This study was carried out to investigate the protective effect of honokiol and magnolol on tert.-butyl hydroperoxide (tBH) or D-galactosamine (GalN)-induced injury in primary cultures of rat hepatocytes. The cellular leakage of LDH and AST by 1 hour treatment of 1.5 mM tBH were significantly inhibited by treatment of honokiol (40 and 20 μM) or magnolol(40 μM). Hepatocyte death induced by tBH was significantly inhibited by treatment of honokiol (40 and 20 μM) or magnolol (40 μM). Treatment with honokiol or magnolol significantly inhibited lipid peroxidation in cells and in medium, generation of intracellular reactive oxygen species (ROS), and intracellular glutathione (GSH) depletion induced by tBH in primary cultured hepatocytes. In GalN-inhuced hepatocyte injury, the cellular leakage of LDH and AST by 24 hour treatment of 30 mM GalN were significantly inhibited by treatment of honokiol (20, 5, 1 and 0.2 μM) or magnolol (20, 5, 1 and 0.2 μM). Hepatocyte death was also significantly inhibited by treatment of honokiol or magnolol. Treatment with honokiol (20, 5 and 1 μM) or magnolol (20 and 5 μM) significantly inhibited the intracellular GSH depletion induced by GalN in primary cultured hepatocytes. These hepatoprotective effects of honokiol and magnolol on oxidative stress induced by tBH were probably via their antioxidant activity such as their ability of reducing intracellular ROS generation, preserving intracellular antioxidant defense system as shown by GSH preservation and inhibiting lipid peroxidation. Honokiol and magnolol also had protective effect on GalN-induced hepatotoxicity which model we used in this study as other than oxidative stress via inhibiting intracellular GSH depletion.

[PE1-3] [04/19/2002 (Fri) 10:00 - 13:00 / Hall E]

Nasal Stability of PEGylated Salmon Calcitonins

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The aim of this study was to evaluate the stabilization effect by PEGylation of salmon calcitonin (sCT) in nasal enzyme systems. It was investigated and compared to native sCT that the enzymatic degradation of positional isomers of mono-PEGylated sCT (mono-PEG-sCT) in rabbit nasal membrane homogenate and cultured human nasal epithelial cell (RPMI 2650) components. The three different positional isomers of mono-conjugated sCT with SP-mPEG (M.W. 2000), e.g., N-terminus-, Lys11-, Lys18- were directly separated by using reverse-phase HPLC and characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Each sample was mixed with pre-incubated nasal membrane homogenate and RPMI 2650 cell components at 37°C, after various time intervals, the incubation was stopped by adding of ice-cold methanol solution. The residual amount and degradation products of each sample were quantified by HPLC and characterized by MALDI-TOF MS. The survival of mono-PEG-sCTs in nasal enzyme systems were increased so notable that the elimination rate constants of mono-PEG-sCTs were greater than that of native sCT by more 100 times. This finding suggests that the nasal application of mono-PEG-sCT would exhibit superior bioavailability and enhanced subsequent therapeutic effect.

[PE1-4] [04/19/2002 (Fri) 10:00 - 13:00 / Hall E]

Increased expression of nephroblastoma overexpressed gene in activated hepatic stellate cells

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The activation of the hepatic stellate cell (HSC) is a key step in liver fibrogenesis. The aim of this study is to obtain a deeper understanding of the molecular mechanisms of HSC activation. Utilizing large scale sequencing of a 3'-directed cDNA library, we investigated expression profiles of quiescent and activated rat HSCs. During the activation process, nephroblastoma overexpressed gene (NOV) was identified as one of the significant upregulated factors. Upregulation of NOV in cultured HSCs was confirmed by northern blot. NOV expression in models of experimental fibrosis and fibrotic human livers were investigated at the mRNA level using reverse transcription-polymerase chain reaction.
In cultured HSC, a striking induction of NOV expression was observed after dexamethasone treatment and occurred in a time-dependent manner.

In conclusion, this study shows that NOV is strongly expressed during liver fibrogenesis, and hepatic stellate cells seems to be the major cellular sources of NOV in the liver.

Poster Presentations - Field E2. Pharmacokinetics

[PE2-1] [04/19/2002 (Fri) 10:00 - 13:00 / Hall E]

Quantitification of costunolide in rat plasma by HPLC

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Simple and precise high-performance liquid chromatographic (HPLC) assay was developed and validated for the determination of a sesquiterpenelactone material, costunolide in rat plasma. The method involved liquid liquid extraction of costunolide and internal standard partenolide. Samples were analyzed by reversed-phase HPLC using Capcell-Pak C18 column with ultraviolet detection at 230nm. The quantitation limit of costunolide was 0.05ug/ml and the calibration curve was linear over the range of 1-50ug/ml (r2>0.999) with human plasma. The analytes of quality control samples indicated that the normal values could be predicted with an accuracy >97%. The intra- and inter-day coefficients of variation for the analytes were <10%. We are undergoing the invivo pharmacokinetic study using these validation method.

[PE2-2] [04/19/2002 (Fri) 10:00 - 13:00 / Hall E]

Determination of YH3945 in beagle dog plasma by high performance liquid chromatography: validation and longterm stability

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YH3945, a non-peptide farnesyltransferase inhibitor, is being developed by Yuhan Research Institute for the treatment of cancer. The development and validation study of a sensitive, rapid, reproducible, accurate and precise high performance liquid chromatographic (HPLC) method for YH3945 in beagle dog plasma has been carried out and the longterm stability of YH3945 in beagle dog plasma has been investigated. Plasma was extracted with acetonitrile containing the internal standard. An aliquot of the extract was injected onto a reverse C18 column. Retention times of YH3945 and the internal standard were 6.9 and 10.6 min, respectively. The chromatograms showed no endogenous peaks from blank plasma at the retention time of YH3945. Standard curves of YH3945 was linear over the range of 100 ng/ml to 10000 ng/ml (r=0.9995). The lower limit of quantification of YH3945 in plasma was 100 ng/ml. This assay also showed good inter- and intra-precision and accuracy throughout the concentration range. Precision expressed as C.V. was in the 1.2 to 4.6% range. Accuracy expressed as mean R.E. was between -0.9 and 5.5%. The extracted samples of YH3945 were stable at room temperature for 72 hours. The spiked plasma samples of YH3945 remain stable under frozen condition for 6 months, under ambient condition for 4 hours, and under a period of three freeze/thaw cycles. This sensitive, accurate and precise method can be applied to determine concentration of YH3945 in plasma for pharmacokinetic studies in beagle dogs.
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[PE2-3] [04/19/2002 (Fri) 10:00 - 13:00 / Hall E]

Pharmacokinetics and Bioequivalence of Tiropramide in Human Volunteers