N-Glycosylation plays important role on folding of human UGT1A9

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N-Glycosylation is known to play roles in modulating protein structure, function, and stability. Mammalian UGTs are type I endoplasmic reticulum resident membrane proteins. It has been reported that some UGTs are N-glycosylated. In this study, we investigated the role of N-glycosylation of human UGTA9. When the UGT1A9 expressed in HEK293 cells was analyzed by SDS-PAGE, the size was 53 kDa. It was converted to a faster migrating protein of about 50 kDa when treated with endoglycosidase H (Endo H), indicating that UGT1A9 is N-glycosylated. The UGT1A9 has three potential N-glycosylation sites with the sequence NX(S/T). Mutations of asparagine (N) residues to glutamine (Q) were introduced by the site-directed mutagenesis. The molecular mass of the expressed mutants N71Q and N344Q was lower than that of the wild type, but there was little difference in the relative mobility of N292Q. 4-Methylumbelliferone O-glucuronosyltransferase activities of the mutants N71Q and N344Q were considerably lower than those of the wild type. In order to produce unglycosylated protein, wild type expression vector-transfected cells were exposed to tunicamycin, an inhibitor of oligosaccharyltransferase. It was resulted in the synthesis of a faster migrating and less active protein of UGT1A9. Exposure to castanospermine or deoxynojirimycin, inhibitors of α-glucosidase, resulted in slight changes of the kinetics, indicating that the trimming of terminal glucose affects the enzyme activity of UGT1A9. In contrast, the kinetics of UGT1A9 treated with or without Endo H were almost the same, indicating that the deglycosylation of N-glycosylated UGT1A9 did not affect the enzyme activity. These results suggested that N-glycosylation play a great important role on the folding of UGT1A9.

Mutual inhibition between carvedilol enantiomers on glucuronidation mediated by human liver microsome and UDP-glucuronosyl transferases (UGTs)

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Carvedilol, an α₁, β-receptor antagonist, is used as a therapeutic drug for chronic heart failure. Though carvedilol is administered orally as a racemic mixture, it is known that blood level of R-carvedilol is about two folds higher than that of S-carvedilol. However, it is not clear why blood levels of these enantiomers are different. Carvedilol is known to be metabolized by UGTs and CYPs. Glucuronidation of unchanged form is main route of metabolism. The aim of this study is to clarify if glucuronidation is responsible for the difference of blood levels of carvedilol enantiomers. When human liver microsome (HLM) was used and racemic carvedilol was added as a substrate in the reaction mixture, S-carvedilol was more rapidly glucuronized than R-carvedilol. However, superiority of S-carvedilol glucuronidation to R-carvedilol glucuronidation disappeared when single enantiomer was used as a substrate. These results suggest that the difference of the rate of glucuronidation mediated by HLM and difference of blood level between carvedilol enantiomers are due to mutual inhibition between carvedilol enantiomers on glucuronidation reaction. Glucuronidation of R-carvedilol was inhibited strongly by S-carvedilol with IC₅₀ value of 9.5 μM, whereas inhibition of glucuronidation of S-carvedilol by R-carvedilol was relatively weak with IC₅₀ value of more than 100 μM. We also examined using recombinant enzyme proteins. Glucuronidation of R-carvedilol mediated by UGT1A1, a R-carvedilol selective isoform, was inhibited by S-carvedilol with IC₅₀ value of 22 μM. Glucuronidation of S-carvedilol mediated by UGT2B7, a S-carvedilol selective isoform, was inhibited by R-carvedilol with IC₅₀ value of 55 μM. As these two isoforms showed similar affinity, another isoform such as UGT2B4 might responsible for the difference of glucuronidation by HLM.