30PE-25

**ANALYTICAL VALIDATION FOR RAPID INHIBITION SCREENING OF CYTOCHROME P450 ENZYME ACTIVITIES IN HUMAN LIVER MICROSOMES BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY**

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In drug development, it is important to assess its inhibition/induction effect on cytochrome P450 (CYP) isozymes in order to predict only the potential clinical drug–drug interactions. Inhibitory effects on CYP isozymes are evaluated individually to detect the effect on the *in vitro* biotransformation of each CYP probe substrate using human liver microsomes. A rapid and sensitive liquid chromatography–tandem mass spectrometry (LC/MS/MS) method was developed for routine screening of drug candidates for inhibition of major human CYP isozymes, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. In this 'cocktail' approach, the 50% *inhibition* concentrations (IC₅₀ values) were determined to analyze the major metabolites of CYP probe substrates. A cocktail assay containing these isozymes with probe reactions shortened the analysis time and decreased amounts of samples from human tissues. Moreover, the high quality assays provide information for decision compound selection and optimization in drug discovery. This technique can be applied to other *in vitro* screening such as enzyme induction using human hepatocytes.

30PE-26

**SYNERGISTIC EXPRESSION OF 3-METHYLCHOLANTHRENE-INDUCED CYP1A GENE FAMILY BY ANDROGRAPHOLIDE IN MALE, BUT NOT IN FEMALE MOUSE LIVER.**

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Andrographolide (Andro) is the major diterpenoid constituent of the plant *Andrographis paniculata*, traditionally employed for centuries in Asia as a folkremedy for a wide spectrum of ailments or a herbal supplement for health promotion. Recently enhanced enzyme activities, which were responsible for CYP1A1/2 and CYP2B, were observed in mouse liver after administration of crude extract of the plant for a few weeks. Furthermore, synergistic expression of mRNA with typical CYP1 inducers by Andro was observed in mouse hepatocytes in primary culture. In the present study, the synergism was confirmed in male C57BL/6 mice, after administration of 3-methylcholanthrene (3-MC) at 20 mg/kg for 3 days with and without Andro at 5mg/kg. Andro treatment continued for further two days. Andro by itself didn’t induce the expression of either CYP1A1, CYP1A2, or CYP1B1 mRNA, but co-administration of 3-MC and Andro enhanced the expression of these. Especially, the expression of CYP1A1 was synergistically induced and that of CYP1A2 and CYP1B1 was lesser extent. On the other hand, in female mice, the effects of Andro were different; CYP1A1 induction by 3-MC was reduced by Andro, and Andro showed no effect on the induction of the expression of CYP1A2 and CYP1B1. These results suggest that sex hormone might be involved in the regulation of CYP1 induction. The mechanism of the synergistic induction of CYP1A1 and of the sex-dependent induction of CYP1 expression in the presence of Andro is under investigation.