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DEVELOPMENT OF A SIMPLE UDP-GLUCURONOSYLTRANSFERASE ASSAY FOR UGT1A1, UGT1A4, UGT1A6, AND UGT2B7

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UDP-glucuronosyltransferases (UGTs) catalyze the glucuronidation which is one of the most common phase II metabolism for many drugs. UGTs are divided into two families (UGT1 and UGT2) on the basis of primary amino acid sequences. In humans, UGT isoforms (1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B11, and 2B15) are expressed in the liver. We have attempted, in this study, to develop a simple UGT assay for UGT1A1, UGT1A4, UGT1A6, and UGT2B7 using labeled probe substrates. 17β-Estradiol (O-glucuronidation), imipramine (N-glucuronidation), serotonin (O-glucuronidation), and 3'-azido-3'-deoxythymidine (AZT, O-glucuronidation) were used as probe substrates. Each probe substrate was individually incubated with pooled human liver microsomes (pooled HLM) or UGT isoforms. Glucuronidation activities were measured by radioactivity detection after HPLC separation. Among UGT isoforms (1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, and 2B15) studied, glucuronidation of 17β-estradiol, imipramine, serotonin, and AZT were catalyzed by UGT1A1 and UGT1A3 (UGT1A1 was predominant), UGT1A4, UGT1A6, and UGT2B7, respectively. In pooled HLM, K_m values of 17β-estradiol, imipramine, serotonin, and AZT glucuronidation were 27.7, 806, 19700, and 1940 µM, respectively, and corresponding V_max values were 0.491, 0.174, 27.1, and 1.21 nmol/mg/min. Diclofenac, a known substrate/inhibitor of glucuronidation, inhibited all glucuronidation in pooled HLM. These results confirmed that 17β-estradiol, imipramine, serotonin, and AZT were the predominant substrates of UGT1A1, UGT1A4, UGT1A6, and UGT2B7, respectively. In conclusion, we have successfully developed a simple UGT assay for UGT1A1, UGT1A4, UGT1A6, and UGT2B7 in pooled HLM.

**2-P-20**

ENZYME-ASSISTED SYNTHESIS OF GLUCURONIDES USING UDP-GLUCURONOSYLTRANSFERASES EXPRESSED IN YEAST.

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Glucuronidation, which is catalyzed by UDP-glucuronosyltransferases (UGTs), is the most common pathway for detoxification and elimination of hydrophobic xenobiotics occurring in tissues of most mammals. Because of their ubiquitous nature and high physiological significance, development of an efficient in vitro synthesis of glucuronides often becomes critical during studies of drug metabolism undertaken in the development of a new pharmaceutical product. In order to synthesize enzymatically the glucuronides as drug metabolites, we have developed several mammalian UGT expression system in yeast cells, Saccharomyces cerevisiae AH2. The expression vectors containing human, rat and mouse hepatic UGT1A and 2B isoforms cDNA were constructed and expressed in yeast. Yeast microsomes containing UGT isoforms were prepared from transformed yeast cells. Each UGT expression in microsomes was confirmed by western blot analysis using C-terminal peptide-specific antibody. In the presence of UDP-glucuronic acid and 7-hydroxycoumarine as model substrate, yeast microsomes containing human and rat UGT1A6 catalyzed glucuronide formation. Optimization of reaction resulted in a process that proceeded with 80% conversion product yield. Quercetin with multiple glucuronidating site was conjugated as UGT isoform-dependent formation, resulting in the regiospecific glucuronidation of several drugs using UGT isoforms. These several mammalian UGT expression system in yeast would be useful for enzyme-assisted synthesis of various glucuronides.