P3: INFLUENCE OF CODING REGION MUTATIONS (G71R, F83L, P229Q AND Y486D) IN THE HUMAN UDP-GLUCURONOSYLTRANSFERASE 1A1 (*UGT1A1*) GENE ON ENZYME ACTIVITY AND SUBSTRATE SELECTIVITY.

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ABSTRACT

UGT1A1 catalyses the glucuronidation of numerous drugs, non-drug xenobiotics and endogenous compounds. In particular, UGT1A1 is the sole enzyme involved in the glucuronidation of bilirubin, and promoter and coding region polymorphisms in *UGT1A1* are associated with inherited disorders related to bilirubin elimination. In Asian populations, three coding region polymorphisms, UGT1A1*6 (G71R), UGT1A1*27(P229Q) and UGT1A1*62 (F83L), have been implicated in Gilbert syndrome while UGT1A1*7 (Y486D) has been linked to the Crigler Najjar syndrome type II. However, the impact of these mutations on UGT1A1 glucuronidation kinetics and substrate selectivity remains unknown. Thus, studies are underway to investigate the effects of these four coding region mutations on UGT1A1 activity and substrate selectivity. The UGT1A1 variants were generated by site-directed mutagenesis using the wild-type cDNA as template. Wild-type UGT1A1 and the UGT1A1 variants were stably expressed in a mammalian (HEK293) cell line, and activity of cell lysates was measured using 4-methylumbelliferone (4MU), 1-naphthol (1NP) and bilirubin as the model substrates. 4MU glucuronidation by UGT1A1, UGT1A1*6 and UGT1A1*27 exhibited Michaelis-Menten kinetics with derived K_m and V_{max} (normalised for UGT1A1 protein expression) values of 96, 123 and 188 μM, and 253, 167 and 253 *p*mol/min. mg, respectively. In contrast, 1NP glucuronidation by UCT1A1, UGT1A1*6 and UGT1A1*27 exhibited sigmoidal kinetics. Respective S_{50} (concentration at $0.5V_{max}$) and V_{max} values, generated using the Hill equation, were 329, 257 and 255 μM, and 109, 62 and 58 *p*mol/min. mg. Bilirubin glucuronidation by UGT1A1, UGT1A1*6 and UGT1A1*27 exhibited substrate inhibition kinetics. Respective K_m, K_{si} and V_{max} values, were 2.9, 2.1 and 2.9 μM, 121, 69, and 73 μM and 430, 193 and 156 *p*mol/min.mg. The approximately 50% reduction in intrinsic clearance (V_{max}/K_m) observed for 4MU and 1NP glucuronidation by UGT1A1*6 and UGT1A1*27 was consistent with the reduction observed for bilirubin glucuronidation by these variants. UGT1A1*7 and UGT1A1*62 exhibited very low activity towards 4MU, 1NP and bilirubin. It is concluded that *UGT1A1* coding region mutations associated with impaired bilirubin elimination also result in reduced xenobiotic glucuronidation.

Key words: UGT1A1, glucuronidation, polymorphisms