The modification of UGT1A10 isoenzyme activity by C-1305 and C-1311 antitumor agents in noncellular system and in HCT-116 colon cell line

Bejrowska Anna

Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Gdansk, Poland

Introduction

Modern cancer treatment provides promising outcomes thanks to combined therapies. However, the coadministration of antitumor drugs can result in drug-drug interactions, what leads to the modification of metabolic pathways responsible for the activation or detoxification of the drugs. Therefore, it is crucial to examine the influence of the potential therapeutic agent on cellular metabolism. Our group developed several new antitumor agents, among which C-1311 was selected to II phase of clinical studies. We revealed previously antitumor acridinone derivatives, C-1305 and C-1311, were metabolized to a great extent by UGT1A10 in the recombinant form and in the tumor cells. The studies on the influence of these compounds on UGT1A10 activity in vitro and in colon cancer line HCT-116 are presented here.

Aim

The purpose of the present studies is to test the ability of acridinone antitumor compounds C-1305 and C-1311 to modulate the activity of UGT1A10 isoenzyme in noncellular system and in colon cancer cell line.

Materials & Methods

Enzyme activity in both models was measured using UGT-specific reaction, 7-hydroxy-4-(trifluoromethyl)-coumarin (TFK) glucuronidation in the presence of C-1305 and C-1311 (0; 0,1; 0,05; 0,01 mM), as well as without the drug (control experiments) by RP-HPLC analysis (UV-Vis detection at 330 nm). We performed series of reactions towards human recombinant UGT1A10 (0,5 mg/ml) and various concentrations of the substrate (0,75; 0,6; 0,5; 0,35; 0,25; 0,1; 0,05; 0,025 mM). After 5 minutes preincubation of TFK and the drug with the enzyme, the reaction was started by addition of the enzymatic cofactor UDPGA (5 mM). After incubatioan in 37 ˚C for 30, 60 or 90 minutes the reaction was stopped by addition of cold methanol to the sample (1:1).

In cellular model we investigated glucuronidation rate by both nontreated and drug-treated cells. Following the 24-hours of drug treatment (0,1; 1; 10; 50; 100 uM C-1305/C-1311/Irinotecan as a reference drug), cells' medium was replaced with fresh medium containing TFK (50uM) for 3 hours. Cell proteins were then precipitated with acetonitrile (1:1).

All samples were cetrifuged (13 400 rpm, 10 mins) and the supernatants were analysed with the aid of RP-HPLC with UV-Vis detection at 330 nm. The experiments were performed in triplicates, the results are expressed as the mean of the three.

Results & Discussion

The results showed that C-1305 and C-1311 act differently towards UGT1A10 activity in dependence on the applied model.

Enzymatic activity of UGT1A10 was reduced by both acridinone derivatives in noncellular system. There was a higher reduction of the maximal velocity of TFK glucuronidation in the presence of C-1305 rather than C-1311, which seems to be a slighter inhibitor in this system.

By contrast, higher level of UGT1A10 activity was observed in HCT-116 cells treated with both studied compounds. C-1305 and C-1311, unlike approved antitumor agent irinotecan cause higher rate of TFK glucuronidation in studied cell line. It is supposed that C-1305 and C-1311 potentially applied in multidrug therapy might modulate the effectiveness of UGT1A10 on the protein and the transcriptional level. This finding provides new insights into potential pharmacokinetic drug-drug interactions between C-1305 and C-1311 and other substrates of UGT1A10.

Conclusions

-C-1305 and C-1311 modulate the activity of UGT1A10 both in cellular and noncellular systems.

-The studied compounds inhibit human recombinant UGT1A10 activity

-C-1305 seems to be a stronger than C-1311 inhibitor of UGT1A10-dependent TFK-glucuronidation *in vitro*

-Both drugs, unlike irinotecan, enhance the activity of UGT1A10 in HCT-116 colon cancer cell line.

Acknowledgements

This study was supported by grant OPUS 5, 2013/09/B/NZ3/00003 from the National Science Center, Poland.

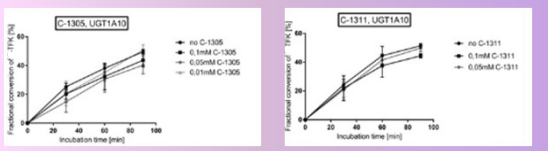

Fig1. Glucuronidation activities of human recombinant UGT1A10 toward TFK in the presence or absence of C-1305 and C-1311. Activities are expressed in fractional conversion of the substrate.

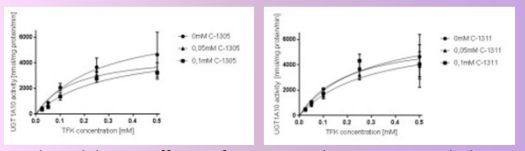

Fig2. The inhibitory effects of C-1305 and C-1311 towards human recombinant UGT1A10 expressed in Michaelis-Menten substrate concentration-velocity plots.

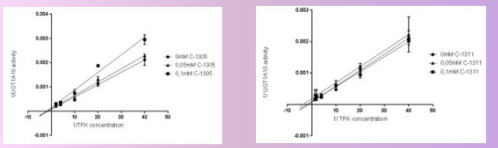

Fig3. The inhibitory effects of C-1305 and C-1311 towards human recombinant UGT1A10 expressed in Lineweaver–Burk plots of the effects of the drugs on TFK glucuronidation.

Tab1. Enzyme kinetic parameters – effects of C1305 and C-1311 on Km and Vmax of TFK glucuronidation by UGT1A10.

Drug concentration [mM]	Vmax [nmol/mg protein/min]		Km [mM]	
	C-1305	C-1311	C-1305	C-1311
0	7294±1675	7294±1675	0.271±0.129	0.271±0.129
0.05	4794±601.2	6322±1629	0.256±0.048	0.287±0.15
0.1	5263±593.5	6275±1118	0.283±0.065	0.302±0.082

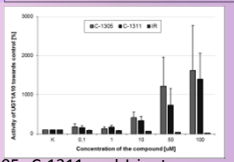

Fig4. Effect of C-1305, C-1311 and Irinotecan on UGT1A10 activity in HCT- colon cancer cell line.