Inhibition of UGT1A9 activity demonstrated for acridine antitumor agent C-1748 may implicate drug-drug interactions

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Introduction

UDP-glucuronosyltransferases (UGTs) are phase II conjugating enzymes catalysing glucuronidation reaction of many exogenous and endogenous substances. Glucuronides are more polar than the native compounds what facilitates their excretion *via* bile and urine. UGT isoenzymes are in the spotlight in respect to antitumor therapy as glucuronidation is the major route of elimination for a lot of anticancer drugs. Therefore, UGT activity modulation can influence the final therapeutic effect of coadministered drug, what is known as drug-drug interactions.

The compound 9-(2'-hydroxyethylamino)-4-methyl-1-nitroacridine, C-1748, is one of the most potent 1-nitroacridine derivative developed in our department. Strong cytotoxic activity against colon cancer cell lines and high antitumor activity against prostate carcinoma xenografts, along with low mutagenic potential are the promising features, which allowed C-1748 to be selected for preclinical studies. Preliminary results indicated therapeutic potential of C-1748 also against pancreatic cancer.

Aim

The objective of the present studies was to explore the inhibition properties of C-1748 toward one of the major human hepatic UGT isoenzyme - UGT1A9 *in vitro* in respect to the potential for drug–drug interactions.

Materials & Methods

The glucuronidation reactions of standard substrate 7-hydroxy-4-(trifluoromethyl)coumarin (TFK) were conducted in the absence or presence of C-1748. We used various concentrations of TFK (0.025, 0.05, 0.1, 0.25 mM) and C-1748 (0, 0.025, 0.05, 0.1, 0.25 mM). TFK was preincubated with 0.2 mg/ml human recombinant UGT1A9 and after 5 min 2 mM UDPGA was added. The incubation was carried out in 50 mM Tris-HCl buffer, pH 7.4 with 8 mM $MgCl_2$ and 25 mg/ml alamethicin at 37 °C. After 0 and 20 min the reactions were stopped by mixing 50 ml of reaction mixture with 50 ml of cold methanol containing 0.01 mM 7-hydroxycoumarin as an internal standard. Samples were incubated in ice for 10 min, followed by centrifugation for 10 min at 13400 rpm. Supernatants were analysed by RP-HPLC/UV-Vis method with detection at 330 nm. The elution was carried out at a flow rate of 1 ml/min, with the following mobile phase: a linear gradient from 30 to 80% eluent B (methanol + 5% v/v water) in eluent A (ammonium formate (0.05 M, pH 3.4) + 5% v/v methanol) for 20 min, followed by linear gradient from 80 to 100% eluent B in eluent A for 5 min.

Results & Discussion

The glucuronidation reactions of standard substrate 7-hydroxy-4-(trifluoromethyl)coumarin were conducted in the absence or presence of C-1748 and monitored by HPLC/UV-Vis method. Our studies showed that C-1748 is not a substrate for UGT1A9, however it exerted dose-dependent inhibition toward this isoenzyme. The concentration of 0.25 mM of C-1748 inhibited more than 70% activity of UGT1A9 with the IC_{50} value of 0.018 mM. The inhibition kinetic type was determined on the basis of Lineweaver-Burk and Dixon plots. The intersection points at Lineweaver-Burk and Dixon plots were located in the horizontal axis indicating the noncompetitive inhibition. The inhibition kinetic parameter (Ki) was calculated to be 0.16 mM using the slopes from the Lineweaver-Burk plot versus the concentration of C-1748.

This finding provides new insights into potential pharmacokinetic drug-drug interactions between C-1748 and other drugs being the substrates of UGT1A9. The clinical use of C-1748 might disrupt the metabolism of other drugs or endogenous substances which are glucuronidated by this isoenzyme.

Conclusions

- **C-1748 is not a substrate for UGT1A9, however it exerted dose-dependent inhibition toward this isoenzyme.**
- **The presented UGT1A9 significant inhibition indicates the high possibility for drug-drug interactions in combined therapies using C-1748 anticancer agent.**
- **Clinical monitoring should be applied in the case of C-1748 coadministration with drugs undergoing UGT1A9-mediated metabolism.**

Acknowledgements

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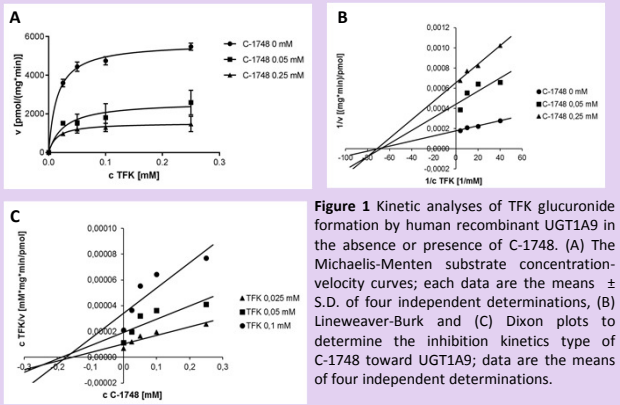

Figure 1 Kinetic analyses of TFK glucuronide formation by human recombinant UGT1A9 in the absence or presence of C-1748. (A) The Michaelis-Menten substrate concentration-velocity curves; each data are the means ± S.D. of four independent determinations, (B) Lineweaver-Burk and (C) Dixon plots to determine the inhibition kinetics type of C-1748 toward UGT1A9; data are the means of four independent determinations.

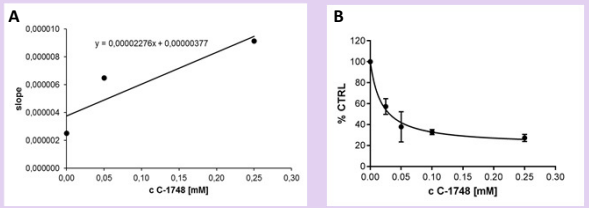

Figure 2 Determination of inhibition parameters for UGT1A9-catalyzed TFK glucuronidation in the presence of C-1748. (A) The slopes from the Lineweaver-Burk plot versus the concentration of C-1748 to determine Ki value and (B) the IC_{50} curve; data are the means ± S.D. of four independent determinations.

A

c C -1748 [mM]	K_m [mM]	V_{max} [pmol/(mg*min)]
0.00	0.01472 ± 0.001517	5675 ± 114
0.05	0.02673 ± 0.01227	2628 ± 322
0.25	0.01424 ± 0.005475	1534 ± 113

B

K_i [mM]	IC_{50} [mM]
0.1656	0.01852 ± 0.004884

Table 1 Enzyme kinetic and inhibition parameters. (A) Km and Vmax values of TFK glucuronidation by UGT1A9 in the absence or presence of C-1748, (B) Ki and IC_{50} values of C-1748 for UGT1A9-catalyzed TFK glucuronidation. Data are the means ± S.D. of four independent determinations.