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SHORT COMMUNICATION

The small-molecule tyrosine kinase inhibitor nilotinib is a potent noncompetitive inhibitor of the SN-38 glucuronidation by human UGT1A1

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Abstract
Purpose Inhibition of the UDP-glucuronosyltransferase (UGT) 1A1 by nilotinib was examined in vitro with SN-38 as a substrate, to estimate the possibility of drug–drug interaction of nilotinib with other medicines predominantly detoxified by UGT1A1.
Methods Inhibition of UGT1A1-catalyzed SN-38 glucuronidation by nilotinib was examined with human liver microsomes (HLM) and recombinant human UGT1A1 as enzyme sources. Inhibition constants (K_i) were estimated with kinetic analysis.
Results Nilotinib potently inhibited the SN-38 glucuronidation by human liver microsomal UGT1A1 and recombinant UGT1A1 in a noncompetitive manner, with K_i values of 0.286 ± 0.0094 and 0.079 ± 0.0029 μM, respectively. If a drug that serves as a substrate of UGT1A1 is administered with nilotinib, the area under the plasma concentration–time curve of a drug estimated by using these K_i values would be two times or higher than that without nilotinib, suggesting drug–drug interactions involving UGT1A1. These in vitro data and the prediction of drug–drug interaction are helpful for the clinical management of the nilotinib use.
Conclusion We found that nilotinib is a potent noncompetitive inhibitor of human UGT1A1 activity.

Keywords Nilotinib · UGT1A1 · Inhibition · Drug–drug interaction · SN-38

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Introduction

Drug–drug interactions have received increasing attention over the past few decades. A recent survey indicated that more than 30% of the US population over 57 years of age takes at least five prescription drugs at any given time. Drug–drug interactions contributed to the toxicity of some drugs that were withdrawn from the US market. Many of these interactions involved inhibition of drug-metabolizing enzymes and transporters, resulting in increased systemic exposure and subsequent adverse drug reactions [1]. Therefore, the evaluation of drug–drug interaction potential is an essential part of risk assessment for the better clinical management of the use of medicines.

Nilotinib is a small-molecule multiprotein tyrosine kinase inhibitor, targeting the Bcr-Abl fusion protein, c-Kit, platelet-derived growth factor receptor (PDGFR) α, and PDGFR β [2, 3], which is approved for the treatment of Bcr-Abl positive CML in adult patients resistant to or intolerant of prior therapy that included imatinib [4]. One study has suggested that nilotinib might inhibit UDP-glucuronosyltransferase (UGT) 1A1 to cause hyperbilirubinemia [5]. If nilotinib is a potent inhibitor of UGT1A1, physicians should pay attention to the drug–drug interactions between nilotinib and other medicines that served as

substrate of UGT1A1. The inhibition of estradiol glucuronidation by nilotinib was previously studied in vitro (European Medicines Agency; http://www.ema.europa.eu/ema/index.jsp?curl=pages/home/Home_Page.jsp&murl=&mid=). However, there has been no report demonstrating the details of the in vitro study. Furthermore, there is no quantitative estimation of the nilotinib-induced drug–drug interactions with the K_i value obtained in the in vitro study.

Therefore, in the present study, we assessed the inhibition of UGT1A1 activity by nilotinib in vitro using SN-38 as a substrate. Human liver microsomes (HLM) and recombinant human UGT1A1 were used as the source of UGT1A1. We further quantitatively estimated the possibility of nilotinib-induced drug–drug interaction.

Methods

Chemicals

Nilotinib, SN-38, and SN-38 glucuronide (SN-38G) were purchased from Toronto Research Chemicals (North York, Canada). Camptothecin was from Wako (Tokyo, Japan). UGT reaction mix solution A (25 mM UDP-glucuronic acid [UDPGA] cofactor) and UGT reaction mix solution B (5×-UGT buffer mix with alamethicin, 250 mM Tris–HCl, 40 mM $MgCl_2$, and 0.125 mg/mL alamethicin in water) were from BD Biosciences (Woburn, MA). All chemicals and solvents were of the highest grade commercially available.

Human liver microsomes and recombinant human UGT1A1

Pooled HLM were purchased from BD Biosciences (Woburn, MA). The pooled HLM were derived from 24 donors (92% Caucasian, 4% Hispanic, and 4% African-American; 17 men and 7 women) with a median age of 45 years (range, 16–77). Microsomes were diluted in 250 mM sucrose. Microsomal protein content was 20 mg/mL. Estradiol 3-glucuronidation by the liver microsomes measured by BD Biosciences (Woburn, MA) was 720 pmol/min/mg prot. Recombinant human UGT1A1 supersomes expressed in baculovirus-infected insect cells were obtained from BD Biosciences (Woburn, MA). Microsomal protein content was 5.0 mg/mL.

Inhibition assay of SN-38 glucuronidation

Based on the linear relation between the human liver microsomal protein concentration and the reaction time versus the amount of metabolite formation, the protein content and the reaction time were set at 0.5 mg/mL and 5 min, respectively. In the case using the recombinant human UGT1A1 as an enzyme source, the protein content and the reaction time were at 0.2 mg/mL and 30 min, respectively.

The effects of nilotinib on SN-38 glucuronidation by HLM or the recombinant human UGT1A1 were assessed as follows: After preincubation of the incubation mixture with nilotinib or solvent dimethyl sulfoxide (DMSO) at 37°C for 5 min, the substrate SN-38 dissolved in 1% DMSO was added. Nilotinib was dissolved in DMSO. The final concentration of the solvent in the reaction mixture was 1.1%. SN-38 glucuronidation by HLM or recombinant human UGT1A1 was assayed using BD Biosciences (Woburn, MA) products, as described in the UGT reaction mix solution A or B protocol. The typical incubation mixture consisted of 50 mM Tris–HCl buffer (pH 7.5), 8 mM $MgCl_2$, 25 μg/mL alamethicin, 2 mM UDPGA, and microsomal fractions of human liver or the recombinant human UGT1A1 in a final volume of 0.2 mL. The reaction was terminated by adding a twofold volume of acetonitrile. Each assay was performed three times in duplicate.

HPLC analysis for SN-38 glucuronide

SN-38G was analyzed by HPLC using a computerized HPLC system (Hitachi model 7000 series, Hitachi, Tokyo, Japan) equipped with a TSK-gel ODS-120T analytical column (4.6 × 250 mm; 4 μm; TOSOH, Tokyo, Japan), as described elsewhere [6]. The mobile phase consisted of 75 mM ammonium acetate (pH 4.75) for solvent A and acetonitrile for solvent B. The metabolite was separated using a linear gradient of 85–65% solvent A, a time of 0–25 min, and a flow rate of 1.0 mL/min. The metabolite was quantified by comparing the HPLC peak area to that of the internal standard camptothecin. The lower limit of quantification was 0.49 nM for SN-38G. The intra- and inter-assay coefficients of variation at 4.0 nM were less than 4.2 and 12.2%, respectively.

Estimation of enzyme and inhibition kinetics

When the HLM were used to estimate the enzyme kinetics (K_m and V_{max}) of SN-38 glucuronidation by UGT1A1 or to determine the K_i of nilotinib for UGT1A1-catalyzed SN-38 glucuronidation, SN-38 concentrations ranged from 2.5 to 40 μM in the absence or presence of an inhibitor (0.125–1 μM). In the case of using the recombinant human UGT1A1, SN-38 concentrations ranged from 1.25 to 40 μM in the absence or presence of an inhibitor (0.025–0.2 μM).

Michaelis–Menten equation was fitted to data points to estimate the K_m and V_{max} values by nonlinear least-squares regression analysis, performed with GraphPad Prism version 5 software (GraphPad Software). The K_i values were also calculated by nonlinear regression analysis with

GraphPad Prism version 5 software (GraphPad Software), using the equations for competitive inhibition, noncompetitive inhibition, or mixed inhibition [7]. The type of inhibition was determined from the enzyme inhibition models fitted to the data. Goodness of fit to the inhibition models was estimated from the F statistics, R^2 values, parameter standard error estimates, and 95% confidence intervals. Kinetic constants (K_m, V_{max}, and K_i) were reported as the means ± standard error.

Results

Inhibition of UGT1A1 by nilotinib

The substrate concentration versus SN-38 glucuronidation plots in the presence or absence of nilotinib is shown in Fig. 1a. Apparent K_m and V_{max} were calculated to be 6.80 ± 0.32 μM and 110 ± 1.8 pmol/min/mg prot., respectively. Nilotinib inhibited the SN-38 glucuronidation by UGT1A1 in a noncompetitive manner (Fig. 1b). The Eadie-Hofstee plots of SN-38 glucuronidation showed linearity with an R^2 value of 0.96, indicating that SN-38 glucuronidation was catalyzed by a single enzyme of UGT1A1 expressed in the HLM (Fig. 1c). Nonlinear regression analysis also revealed noncompetitive inhibition with R^2 of 0.99 (Fig. 1a). The K_i value was estimated to be 0.286 ± 0.0094 μM (Fig. 1a).

Apparent inhibition kinetics of nilotinib toward UGT1A1-mediated SN-38 was further examined with the recombinant human UGT1A1. Apparent K_m and V_{max} were calculated to be 4.18 ± 0.19 μM and 85.6 ± 1.3 pmol/min/mg prot., respectively (Fig. 2a). The K_m value obtained with the recombinant UGT1A1 was almost similar to that with HLM. Nilotinib inhibited the SN-38 glucuronidation by UGT1A1 in a noncompetitive manner (Fig. 2b). Nonlinear regression analysis also demonstrated noncompetitive inhibition with R^2 of 0.98 (Fig. 2a). The K_i value was estimated to be 0.079 ± 0.0029 μM (Fig. 2a).

Estimation of nilotinib-induced drug–drug interaction through UGT1A1

Inhibition of UGT1A1 activity by nilotinib may cause drug–drug interactions involving UGT1A1-catalyzed metabolism. The increase in the area under the plasma concentration–time curve (AUC) of other drugs by nilotinib can be estimated by using the K_i values obtained in this study using the method described by Ito et al. [8]. The average systemic plasma concentration of nilotinib after repeated oral administration ($[I]_{av}$) and the maximum unbound hepatic input concentration of nilotinib ($[I]_{in,u}$) are calculated as follows:

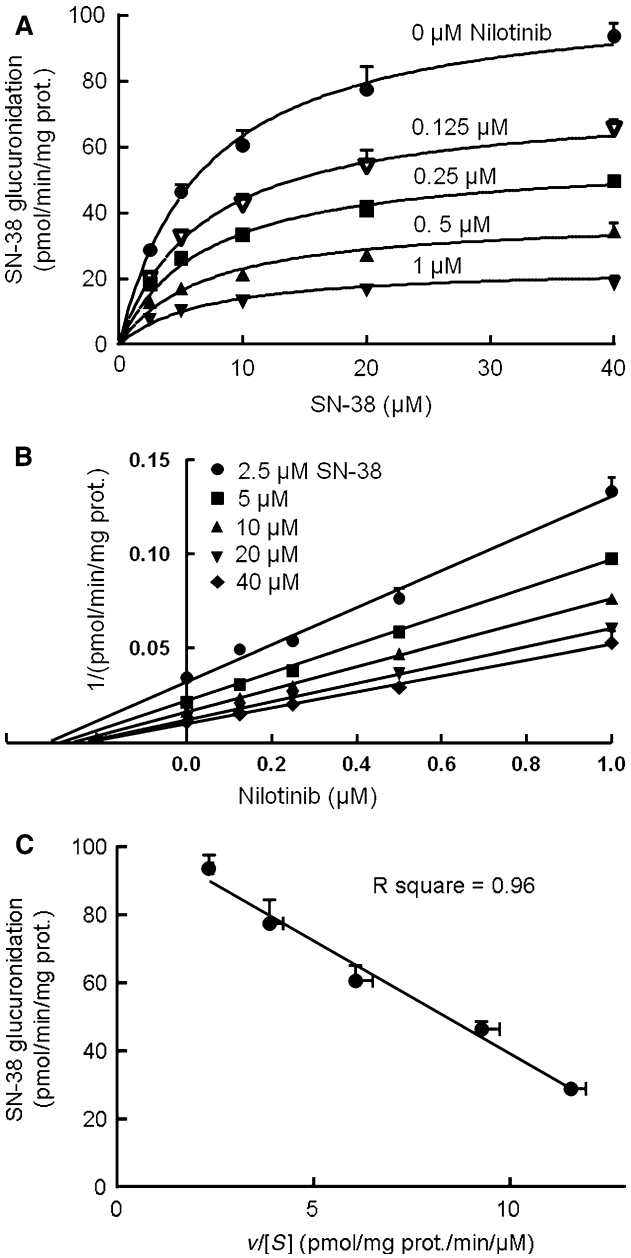

Fig. 1 Inhibition kinetics of nilotinib on SN-38 glucuronidation by UGT1A1 examined with human liver microsomes. **a** SN-38 concentration versus SN-38 glucuronidation plots. **b** Dixon plots. **c** Eadie-Hofstee plots of SN-38 glucuronidation in the absence of nilotinib. *v*, *Velocity* of glucuronidation; [*S*], SN-38 concentration. Each point shows the mean of three independent experiments with standard deviation

$$[I]_{av} = D/\tau/(CL/F) \quad (1)$$

$$[I]_{in,u} = fu\ ([I]_{av} + ka\ Fa D/Qh) \quad (2)$$

where D, τ, and CL/F are the dose, the dose interval and oral clearance of nilotinib, fu is the plasma unbound fraction, ka is the absorption rate constant, Fa is the extent of absorption, and Qh is the hepatic blood flow rate. The package inserts of nilotinib in the United States and Japan

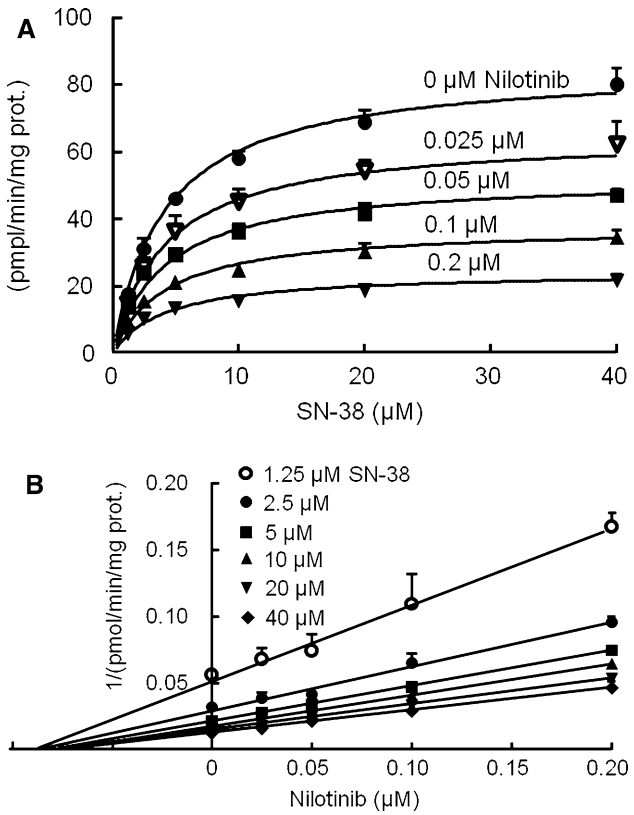

Fig. 2 Inhibition kinetics of nilotinib on SN-38 glucuronidation by recombinant human UGT1A1 **a** SN-38 concentration versus glucuronidation of SN-38. **b** Dixon plots. Each point represents the mean of three independent analyses with standard deviation

state that D and τ are 400 mg (0.685 mmol) and 12 h, respectively. CL/F obtained in fasting, Fa, and fu were reported to be 32.8 L/h, 0.3, and 0.02, respectively [9]. Since the ka value for nilotinib has not yet been reported, the value was assumed to be 0.1 min^{-1}, as proposed by Ito et al. [8]. The Qh was assumed to be 1,610 mL/min [8]. The AUC ratio in the presence or absence of nilotinib was used to estimate the potency of nilotinib to increase the AUC of a simultaneously administered drug that serves as a substrate of UGT1A1.

$$\text{AUC ratio} = 1 + [\text{I}]_{\text{in,u}}/K_i \quad (3)$$

The increase in the AUC was calculated to be 2.0 and 4.7 by using the K_i values obtained with HLM and recombinant human UGT1A1, respectively, even though we used the plasma unbound nilotinib concentration to reduce the possibility of false-positive estimates, suggesting the high risk of drug–drug interactions involving nilotinib [8].

Discussion

Our in vitro study showed that nilotinib was a potent noncompetitive inhibitor of human UGT1A1. The K_i values of UGT1A1 inhibition by nilotinib obtained with HLM and recombinant human UGT1A1 were 0.286 and 0.079 μM, respectively.

Singer et al. [5] suggested that the combined impact of the inhibition of UGT1A1 activity by nilotinib and genetic polymorphisms of *UGT1A1*, which were related to reduced expression or activity of UGT1A1, increased the incidence of hyperbilirubinemia. However, there has been no direct evidence on the inhibition of UGT1A1 by nilotinib [4, 5]. Our present results provide the first direct evidence for the in vitro inhibition of UGT1A1 activity by nilotinib and might support the mechanism of hyperbilirubinemia proposed by Singer et al. [5].

The increase in the AUC ratio of other drug to nilotinib was estimated to be higher than 2.0, suggesting the high risk of drug–drug interactions involving nilotinib [8]. Food intake has been shown to significantly affect the absorption of nilotinib [9]. Increased absorption of nilotinib was most pronounced after a high-fat meal, associated with an 82% increase in the AUC. Therefore, the AUC ratio may exceed 2.0, when nilotinib is taken after ingesting such foods. Caution should therefore be exercised when a drug serving as a substrate of UGT1A1 is administered with nilotinib.

The genetic polymorphisms in *UGT1A1* that cause lower expression of the protein (e.g., **28*) or lower catalytic activity of the enzyme (e.g., **6*) seen in patients with Gilbert's syndrome may further increase the AUC ratio as suggested by Singer et al. [5].

Interestingly, the FDA report [4] proposed that nilotinib competitively inhibited UGT1A1 activity, which does not agree with our findings. The reason for this difference is unclear because the FDA report did not include any experimental data.

Apparent K_m for SN-38 glucuronidation by UGT1A1 expressed in the HLM was concordant with the previous results [10]. V_{max} for the enzymatic reaction was about twice as high as that previously reported [10].

We compared the inhibition potency of SN-38 glucuronidation by nilotinib with several other small-molecule tyrosine kinase inhibitors. The lowest concentration that inhibited 50% of the maximal SN-38 glucuronidation by human UGT1A1 was obtained with nilotinib (0.146 μM), followed by sorafenib (0.446 μM), erlotinib (0.457 μM), lapatinib (1.47 μM), dasatinib (1.52 μM), gefitinib (3.50 μM), imatinib, and sunitinib (>10 μM). The results indicate that nilotinib is the most potent UGT1A1 inhibitor among small-molecule tyrosine kinase inhibitors tested.

We found that nilotinib is a potent noncompetitive inhibitor of UGT1A1. The result is applicable for the appropriate clinical management of the use of nilotinib with medicines predominantly metabolized by UGT1A1.

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