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Research Article

Keywords: Dabrafenib, Irinotecan, SN-38 glucuronidation, UGT1A1, Drug-drug interaction

Posted Date: April 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-348479/v1>

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**Combination of dabrafenib with irinotecan might trigger off a higher frequency
of adverse events by pharmacokinetic interaction**

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Summary

Dabrafenib and irinotecan are two drugs that can be utilized to treat melanoma. A previous *in vivo* study has shown that dabrafenib enhances the antitumor activity of irinotecan in a xenograft model with unclear mechanism. This study aims to investigate the inhibition of dabrafenib on SN-38 (the active metabolite of irinotecan) glucuronidation using human liver microsomes (HLMs) and recombinant human UGT1A1, trying to elucidate the possible mechanism underlying the synergistic effect. Our data indicated that dabrafenib noncompetitively inhibited SN-38 glucuronidation in pooled HLMs and recombinant UGT1A1 with a $K_{i,u}$ value was 12.43 ± 0.28 and 2.64 ± 0.27 μM , respectively. Based on the *in vitro* $K_{i,u}$ value and estimation of kinetic parameters, dabrafenib administered at 150 mg twice daily may result in about 17%-81.9 % increase in the area under the curve (AUC) of SN-38 *in vivo*. Moreover, the ratios of $[I]_{\text{gut}}/K_{i,u}$ are 0.70 and 3.32 in HLMs and recombinant UGT1A1, respectively, indicating a high risk of drug-drug interactions (DDIs) when dabrafenib was used in combination with irinotecan. Our study provides a basis for further development and optimization of this combination in clinical research.

Keywords: Dabrafenib; Irinotecan; SN-38 glucuronidation; UGT1A1; Drug-drug interaction

1. Introduction

Malignant melanoma is a major worldwide public health problem with increasing

incidence. Specific oncogenes mutation, such as the BRAF mutation, as an important underlying cause of the melanoma, is present in approximately 60% of melanoma patients and has been developed as a therapeutic target [1-3]. Dabrafenib is a potent and selective ATP-competitive inhibitor for the BRAF kinase and has been approved for the treatment of unresectable or metastatic BRAF V600-mutat melanoma [4]. As a selective single agent, dabrafenib shows limited clinical activity. Therefore, several combination therapy studies of dabrafenib have been evaluated recently to improve the efficacy in melanoma patients [5-7]. Pharmacokinetic studies have shown that dabrafenib is metabolized by cytochrome P450 (CYP) 2C8 and CYP3A4, and is also an inducer of CYP3A4, CYP2B6, and CYP2Cs, implying possibility of drug-drug interactions (DDIs). However, only a few studies have described the potential for DDIs of dabrafenib [8,9].

Irinotecan, a commonly used chemotherapeutic agent, has been recognized in the treatment of cancers including melanoma [10]. SN-38 is the pharmacologically active metabolite of irinotecan and is mainly cleared by the hepatic UDP-glucuronosyltransferase 1A1 (UGT1A1) to SN-38 glucuronide (SN-38G), with weaker contributions of UGT1A3, 1A6, 1A7 and 1A9 [11]. The inhibition of UGT1A1 *in vivo* may increase systemic exposure to SN-38, which is responsible for the irinotecan-induced severe diarrhea and neutropenia [12]. Several tyrosine kinase inhibitors (TKIs), i.e., gefitinib, sunitinib and pazopanib, have been investigated in the combination with irinotecan and may cause clinical toxicity of irinotecan by inhibiting UGT1A1 [13-15]. An *in vivo* study has indicated that the combination of dabrafenib plus irinotecan can

result in increased anti-tumor activity in a xenograft model [16], suggesting a pharmacokinetic or pharmacodynamic interaction. Considering that dabrafenib has been proven to be a potent inhibitor of UGT1A1 [17], the combination of dabrafenib and irinotecan may produce DDIs by inhibiting UGT1A1-mediated SN-38 glucuronidation.

In this study, we investigated the effects of dabrafenib on SN-38 glucuronidation using human liver microsomes (HLMs) and recombinant human UGT1A1. The area under the plasma concentration-time curve (AUC) ratio was used to estimate the likely magnitude of drug interactions between dabrafenib and SN-38.

2. Materials and methods

2.1 Chemicals

Dabrafenib (purity>99%) was purchased from Selleck Chemicals (Houston, USA). SN-38 and its glucuronide SN-38G, 7-hydroxycoumarin and UDPGA (trisodium salt) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Alamethicin and Tris-HCl were acquired from Aladdin Industrial Corporation (Shanghai, China). All other reagents were of the highest grade and obtained from commercial sources.

2.2 Human liver microsomes and recombinant UGT1A1

Pooled human liver microsomes (HLMs) (protein content: 20 mg/mL) were obtained from Research Institute for Liver Diseases (RILD, Shanghai, China). Recombinant human UGT1A1 supersomes (protein content: 5 mg/mL), expressed in baculovirus-

infected insect cells, were obtained from BD Gentest (Woburn, MA, USA).

2.3 SN-38 glucuronidation inhibition assay

SN-38 glucuronidation inhibition assay was conducted by using a previously published method [18], with minor modifications. A typical incubation mixture (200 μ L final volume) consists of 50 mM Tris-HCl buffer (pH=7.4), 10 mM $MgCl_2$, 5mM UDPGA, 50 μ g/mg protein alamethicin, 1-10 μ M SN-38, 0.25 mg/mL HLMS or 0.2 mg/mL recombinant human UGT1A1 in the absence or presence of different concentrations dabrafenib (0.01, 0.05, 0.1, 1, 10, 50 and 100 μ M). Incubation period was 30 min for HLMS and recombinant UGT1A1. The reaction was terminated by the addition of 200 μ L ice-cold acetonitrile including 7-hydroxycoumarin as an internal standard, and then centrifuged at $20,500 \times g$ for 15 minutes to remove protein. SN-38 and SN-38 glucuronide were analyzed using a Waters ACQUITY UPLC combined with Applied Biosystems Qtrap 6500. Chromatographic separation was performed on a BEH C18 column (50 mm $\times$ 2.1 mm, 1.7 μ m Waters Corporation, Milford, MA). The column temperature and injection volume were set at 35 $^{\circ}$ C and 2 μ L, respectively. The mobile phase comprised 0.1% formic acid in water (A) and acetonitrile with 0.1% formic acid (B) at a flow rate of 0.3 mL/min. The following gradient: 0 to 2min, 5% B; 2 to 2.4 min, 5% B to 50% B; 2.4 to 3 min, 50% B; and 3 to 4 min, balanced to 5% B. The mass spectrometer operated in the multiple reaction monitoring mode (MRM) with proton adducts at 569.2/393.2 and 163.1/107.1 for SN-38G and internal standard (7-hydroxycoumarin), respectively.

2.4 Inhibition kinetic assay

Inhibition kinetic parameters (K_i) and inhibition type were determined using multiple concentrations of SN-38 (1, 2.5, 5 and 10 μM) in the absence or presence of different concentrations dabrafenib (0, 5, 10, 20 and 40 μM for pooled HLMs; 0, 0.5, 1, 2 and 5 μM for recombinant UGT1A1). K_i values were estimated by the nonlinear regression analysis of Eq. (1) for competitive inhibition, Eq. (2) for non-competitive inhibition, Eq. (3) for uncompetitive inhibition, and Eq. (4) for mixed inhibition [19]:

$$v = \frac{V_{max}S}{K_m(1+I/K_i)+S} \quad (1)$$

$$v = \frac{V_{max}S}{(K_m+S)(1+I/K_i)} \quad (2)$$

$$v = \frac{V_{max}S}{K_m+S(1+I/K_i)} \quad (3)$$

$$v = \frac{V_{max}S}{(K_m(1+I/K_i)+S(1+I/\alpha K_i))} \quad (4)$$

where v and V_{max} are the velocity and maximum velocity of the reaction, respectively; S and I are the substrate and inhibitor concentrations, respectively; K_m is Michaelis constant (substrate concentration at half of V_{max} of the reaction); K_i is inhibition constant describing the affinity of the inhibitor to the enzyme; and αK_i describing the affinity of the inhibitor to the complex of enzyme and substrate.

2.5 Quantitative prediction of DDI risk (AUC_i/AUC)

Methods for the prediction of the inhibitor concentration at the active site of the enzyme and the magnitudes of inhibitory interactions of dabrafenib and SN-38 used in the analysis are shown below.

The average systemic plasma concentration of dabrafenib after repeated oral administration ($[I]_{av}$), the maximum systemic plasma concentration of dabrafenib after repeated oral administration ($[I]_{max}$) and the maximum concentrations of dabrafenib in human gut lumen after a single oral administration ($[I]_{gut}$) were calculated for use in the analyses (Eq. (5), Eq. (6), and Eq. (7)) [20,21].

$$[I]_{av} = \frac{D/\tau}{CL/F} \quad (5)$$

$$[I]_{max} = \frac{[I]_{av}k\tau}{1-\exp(-k\tau)} \quad (6)$$

$$[I]_{gut} = \frac{D \cdot (1-F_a)}{V_L \cdot MW} \quad (7)$$

where D is the dose of dabrafenib used in the in vivo interaction study (D=150 mg); τ is the dosing interval ($\tau=12$ h); CL/F is the apparent clearance (CL/F=17 L/h); k is the elimination rate constant ($k=0.087$ h⁻¹); F_a is the oral bioavailability ($F_a=0.95$); MW is the molecular weight (MW=519.56 g/mol); and V_L is the average human gut volume ($V_L=1.65$ L/70kg) [22-24].

Nonspecific binding was accounted for when inhibitor constants ($K_{i,u}$) were calculated from *in vitro* inhibition studies. The fraction unbound of inhibitor (fu_{mic}) was calculated at a microsome concentration using Eq. (8) [25].

$$fu_2 = \frac{1}{\frac{C_2(1-fu_1)}{C_1 \cdot fu_1} + 1} \quad (8)$$

where fu_2 is the fu_{mic} calculated for HLM concentration (C_2) in the present studies; fu_1 is the fu_{mic} obtained from literature at a specified HLM concentration (C_1).

The magnitudes of inhibitory interactions of dabrafenib and SN-38 can be estimated as the ratio of the area under the plasma concentration-time curve (AUC) of SN-38 in the presence and absence of dabrafenib. This ratio was calculated using Eq. (9) for drugs with negligible renal clearance [26].

$$\frac{AUC_i}{AUC} = 1 + \frac{[I]}{K_{i,u}} \quad (9)$$

$K_{i,u}$ is unbound inhibitor constant; $[I]$ is the concentration of dabrafenib at UGT1A1 catalytic site ($[I]_{\max}$ and $[I]_{\text{gut}}$).

3. Results

3.1 Inhibition of SN-38 glucuronidation by dabrafenib in pooled HLMs and recombinant UGT1A1

To investigate the effect of dabrafenib against SN-38 glucuronidation, concentration-dependent inhibition of dabrafenib on the activity of SN-38 glucuronidation was determined using multiple concentrations of dabrafenib in pooled HLMs and recombinant UGT1A1. Nonlinear regression analysis showed that dabrafenib exhibited UDPGA- and concentration-dependent inhibition against SN-38 glucuronidation activity in HLMs (Fig. 1A) and recombinant UGT1A1 (Fig. 2A). The IC_{50} (half-maximal inhibitory concentration) values for HLMs and recombinant UGT1A1 were calculated to be 12.99 and 2.23 μM , respectively. The results demonstrated that dabrafenib exhibited potent inhibition of SN-38 glucuronidation in HLMs and recombinant UGT1A1.

3.2 Inhibition kinetic analysis in pooled HLMs and recombinant UGT1A1

Inhibition kinetic analysis was performed to further characterize the inhibition type and the inhibition constant (K_i) of dabrafenib against SN-38 glucuronidation. The representative Lineweaver-Burk plots (Fig. 1B, 2B) and Dixon plots (Fig. 1C, 2C)

shows the changes in both $V_{\max}$ and K_m values. Nonlinear regression analysis of the enzyme inhibition mode suggested that dabrafenib noncompetitively inhibited SN-38 glucuronidation in pooled HLMs and recombinant UGT1A1 with a K_i of 15.36 ± 0.35 and 4.63 ± 0.48 μM , respectively. The unbound fraction of dabrafenib in the incubation mixture containing 0.25 mg/mL HLMs has been reported to be 0.81 ± 0.06 [27]. Assuming that dabrafenib has the same unbound fraction in both supersomes and HLMs, the calculated unbound fraction of dabrafenib in 0.2 mg/mL UGT1A1 was 0.57. The calculated $K_{i,u}$ value was 12.43 ± 0.28 and 2.64 ± 0.27 μM for pooled HLMs and recombinant UGT1A1, respectively.

3.3 Potential for dabrafenib induced UGT1A1-mediated DDIs

To determine whether dabrafenib can cause significant DDIs when combined with irinotecan by inhibiting UGT1A1-mediated SN-38 glucuronidation, the inhibition potential *in vivo* was evaluated by estimating the alteration of AUC of SN-38 using the $K_{i,u}$ values obtained in the present study, $[I]_{\max}$ and $[I]_{\text{gut}}$ by using Eq. (5), Eq. (6), Eq. (7), Eq. (8) and Eq. (9). As shown in Table 1, the calculated $[I]_{\max}$ after oral administration of 150 mg twice daily dabrafenib was 2.16 μM , which was similar to the reported $C_{\max}$ (2.85 μM) [28]. Then, after oral administration of dabrafenib of 150 mg twice daily, the AUC of coadministered SN-38 was predicted to increase by 17%-82%. Moreover, the ratios of $[I]_{\text{gut}}/K_{i,u}$ are 0.70 and 3.32 in HLMs and recombinant UGT1A1, respectively, indicating a high risk of DDI when dabrafenib was used in combination with irinotecan.

4. Discussion

A previous *in vivo* study has shown synergistic effect of dabrafenib and irinotecan, but the mechanism is unclear [16]. Our results confirm that dabrafenib is a potent inhibitor of UGT1A1 and shows a concentration-dependent inhibition of SN-38 glucuronidation in HLMs and recombinant human UGT1A1, indicating potential dabrafenib-irinotecan interaction.

Fatal adverse events (up to 5.3%) have been reported among people receiving irinotecan monotherapy [29]. Exposure to SN-38, expressed as AUC of SN-38, is considered to be associated with the dose-limiting toxicities of irinotecan. The duration and severity of neutropenia were significantly correlated to prolonged systemic SN-38 exposure [30,31], and a higher incidence of severe diarrhea has been found to be correlated with a higher biliary index (the AUC of irinotecan multiplied by the ratio of SN-38 to SN-38G AUC) [32-34]. UGT1A1 is the main enzyme involved in the metabolic clearance of SN-38, and the prevalence of severe toxicity in cancer patients receiving irinotecan monotherapy can be well predicted by UGT1A1 genetic variation [35]. Both UGT1A1*6 and *28 polymorphisms, with approximately 70% reduction of UGT1A1 activity [36,37], have been shown to be associated with the increased systemic exposure to SN-38 in patients homozygous for these variants and increased clinical irinotecan-associated toxicity [38-41]. Considering that the UGT1A1 activity, the exposure level of SN-38 and irinotecan toxicity are closely related clinically, our results can help to optimize the dosage of the combination of dabrafenib and irinotecan,

thereby improving efficiency and reducing toxicity.

In vitro-in vivo extrapolation has become a common method for evaluating potential DDIs in drug development [42]. As reported previously [25], inhibitor constant ($K_{i,u}$), an important parameter of the extrapolation, was corrected for non-specific microsomal binding in our study, and therefore evaluated the potential of DDIs *in vivo* more accurately. Another important parameter was the inhibitor concentration at the enzyme active site ($[I]$). In view of the fact that the accumulation of SN-38 in the intestine is a significant contributor to irinotecan toxicity [12] and UGT1A1 is highly expressed in both human liver and gastrointestinal tract [43], the maximum systemic plasma concentration after repeated oral administration of dabrafenib ($[I]_{\max}$) and the maximum concentration in human gut lumen ($[I]_{\text{gut}}$) instead of the maximum hepatic input concentration were used to evaluate the effects on intestinal SN-38 glucuronidation. However, many other factors, including uptake and secretory transporters, comorbid conditions and dietary factors, may also influence the pharmacokinetic data [42]. It is necessary to conduct further pharmacokinetic study to acquire more accurate knowledge of potential DDIs associated with the combination of dabrafenib and irinotecan.

In conclusion, the present study reveals that dabrafenib has a significant inhibitory effect on UGT1A1-mediated SN-38 glucuronidation and highlights the DDI potential associated with the combination of dabrafenib and irinotecan. Our study provides a useful reminder for further development and optimization of this combined therapy in clinical research.

Author contributions

ZW performed the inhibition kinetic assay and *in vitro-in vivo* extrapolation. And she was a major contributor in writing the manuscript. ZW and XYW performed the LC-MS/MS assay. XYF and MRY prepared the Tris-HCl buffer, MgCl₂, UDPGA and alamethicin. LLJ and YLX plotted the data. YL and JC designed the concept and corrected and finalized the manuscript. All authors read and approved the final manuscript.

Funding

This study was financially supported by the National Key Research and Development Program of China (2017YFC1702006).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Ethical standards

No human and/or animal studies were performed in this study.

Code availability

Not applicable.

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Table 1. Quantitative prediction of DDI risk *in vivo* inhibition on UGT1A1 by dabrafenib.

Enzyme	$K_{i,u}$ (μM)	Dabrafenib (150 mg twice daily)			$[I]_{\text{max}}/K_{i,u}$	$[I]_{\text{gut}}/K_{i,u}$
		$[I]_{\text{av}}(\mu\text{M})$	$[I]_{\text{max}}(\mu\text{M})$	$[I]_{\text{gut}}(\mu\text{M})$		
HLM	12.43 ± 0.28	1.34	2.16	8.75	0.17	0.70
UGT1A1	2.64 ± 0.27	1.34	2.16	8.75	0.82	3.32

Figure captions

Fig.1 Inhibitory effect of dabrafenib against SN-38 glucuronidation in pooled HLMs.

(A) Dose-dependent inhibition of SN-38 glucuronidation by dabrafenib in pooled HLMs; (B) Dixon plot for dabrafenib inhibition of SN-38 glucuronidation in pooled HLMs; (C) Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in pooled HLMs; (D) Slopes of Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in pooled HLMs. All data points shown represent the mean $\pm$ standard error of the parameter fit from triplicate measurements.

Fig.2 Inhibitory effect of dabrafenib against SN-38 glucuronidation in recombinant UGT1A1. (A) Dose-dependent inhibition of SN-38 glucuronidation by dabrafenib in recombinant UGT1A1; (B) Dixon plot for dabrafenib inhibition of SN-38 glucuronidation in recombinant UGT1A1; (C) Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in recombinant UGT1A1; (D) Slopes of Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in recombinant UGT1A1. All data points shown represent the mean $\pm$ standard error of the parameter fit from triplicate measurements.

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Figures

Figure 1

Inhibitory effect of dabrafenib against SN-38 glucuronidation in pooled HLMs. (A) Dose-dependent inhibition of SN-38 glucuronidation by dabrafenib in pooled HLMs; (B) Dixon plot for dabrafenib inhibition of SN-38 glucuronidation in pooled HLMs; (C) Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in pooled HLMs; (D) Slopes of Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in pooled HLMs. All data points shown represent the mean $\pm$ standard error of the parameter fit from triplicate measurements.

Figure 2

Inhibitory effect of dabrafenib against SN-38 glucuronidation in recombinant UGT1A1. (A) Dose-dependent inhibition of SN-38 glucuronidation by dabrafenib in recombinant UGT1A1; (B) Dixon plot for dabrafenib inhibition of SN-38 glucuronidation in recombinant UGT1A1; (C) Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in recombinant UGT1A1; (D) Slopes of Lineweaver-Burk plot for dabrafenib inhibition of SN-38 glucuronidation in recombinant UGT1A1. All data points shown represent the mean $\pm$ standard error of the parameter fit from triplicate measurements.