R β by western blot in cultured VSMCs. Pretreatment of VSMCs with luteolin resulted in a significant inhibition of the PDGF-BB-induced phosphorylation of PDGF-R β . Downstream of PDGF-R β such as extracellular signal-regulated kinase 1/2 (ERK1/2), phospholipase