of cytosolic Ca^{2+} mobilization in activated platelets. In the present study, the effect of NQ301 on arachidonic acid cascade in activated platelets was examined. NQ301 concentration-dependently inhibited washed rabbit platelet aggregation induced by collagen (10 μg/ml), arachidonic acid (100 μM) and U46619 (1 μM), a thromboxane A_2 receptor agonist, with IC_{50} values of 0.60±0.02, 0.79±0.04 and 0.58±0.04 μM, respectively. NQ301 also produced a shift to the right of the concentration-effect curve of U46619, suggesting a competitive type of antagonism. NQ301 slightly but concentration-dependently inhibited collagen-induced arachidonic acid liberation. In addition, NQ301 potently suppressed thromboxane (TX) B_2 formation by platelets that were exposed to arachidonic acid in a concentration-dependent manner, but had no effect on the production of prostaglandin (PG) D_2, indicating an inhibitory effect on TXA_2 synthase. This was supported by a TXA_2 synthase activity assay that NQ301 concentration-dependently inhibited TXB_2 formation conversed from PGH_2. Moreover, NQ301 also concentration-dependently inhibited 12-hydroxy-5,8,10,14-eicosatetraenoic (12-HETE) acid formation by platelets that were exposed to arachidonic acid. Taken together, these results suggest that NQ301 has a potential to inhibit TXA_2 synthase activity with TXA_2/PGH_2 receptor blockade, and modulate arachidonic acid liberation and 12-HETE formation in platelets. This may also be one convincing mechanism for the antithrombotic actions of NQ301.

[PA3-24] [2003-10-11 09:00 - 12:30 / Grand Ballroom Pre-function]

Proteomic Analysis of Cytokine-Like Proteins Secreted from Human Bronchial Epithelial Cells in Response to Pathogenic Bacterial Infection

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Bacterial infection is a very complex process in which both pathogens and host cells play crucial roles, and the host cells undergo drastic changes in their physiology, releasing various proteins in response to the pathogenic infection. Human airway epithelial surface serves as a first line of defense against microorganisms and the external environment. It is well known that bronchial epithelial cells secrete various chemokines and cytokines such as IL-6 and IL-8 to cope with various respiratory pathogens. Although many kinds of these cytokine proteins are identified and characterized for their biological roles, such cytokine-like proteins as a functionally unknown protein could be found through high-throughput identification of the proteins in the extracellular space. In this study, the proteomics approach was employed to compare the proteins from pathogenic bacteria-infected human bronchial epithelial cells with uninfected cells and to identify the proteins that specifically secreted to the culture medium. We used a strategy that combined a high-resolution two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). At least twenty different proteins stained by Coomassie G, were identified by mass spectrometry analyses after in-gel tryptic digestion. Some of them were associated with inflammation, transcription and the other proteins were revealed as novel proteins to be functionally studied.

[PA4-1] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Quantative Analysis of dextromethorphan, Carisoprodol and their metabolites in hair by GC/MS

Yang Wonkyung°, Han Eunyoung, Lee Jaesin, Park Yonghoon, Choi Hwakyung, Lim Miae, Chung Heesun

National Institute of Scientific Investigation

Dextromethophan and carisoprodol have been abused to obtain a hallucination for longer than 10 years in Korea. Due to their seriousness of abuse liablility, recently government decided to control them as a psychotropic agents. As these are controlled, it is necessary for us to establish the analysis of these medicine and their metabolites in hair to prove the abuse of these drugs. This study is described for the determination of dextrometorphan and carisoprodol in hair. The method is applied to simultaneous quantify those drugs and

metabolites (dextromethorphan, dextrorphan, 3-methoxymorphinan, 3-hydroxymorphinan, carisoprodol and meprobamate). Analytes were extracted in 1% HCl in methanol from fine cutting hair for 16 hours . After evaporation under N2, residues were added 50ul Ethanol and were seperated on HP-5MS column during a 16 min program and identified by mass spectrometry with the SIM mode(EI-GC-MS).This method was validated recovery, linearity of calibration,within-and between-day precision, accuracy, limit of detection and quantification.. Calibration curves exhibited correlation coefficients > 0.99. Within and between-run precision were calculated at 8, 80 and 160 ng/mg in hair with coefficients of variation less than 10 %. Accuracy at the same concentrations were ±5% of target for all analytes. Recoveries at 10 and 100 ng in hair were over 90 %. After the method validation, we performed that quantatiation analysis of dextromethorphan and carisoprodol in abuser's hairs. Dextromethorphan and metabolites were quantitated 8~130ng/mg and 1~27ng/mg, respectively. Carisoprodol and meprobamate were also quantitated 6~33ng/mg and 39 ~304 ng/mg. We present a validated, sensitive and specific GC-MS method to simultaneously quantify dextromethorphan, carisoprodol and metabolites in hair.

[PA4-2] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Determination of MDMA and MDA in 44 hair samples during 2002 to 2003

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The qualitative and quantitative analysis of MDMA and MDA in hair samples by GC/MS were reported. Hairs were collected from subjects aged 22-37 years, who were suspected of abusing MDMA. After washing with methanol, hair samples were cut into small pieces, extracted with methanol containing 1 percent hydrochloric acid for 20h, and the solution was evaporated. MDMA or MDA in the extract were determined by GC/MS using selected ion monitoring after derivatization with trifluoroacetic anhydride. During 2002 to 2003, 791 hair samples submitted from the police were analyzed for the determination of abused drug in this institute. Among them, MDMA and its metabolites, MDA, were simultaneously detected in 40 samples by GC/MS whereas in the 4 samples, MDA only was found. Of these 44 subjects, 35 were negative for both MDMA and MDA in urine, while 9 were positive. We also evaluated concentrations of MDMA and MDA, and metabolite to parent drug ratio. This study proved that the abuse of MDMA and MDA is prevalent among young people in Korea. In addition, MDMA seemed to be more abused than methamphetamine to younger people.

[PA4-3] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Effect of B-ring –OH numbers of 5,7-dihydroxyflavone on the activity of CYP 1 enzymes

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CYP1 enzymes, CYP1A1, CYP1A2 and CYP1B1, are known to bioactivate procarcinogens particularly polyaromatic compounds. Flavonoids are a class of natural compounds that are present in edible plants. Structurally, these compounds are polyphenols with two aromatic rings (A, B) and a heterocycyclic ring (C). We observed the differential inhibition of 5,7-dihydroxyflavones which are different in numbers of B-ring –OH, to the activity of ethoxyresorufin O-deethylase (EROD) in human hepatic microsomes with the IC50 values, ie, 0.57 mM, 1.28 mM, and 3.62 mM, chrysin, apigenin, and Luteolin, respectively. Thus, the effect of B-ring -OH numbers of 5,7-dihydroxyflavone on the activity of CYP enzymes was observed in this study. CYP1A2 was inhibited in the order of chrysin (no –OH, IC50, 0.42 mM), apigenin (one –OH, 5.14 mM) and luteolin (two -OHs, 8.85 mM), but CYP1A1 was inhibited with reverse ranks. CYP1B1 was strongly inhibited all of them with less than 0.5 mM of IC50. All of them were shown the mixed type inhibition judging by Dixon plot. Thus, the increase of B-ring –OH number in 5,7-dihydroxyflavones was more strongly inhibited CYP1A1 compared to CYP1A2 and decreases of –OH numbers was shown the stronger inhibition of CYP1A2. These differential inhibition of CYP1 enzymes by B-ring –OH numbers of 5,7-dihydroxyflavone might due to different amino acid residue at the active site of enzymes.