DRUG METABOLISM & DISPOSITION

CORRECTIONS TO "HUMAN UDP-GLUCURONOSYLTRANSFERASES: ISOFORM SELECTIVITY AND KINETICS OF 4-METHYLUMBELLIFERONE AND 1-NAPHTHOL GLUCURONIDATION, EFFECTS OF ORGANIC SOLVENTS, AND INHIBITION BY DICLOFENAC AND PROBENECID"

We wish to report errors in the UGT1A6 activity and inhibition data published in the above article [Uchaipichat V, Mackenzie PI, Guo XH, Gardner-Stephen D, Galetin A, Houston JB, and Miners JO (2004) *Drug Metab Dispos* **32:**413–423]. It has come to our attention that the recombinant UGT1A6 enzyme preparation used in this work also contained UGT1A9. Accordingly, we have repeated the studies outlined in the original publication with lysate from HEK293 cells expressing only UGT1A6. The expression of UGT1A6 was confirmed by Western blot analysis. The expression (relative to UGT1A1) was not markedly changed from that reported in the original article: 1.31:1 versus 1.39:1 (previously). Incubation conditions were as described previously, but it was necessary to alter the HEK293 cell lysate protein concentrations and reaction times to ensure that glucuronide formation was linear with respect to both variables. Substrate [4-methylumbelliferone (4MU) and 1-naphthol (1NP)] concentration ranges were also modified for the optimal assessment of kinetic parameters. The altered incubation conditions are shown in Table 1. Although the kinetic models reported in the original publication for 4MU (Michaelis-Menten) and 1NP (sigmoidal; fitted to Hill equation) glucuronidation were unchanged (see Tables 2 and 3 of original article), the derived kinetic parameters (especially V_{max}) were different. The recalculated K_m and V_{max} values for 4MU glucuronidation by UGT1A6 are 109 ± 3.7 μM (previously 8.8 ± 0.4 μM) and 143,897 ± 1713 pmol/min · mg protein (previously 7333 ± 116 pmol/min · mg protein), respectively, whereas the recalculated S_{50}, n (Hill coefficient), and V_{max} values for 1NP glucuronidation are 3.1 ± 0.02 μM (previously 1.8 ± 0.1 μM), 1.3 ± 0.01 (previously 1.9 ± 0.2), and 19,596 ± 74 pmol/min · mg protein (previously 43 ± 2 pmol/min · mg protein), respectively. 1NP glucuronidation by UGT1A6, which exhibited sigmoidal kinetics, was additionally refitted to the two-site model. K_s, V_{max}, and α values generated from this model were 6.7 ± 0.2 μM (previously 17 ± 3 μM), 19,646 ± 127 pmol/min · mg protein (previously 45 ± 1 pmol/min · mg protein), and 0.22 ± 0.02 (previously 0.01 ± 0.005), respectively. Given the higher values of V_{max}, the recalculated intrinsic clearances (CL_{int}) or maximal clearances (CL_{max}) are also higher than those reported in Table 4 of the original article: 1320 μl/min · mg protein (previously 829 μl/min · mg protein) and 3602 μl/min · mg protein (previously 12.2 μl/min · mg protein) for 4MU and 1NP glucuronidation, respectively. Although the conclusion from the original article that UGT1A6, 1A7, 1A8, 1A9, and 1A10 generally exhibit highest activity toward 4MU and 1NP remains unchanged, it should be noted that both phenolic compounds (especially 1NP) were glucuronidated most effectively by UGT1A6.

The inhibitory effects of diclofenac, probenecid, and organic solvents on UGT1A6 were reassessed with 4MU as the "probe" substrate at the appropriate recalculated K_m value (viz. 100 μM). As in the original report, diclofenac was a competitive inhibitor of UGT1A6 (K_i value of 60 ± 0.1 μM; previously 23 ± 0.1 μM). However, the recharacterized inhibition mechanism for probenecid was noncompetitive (previously competitive), with a derived K_i value of 2790 ± 200 μM (previously 1429 ± 3 μM; Table 5 of original article). The effects of organic solvents were also different from those reported in the original article, and the corrected data are shown in Fig. 1 (cf. Fig. 3 of original article). Notably, dimethyl sulfoxide has a minor effect on UGT1A6 activity, whereas acetone, acetonitrile, and ethanol (at a concentration of 1% v/v) and methanol (1, 2, and 4% v/v) decreased UGT1A6 activity by >20%.

The online version of this article has been corrected in departure from the print version.

The authors apologize for any confusion and inconvenience caused by the erroneous data reported previously for UGT1A6. Data reported for all other isoforms are accurate.

TABLE 1

Protein amount, incubation time, and substrate concentration ranges used for the measurement of 4MU and 1NP glucuronidation by UGT1A6

	Protein Amount	Incubation Time	Concentration Range
	μg/incubation	*min*	*μM*
4MU	1.5	30	10–600
1NP	0.5	60	0.5–20

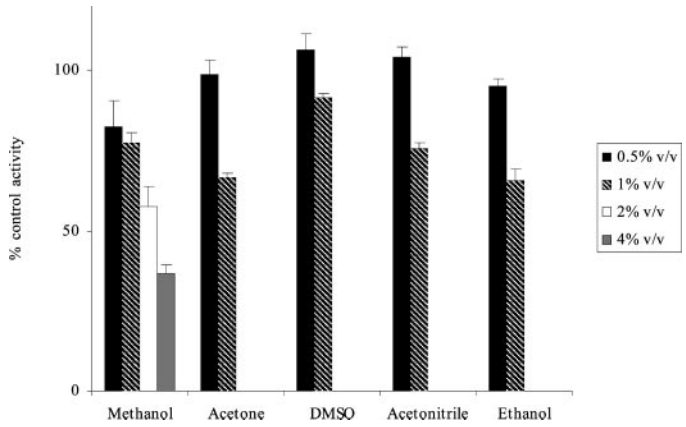

FIG. 1. Effects of organic solvents on 4MU glucuronidation by UGT1A6. The concentration of 4MU was 100 μM. Each bar represents the mean percentage activity relative to control from three replicates; error bars indicate standard deviations.