ABSTRACT

The anxiolytic agent lorazepam (LZP) and anticonvulsant valproic acid (VPA) are mainly eliminated in humans by glucuronidation. This study aimed to characterize UDP-glucuronosyltransferases responsible for (R,S) lorazepam glucuronidation and quantitatively predict *in vivo* metabolic drug-drug interaction between LZP and VPA. Formation of the glucuronide metabolites (R-LZPG and S-LZPG) were quantified by HPLC. Recombinant human UGT enzymes were expressed in HEK293 or baculovirus-insect cells. Kinetics of R- and S-LZP glucuronidation by HLMs (n=5) exhibited substrate inhibition. Mean derived binding affinity (K_m) and V_{max} of R- and S-LZPG kinetics were 36.7 ± 11.4 μM and 7.3 ± 2.1 pmol/min.mg, and 50.3 ± 18.2 μM and 11.7 ± 5.2 pmol/min.mg, respectively. Substrate inhibition with both metabolites was weak (K_m 10- to 17-fold lower than K_{si}). Twelve recombinant UGTs were screened for LZP glucuronidation activity at LZP concentrations of 10, 50 and 250 μM. UGT 2B4, 2B7 and 2B15 catalyzed S-lorazepam, but the highest activity is observed for UGT2B15. R-lorazepam was catalyzed by UGT 2B4, 2B7 and 2B15. Notably, UGT 1A7 and 1A10 which are expressed in gastrointestinal tract metabolized only R-lorazepam. To identify UGTs responsible for lorazepam glucuronidation in the liver, the kinetic studies of hepatically UGTs which exhibited measurable activities were further investigated. Derived K_m or S_{50} values observed with recombinant UGT 2B4, 2B7 and 2B15 were in the range of 13-46 μM which is comparable to those observed by using HLMs. VPA exhibited non-competitive inhibition on R- and S-lorazepam glucuronidation by HLMs with respective K_i values of 3.9 and 3.2 mM. In conclusion, UGT 2B4, 2B7 and 2B15 are likely to be the major enzymes responsible for human liver microsomal R,S-lorazepam glucuronidation. Based on K_i value, 20% increased of LZP area under the plasma concentration time curve was predicted when co-administered with VPA.

Keywords: lorazepam, valproic acid, glucuronidation, UDP-glucuronosyltransferase, drug-drug interaction