[PA1-1] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Determination of bioavailability of tolperisone HCl by HPLC

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Tolperisone hydrochloride is used as a muscle relaxant. Very few assay methods of tolperisone were reported, such as potentiometry, spectrophotometry and high performance thin layer chromatography. In addition, there is no report related to HPLC method to determine the tolperisone level in biological sample. In this study, A very sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for the determination of tolperisone HCl in plasma has been developed. Tolperisone HCl was isolated from plasma by extraction with dichlormethane. The drug was separated on a C18 column (4.6 mm x 150 mm) using 0.05% 1-hexanesulfonic acid in 45/55 v/v methanol/water as mobile phase 1 ml/min and UV detection at 260 nm. The detection limits for tolperisone HCl was 1 ng/ml and the quantitation limits was 5 ng/ml. Linear calibration curves over 5-750 ng/ml of tolperisone HCl was established. The average recovery of added tolperisone HCl was above 95%. The proposed method was applied to the determination of bioavailability of a tolperisone HCl tablet in 8 volunteers

[PA1-2] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Determination of Optical Purity of a-Arylmethylpropionic acds by Normal Phase Liquid Chromatography

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A various 2-arylmethylpropionic acds(profen) have been widely used as non-steroidal anti-inflammatory drugs for the relief of acute and chronic rheumatoid arthritis and osteoarthritis, as well as for other connective tissue disorders and pains. Example is fenoprofen, ibuprofen, ketoprofen, and naproxen. All are chiral and, except for naproxen, are marketed in racemic form. Enantioseparations of profens have been of considerable interest because their anti-inflammatory and analgesic effects have been attributed almost exclusively to their (S)-enantiomer.
A simple method for determination of (+) and (-) - a-arylmethylpropionc acids has been developed . By means of EEDQ, a-arylmethylpropinic acids is coupled to (S)-napthylethylamide, a reaction which is complete in 3hr at room temperature . The diasteoisomeric derivatves are then separated by normal-phase high-performance liquid chromatography.

[PA1-3] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Phosphatidylinositol 3-Kinase Regulates Nuclear Translocation of NF-E2-Related Factor 2 through Actin Rearrangement in Response to Oxidative Stress

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Expression of phase II detoxifying genes is regulated by NF-E2-related factor 2 (Nrf2)-mediated antioxidant response element (ARE) activation. Phosphatidylinositol 3-kinase (PI3-kinase) plays an essential role in ARE-mediated rGSTA2 induction by oxidative stress and controls microfilaments and translocation of actin-associated proteins. This study was designed to investigate the PI3-kinase-mediated nuclear translocation of Nrf2 and the interaction of Nrf2 with actin. Pretreatment of the cells with PI3-kinase inhibitors (wortmannin/LY294002) prevented nuclear translocation of Nrf2 by tert-Butylhydroquinone (*t*-BHQ). *t*-BHQ relocalized Nrf2 in concert with changes in actin microfilament architecture, as visualized by confocal microscopy. Furthermore, *t*-BHQ increased the level of nuclear actin, co-immunoprecipitated with Nrf2, which returned to that of control by pretreatment with PI3-kinase inhibitors. Cytochalasin B, an actin disruptor, alone stimulated actin-mediated nuclear translocation of Nrf2 and induced rGSTA2. These results were blocked by phalloidin that stabilizes actin filaments. Subcellular fractionation and immunoblot analyses allowed us to detect both 57 kDa and 100 kDa Nrf2. Immunoprecipitation assays showed that the 100 kDa protein comprised both Nrf2 and actin. This study demonstrates that the PI3-kinase regulates rearrangement of actin in response to oxidative stress and that depolymerization of actin causes a complex of Nrf2 to translocate into nucleus.

[PA1-4] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Hydrogen Peroxide Activates ERK in Cultured Feline Ileal Smooth Muscle Cells

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H_2O_2 has been shown to act as a signaling molecule involved in many cellular functions such as oxidant-induced stress, apoptosis, proliferation. In this study, we investigated the action mechanisms of H_2O_2 on activation of Extracellular Signal-Regulated Protein Kinase(ERK) in cultured feline ileal smooth muscle cells(ISMC). Western blot analysis done with phospho-specific MAP kinases antibodies demonstrated that potent activation of ERK and moderate activation of SAPK/JNK occurred within 30 min of H_2O_2 treatment. However, p38 MAP kinase was not activated by H_2O_2. The activation of ERK by H_2O_2 was reduced by MEK inhibitor PD98059, removal of extracellular Ca^{2+}, depletion of the intracellular Ca^{2+} pool by thapsigargin, or pretreatment of ISMC with the calmodulin antagonist W-7. In addition, H_2O_2-induced ERK activation was attenuated by a tyrosine kinase inhibitor genistein, but not by downregulation of protein kinase C(PKC) with phorbol-12-myristate-13-acetate(PMA) or by a PKC inhibitor GF109203X. Further, ERK activation by H_2O_2 was blocked by pretreatment with either *N*-acetyl-cysteine, *o*-phenanthroline, or mannitol. Taken together, these data show the factors controlling MAPK activation by H_2O_2 in intestinal smooth muscle cells and suggest that ERK plays a critical role in the oxidant cell injury induced by H_2O_2.

[PA1-5] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Arachidonic Acid Liberated through Activation of $iPLA_2$ Mediates the Production of Reactive Oxygen Species and Apoptosis Induced by N-Ethylmaleimide in HepG2 Human Hepatoma Cellls

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