FUNCTIONAL ANALYSIS OF NOVEL FLAVIN CONTAINING MONOOXYGENASE 3 VARIANTS, THR201LYS AND MET260VAL, FOUND IN A JAPANESE POPULATION

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The flavin-containing monooxygenases (FMOs) are a family of microsomal enzymes that catalyze the oxygenation of a wide variety of nucleophilic compound containing nitrogen, sulfur, phosphorus heteroatoms. FMO3 is considered function form expressed in adult human liver. Recently, we found two novel single nucleotide polymorphisms (SNPs) (c.602 C>A and c.778 A>G) causing amino acid substitutions, Thr201Lys in exon 5 and Met260Val in exon 6, respectively, in a population of self-reported trimethylaminuria Japanese volunteers. The Thr201Lys and Met260Val also presented together with known SNPs (Glu158Lys-Glu308Gly and Val257Met, respectively) in the same alleles of the FMO3 gene to form novel haplotypes. The purpose of this study is to clarify the function of the novel SNPs, Glu158Lys-Thr201Lys-Glu308Gly and Val257Met-Met260Val of FMO3. We determined the oxidation activities for the typical substrate of FMO3, methyl p-tolyl sulfide, benzylamine, and trimethylamine using E. coli expression systems. The intrinsic clearance (Vmax/Km) values for methyl p-tolyl sulfide S-oxidation, benzylamine N-oxidation, and trimethylamine N-oxidation catalyzed by the two mutant type FMO3 enzymes expressed in E. coli membranes were approximately one tenth of those of wild-type FMO3 enzyme and the typical mutant type, E158K-E308G FMO3 enzyme. These results indicate that these two novel SNPs in the FMO3 affect the disposition of food derived trimethylamine and several xenobiotics that are metabolized by FMO3.

IN VITRO GLUCURONIDATION OF ANTIALLERGIC DRUG, TRANILAST, IN HUMAN

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The major metabolic pathway of tranilast, an oral antiallergic drug, has been reported to be glucuronidation and demethylation. Demethylation of tranilast is catalyzed by CYP2C9. However, the glucuronidation of tranilast is still unknown. The purpose of the present study is to identify the UDP-glucuronosyltransferase (UGT) isoforms catalyzing tranilast glucuronidation in human. Tranilast was incubated with human liver microsomes, human jejunal microsomes, and recombinant human UGTs. Tranilast glucuronide was quantified by HPLC. The activity of tranilast glucuronidation was observed in both human liver microsomes and human jejunal microsomes. The Vmax/Km value of jejunal microsomes (848 nl/mg/min) was 3.8-fold higher than that of liver microsomes (224 nl/mg/min). Tranilast glucuronosyltransferase activities in microsomes from 16 human livers were correlated with estradiol 3-glucuronosyltransferase activity, which was a specific activity of UGT1A1 (r=0.95, p<0.0001). In an in vitro study using recombinant human UGT isoforms, UGT1A1 was most responsible for tranilast glucuronidation at 100 µM and UGT1A3, UGT1A8, UGT1A9, and UGT1A10 partly involved. Tranilast glucuronosyltransferase activity was strongly inhibited by bilirubin, a UGT1A1 substrate, in human liver microsomes (IC50=124 µM) and human jejunal microsomes (IC50=81 µM). On the other hand, bilirubin glucuronosyltransferase activity was inhibited by tranilast. Therefore, tranilast glucuronosyltransferase activity was clarified to be mainly catalyzed by human UGT1A1. In addition, the effect of the CYP2C9 metabolite, 4-demethyltranilast, to tranilast glucuronosyltransferase activity will also be reported in our presentation.