**2-D-9-5**

**PROTEIN QUANTIFICATION IS USEFUL FOR ANALYZING THE INTERINDIVIDUAL DIFFERENCES IN HUMAN LIVER FUNCTION**

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**[Purpose]** mRNA quantification is widely used as an indirect indicator for estimating relative expression and relative activity of metabolic enzymes and transporters in various tissues. The purpose of this study was to determine the correlation of protein expression to enzyme activity and mRNA expression of CYP enzymes and transporters in human liver tissue.

**[Methods]** Enzyme activities of CYPs were measured in microsomal fraction using LC-MS/MS. Protein expressions of CYPs were measured in microsomal fraction and those of transporters were in plasma membrane fraction by Multiplexed-MRM analysis using LC-MS/MS. mRNA expressions were measured by quantitative real-time RT-PCR.

**[Results and Discussion]** The correlations were assessed among 17 human liver biopsies. Regarding CYPs, protein expression levels in most cases showed better correlation to enzyme activities than to mRNA expression. CYP3A4 showed good correlation between protein and mRNA levels, while several isoenzymes showed poor correlation. In the cases of transporters detected in human livers, all transporters showed poor correlation between protein and mRNA levels compared to CYPs. These results indicate that protein expression levels, rather than mRNA levels, should be measured for estimating relative activity of CYP and transporters among different human liver specimens.

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**ABSOLUTE EXPRESSION OF CYP, UGT AND TRANSPORTER PROTEINS IN CULTURED PRIMARY HUMAN HEPATOCYTES**

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**[Purpose]** Primary cultured hepatocytes provide a well accepted in vitro tool for studying hepatic drug metabolism, drug-drug interaction potentials as well as the involvement of drug transporters in drug disposition. The purpose of this study was to investigate the effect of different culture conditions on enzyme activities, protein expression and gene expression of CYP and UGT enzymes and transporters in sandwich cultured and primary cultured human hepatocytes.

**[Methods]** LC-MS/MS absolute protein quantification in conjunction with enzymatic activity and gene expression analysis was employed to determine expression of multiple phase I/II enzymes and drug transporters in cultured fresh human hepatocytes from three donors.

**[Results and Discussion]** Four different in vitro culturing conditions were tested and protein expression compared to liver biopsy samples (N=17). As a result, CYP and UGT absolute protein expression in cultured hepatocytes was variable, yet akin to levels found in liver. Higher dexamethasone concentrations (0.1 μM vs. 0.01 μM) in culture medium increased expression levels primarily of CYP enzymes. With regard to transporter expression, cultured hepatocytes showed higher absolute expression of canalicular transporters (BCRP, MRP2) and lower expression of sinusoidal transporters (OCT-1, NTCP, OATPs) compared to human liver. Culturing conditions had only minor effects on transporter expression in human hepatocytes. These results provide useful information to select the appropriate in vitro conditions for studying hepatic drug metabolism and to help interpret data obtained from in vitro transporter studies using cultured human hepatocytes.