Association between severe toxicity of nilotinib and UGT1A1 polymorphisms in Japanese patients with chronic myelogenous leukemia

慢性骨髄性白血病に罹患した日本人患者におけるニロチニブの
重篤な毒性と UGT1A1 遺伝子多型との関連

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Abstract

Background Nilotinib is a BCR-ABL kinase inhibitor approved for the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia (CML). The UDP-glucuronosyltransferase 1A1 (UGT1A1) polymorphism *UGT1A1*28 (*28) /*28* has been linked to an increased risk of hyperbilirubinemia in patients with CML who receive nilotinib. Beside **28*, *UGT1A1*6 (*6)* is another important variant allele in Japanese patients because it is associated with adverse events of irinotecan, metabolized by UGT1A1. We retrospectively investigated the association between severe toxicity of nilotinib and UGT1A1 polymorphisms (**6* and**28)* in Japanese patients with CML.

Patients and methods Eight patients with cytogenetically confirmed CML who were receiving nilotinib were studied to explore the association of UGT1A1 polymorphisms with severe nilotinib-related toxicity. Genotyping analyses were determined for **6* and **28*.

Results All 3 patients with the **6/*6* or **6/*28* genotype had severe toxicity, including QT interval prolongation (grade 3), elevated lipase levels (grade 3) plus hyperbilirubinemia (grade 2), and anemia (grade 3) plus hepatic cyst hemorrhage (grade 2) in 1 patient each. Among the 5 patients with the **6/*1* or **1/*1* genotype, 1 had elevated lipase levels (grade 3) and another had severe pain in the lower extremities

(grade 3).

Conclusion These findings suggested that UGT1A1 polymorphisms are important determinants of severe toxicity of nilotinib in Japanese patients.

Key Words: nilotinib; severe toxicity; *UGT1A1*6*; *UGT1A1*28*; chronic myelogenous leukemia

Introduction

Nilotinib is a small-molecule tyrosine kinase inhibitor targeting the BCR-ABL fusion protein, c-Kit, platelet-derived growth factor receptor (PDGFR) alpha and beta [1]. Nilotinib has been approved for the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia (CML) in adults with newly diagnosed disease or who are resistant to or intolerant of imatinib [2-4]. Severe toxicity has been reported in patients with CML who received nilotinib, including corrected QT (QTc) interval prolongation and hepatotoxicity. The package insert for nilotinib (Tasigna®) in the United States of cautions that nilotinib has been shown to prolong cardiac ventricular repolarization in a dose-dependent manner. Although the frequency of severe QTc interval prolongation associated with nilotinib was very low in large clinical trials [3, 5-7], QTc interval prolongation warrants caution because such cardiotoxicity is often fatal.

A pharmacogenetic study of 97 patients with CML who received nilotinib suggested that the homozygous genotype for UDP-glucuronosyltransferase 1A1 (UGT1A1) promoter polymorphism (**28/*28*) is associated with a significantly higher risk of hyperbilirubinemia than the **28/*1* and **1/*1* genotypes, with a relative risk of 4.5 (95% confidence interval, 1.9 to 10.8) [8]. UGT1A1, a polymorphic enzyme of the

glucuronidation pathway of bilirubin and some drugs, has clinically important variant alleles, such as *28* in the promoter region ($(TA)_6$> $(TA)_7$) and *6* in exon 1 (211G>A). In Japanese patients who receive irinotecan, an anticancer drug used to treat many cancers, previous studies have reported an association between severe toxicity of irinotecan and UGT1A1 polymorphisms (*6* and *28*) [9-11]. These studies demonstrated that the risk of irinotecan-induced severe toxicity, such as leukopenia and diarrhea, is higher in patients with the homozygous (*28/*28* or *6/*6*) or compound heterozygous (*6/*28*) UGT1A1 genotype.

In the present study, we investigated the association between UGT1A1 polymorphisms (*6* and *28*) and severe toxicity of nilotinib, especially adverse events such as QTc interval prolongation and hyperbilirubinemia, in Japanese patients with CML.

Patients and methods

Patients

Patients with cytogenetically confirmed CML who were receiving nilotinib at Nagoya University Hospital from June 2009 to May 2012 were retrospectively studied to explore the association of UGT1A1 polymorphisms with severe nilotinib-related

toxicity. At the time of treatment, all patients were 20 years or older and gave written informed consent for their peripheral blood samples and medical information to be used for research purposes. The study protocol was approved by the Institutional Review Board of Nagoya University Hospital, and the study was conducted in accordance with the Declaration of Helsinki.

Treatment

Patients were given nilotinib orally twice daily on consecutive days in accordance with the Japanese package insert, taking the patient compliance instruction from pharmacists. In principle, the initial dose levels were 400 mg twice daily for patients who had resistant disease or were intolerant of imatinib, and 300 mg twice daily for those with newly diagnosed CML. Additional dose modifications, other supportive treatments, and clinical evaluations in individual patients were essentially left to the discretion of the treating physicians.

Genomic analysis

Blood samples for genetic analysis were obtained, collected into heparinized tubes, and stored at 4°C until analysis. Genotyping analyses for **6* and **28* were performed

with the use of an approved Invader ® UGT1A1 assay kit (Sekisui Medical Co., Ltd., Tokyo, Japan) at Bio Medical Laboratories, Inc., Tokyo, Japan [12] .

Clinical evaluation of toxicity

Clinical information was extracted from the medical records of individual patients who received nilotinib. The severity of toxicity was graded by the treating physicians according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. Severe toxicity in this study was defined as grade 3 or higher toxicity and grade 2 toxicity requiring drug interruption.

Laboratory studies were performed every month in all patients. Electrocardiography (ECG) was performed by laboratory technicians at baseline, 7 days after the initiation of treatment, and subsequently. The details of clinical evaluations in individual patients were essentially left to the discretion of the treating physician. QTc intervals were initially monitored by an automatic analyzing system equipped with the ECG units. In the event of serious QTc interval prolongation, the ECG recordings were confirmed by a cardiologist. Serious QTc interval prolongation was defined as grade 2 or higher toxicity (QTc >480 msec). Severe hyperbilirubinemia was defined as an elevated total bilirubin level of grade 2 or higher (>1.8 mg/dl, corresponding to >1.5 times the upper limit of

normal in the laboratory where the sample was analyzed).

Statistical analysis

We divided the patients into 2 groups according to the classification used for Japanese patients treated with irinotecan: UGT1A1 poor metabolizers (**6/*6, *28/*28*, or **6/*28*) and UGT1A1 extensive metabolizers (**6/*1*, **28/*1*, or **1/*1*) [9-11]. We evaluated the association between these groups and severe toxicity with the use of Fisher's exact test, using IBM® SPSS® Statistics 20 software (SPSS Inc., an IBM Company, Armonk, New York, USA). P values of less than 0.05 were considered to indicate statistical significance.

Results

Eight patients who received nilotinib were studied (Tables 1 and 2). Two patients had newly diagnosed disease, 5 were resistant to or intolerant of imatinib, and the remaining patient was intolerant of imatinib and interferon-alpha. Six patients needed reductions in the initial dose of nilotinib. Dose reduction was necessitated by arrhythmias (atrial fibrillation) in 2 patients, a prior history of aortic valve replacement in 1, overt aortic regurgitation in 1, anemia in 1, and severe toxicity during previous treatment with

imatinib (persistent nausea, fatigue and dizziness, grade2 or higher) in 1. CML-chronic phase (CML-CP) was maintained during treatment in all 8 patients. The median duration of treatment was 29.8 months (range: 8.4 - 45.3 months). Molecular optimal responses were confirmed in all 8 patients. All 8 patients continued to receive a stable dose level to maintain CML-CP. Three patients concurrently received other drugs that can inhibit the activity of CYP3A4, the major drug-metabolizing enzyme of nilotinib, such as warfarin for arrhythmias, amlodipine (a calcium antagonist) for hypertension, and ebastine (a histamine antagonist) for allergic rhinitis. However, these drugs apparently had no clinical effect on the severe toxicity of nilotinib.

Five of the 8 patients had severe toxicity during treatment with nilotinib (Table 2). Nilotinib-induced toxicity included QTc interval prolongation (grade 3), elevated lipase levels (grade 3) plus hyperbilirubinemia (grade 2), anemia (grade 3) plus hepatic cyst hemorrhage with abdominal pain (grade 2), elevated lipase levels (grade 3), and severe bilateral pain in the lower extremities (grade 3) in 1 patient each. Dose reduction during the treatment period was required because of severe toxicity in 5 patients and because of a deterioration of symptoms due to aortic regurgitation in 1 patient.

Pharmacogenetic analysis

Genotyping analyses revealed the homozygous genotype (**6/*6*) in 2 patients, the compound heterozygous genotype (**6/*28*) in 1, the single heterozygous genotype (**6/*1*) in 1, and the wild type genotype (**1/*1*) in 4 (Table 2). All 3 poor metabolizers with the **6/*6* or **6/*28* genotype had severe toxicity. One patient with the **6/*6* genotype had QTc interval prolongation (grade 3). The other patient with the **6/*6* genotype had elevated lipase levels (grade 3) plus hyperbilirubinemia (total bilirubin, 3.0 mg/dl, grade 2) on day 4. The patient with the **6/*28* genotype had anemia (grade 3) plus hepatic cyst hemorrhage with abdominal pain (grade 2). In this patient, anemia developed 1 month after the initiation of nilotinib, and hepatic cyst hemorrhage occurred 2 weeks after increasing the dose from 200 mg twice daily to 300 mg twice daily 6 months after the initiation of nilotinib. Two of the 5 extensive metabolizers with the **6/*1* or **1/*1* genotype had severe toxicity. One patient with the **1/*1* genotype had severe bilateral pain of the lower extremities (grade 3) on day 6. The other patient, who also had the **1/*1* genotype, had elevated lipase levels (grade 3) on day 5.

The poor metabolizers appeared to be more likely to have severe toxicity than the extensive metabolizers, although the incidence of severe toxicity did not differ significantly between the poor metabolizers and the extensive metabolizers ($p = 0.196$).

Discussion

Among the 8 Japanese patients with CML who received nilotinib in our study, all 3 UGT1A1 poor metabolizers with the **6/*6* or **6/*28* genotype had severe nilotinib-related toxicity: QTc interval prolongation (grade 3), elevated lipase levels (grade 3) plus hyperbilirubinemia (grade 2), and anemia (grade 3) plus hepatic cyst hemorrhage (grade 2) occurred in patient each.

Nilotinib is known to often cause QTc interval prolongation and hyperbilirubinemia. In the ENESTnd trial, a pivotal randomized phase 3 trial showing the superiority of nilotinib to imatinib in patients with newly diagnosed CML, the incidences of grade 3 or 4 hyperbilirubinemia (5.7%), elevated lipase levels (7.4%), and hyperglycemia (5.1%) in the 556 patients who received nilotinib were higher than the respective values in the 280 patients who received imatinib (<1.0%, 3%, and 0%, respectively). In the nilotinib group, the QTc interval was prolonged by >480 msec (grade 2 or higher) in 1 patient (<1%), and 3 patients had QTc interval prolongations of >60 msec as compared with the baseline value [5]. In the GIMEMA trial, a phase 2 study in which nilotinib showed promising efficacy in CML, 2 (2.7%) of the 73 patients had QTc interval prolongations of >450 msec (grade 1 or 2) [6]. Although the incidence of severe QTc interval prolongation is very low in patients who receive nilotinib, caution should be exercised

concerning this potentially fatal toxic effect.

In our study, 1 patient with the **6/*6* genotype had QTc interval prolongation while receiving nilotinib at half of the standard dose. The patient, a 66-year-old woman, had undergone aortic valve replacement for severe aortic stenosis 4 years before treatment with nilotinib. She was initially given nilotinib in a low dose of 150 mg daily as compared the standard dose of 300 mg twice daily because of the prior history of heart disease. At the baseline assessment, the QTc interval on ECG was 471 msec (grade 1). Two months after the initiation of nilotinib, the dose was increased to 300 mg daily because there was no severe toxicity. One month after increasing the dose to 300 mg daily, equivalent to half of the recommended dose, the QTc interval was prolonged to 531 msec (grade 3) with no apparent symptoms. The dose of nilotinib was again reduced to 200 mg daily to avoid unexpected, potentially fatal cardiotoxicity. Then, 1 month after the second reduction in the dose of nilotinib, the QTc interval recovered to 470 msec (grade 1), with no potentially fatal cardiac events. Our results thus suggested that QTc interval prolongation occurs in a dose-dependent manner. The mechanism underlying the dose-dependent prolongation of QTc interval is unclear, but our findings suggest that QTc interval prolongation is caused by an increased plasma concentration of nilotinib. The reduced UGT1A1 activity in this patient probably increased the risk of

QTc interval prolongation. Although glucuronidation of nilotinib by UGT1A1 has not been reported, we speculate that metabolic pathway of nilotinib involves direct glucuronidation by UGT1A1.

Drug-induced QTc interval prolongation is acknowledged to be a clinically important cardiotoxic effect, potentially causing sudden death [13]. Such cardiotoxicity can precipitate critical arrhythmias, such as polymorphic ventricular tachycardia and torsade de pointes and has thus received considerable attention over the past few decades. Drug-induced QTc interval prolongation is known to be related to genetic variations such as single nucleotide polymorphisms (SNPs) in potassium-channel genes [14]. A limitation of our study is that we did not investigate SNPs related to QTc interval prolongation in the patient with the **6/*6* genotype who had QTc interval prolongation despite the very low dose of nilotinib.

The occurrence of hyperbilirubinemia in another patient with the **6/*6* genotype suggested that the variant allele **6* is a clinically important variant that should be tested in Asian patients with CML who receive nilotinib, similar to **28*. Several studies have reported that UGT1A1 polymorphisms increase the risk of severe irinotecan-related toxicity, such as diarrhea and leukopenia, by reducing UGT1A1 enzyme activity [15]. Furthermore, the variant allele **6* has been found to be related to severe toxicity in

patients who receive irinotecan [9, 10]. There are significant ethnic differences in the distribution of UGT1A1 polymorphisms. The allele frequency of **28* is 38.8% in whites and 9.7% in Japanese. In contrast, the allele frequency of **6* is 15.7% in Japanese, as compared with only 0.7% in whites [16]. The total frequency of the homozygous and compound heterozygous genotypes (**28/*28*, **6/*6*, and **6/*28*) is 9% in Japanese [10]. Therefore, Japanese and other Asians should undergo genotyping for **6* as well as **28* to accurately predict the risk of severe toxicity of nilotinib.

The mechanism of hyperbilirubinemia has been attributed to reduced UGT1A1 activity caused by nilotinib and UGT1A1 polymorphisms [8]. An in vitro study demonstrated that nilotinib inhibits the glucuronidation of paracetamol, which is glucuronized by UGT1A1 [17]. Another study reported that nilotinib suppresses the glucuronidation of SN-38, an active metabolite of irinotecan, in vitro [18]. At present, however, variant UGT1A1 genotypes have not been demonstrated to reduce the glucuronidation of nilotinib in vitro or in vivo.

In our study, 5 of the 8 patients (63%) had severe toxicity during treatment with nilotinib. The relatively high incidence of severe toxicity may be attributed to the small number of patients studied or differences in patient characteristics between our study and previous clinical trials.

To our knowledge, this is the first study to show an association between severe nilotinib-related toxicity and UGT1A1 polymorphisms (**6* and **28*) in patients with CML who received this drug. The poor metabolizers were apparently at greater risk for severe toxicity than the extensive metabolizers. Our results suggested that the variant allele **6* is an important determinant of severe toxicity, including QTc interval prolongation and hyperbilirubinemia, in Japanese patients who receive nilotinib. Although the small number of patients is a major limitation of this study, we believe that genetic testing for UGT1A1 variants in individual patients who are scheduled to receive nilotinib would facilitate selection of the optimal dosage or suggest the need for using another drug with a lower risk of cardiotoxicity.

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Conflict of interest

Dr. T Naoe received research grants from Janssen, Novartis, Kyowa-Hakko Kirin,

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Table 1 Patient characteristics

Patients:		n = 8
Median age: y (range)		66 (34-74)
Gender: n (%)	male	3 (37.5)
	female	5 (62.5)
Prior treatment: n (%)	0	2 (25.0)
	1	5 (62.5)
	2	1 (12.5)
Reduction in	yes	6 (75.0)
initial dosage: n (%)	no	2 (25.0)
Drug-drug interaction [a]: n (%)	yes	3 (37.5)
	no	5 (62.5)

[a]: Three patients simultaneously received drugs that can inhibit CYP3A4 activity, such as warfarin for arrhythmias, amlodipine (a calcium antagonist) for hypertension, and ebastine (a histamine antagonist) for allergic rhinitis. However, these drugs apparently had no clinical effect on the severe toxicity of nilotinib.

Table 2 UGT1A1 genotypes and severe toxicity in individual patients

Age (y)	Gender	Prior therapy	Complications	Initial dosage (mg)
66	female	none	prior surgery for aortic valve replacement	150 daily
48	female	imatinib	none	400 twice daily
74	female	imatinib	anemia	200 twice daily
74	male	imatinib	atrial fibrillation	200 twice daily
65	male	none	atrial fibrillation chronic renal failure	150 daily
51	female	imatinib	none	300 twice daily
34	female	imatinib	none	400 twice daily
67	male	imatinib interferon-alpha	aortic regurgitation	200 twice daily

(sequel to Table 2)

Molecular response	Total bilirubin [a] at baseline (mg/dl)	QTc interval at baseline (msec)	UGT1A1 genotype	Severe toxicitiy [b]
Major	0.7	471	*6/*6	QTc interval prolongation (grade 3)
Major	0.7	413	*6/*6	elevated lipase levels (grade 3) hyperbilirubinemia (grade 2)
Major	0.5	464	*6/*28	anemia (grade 3) hepatic cyst hemorrhage (grade 2)
Major	0.7	404	*1/*1	none
Major	0.8	408	*1/*1	none
Major	0.6	419	*6/*1	none
Complete	0.9	414	*1/*1	severe pain in extremities (grade 3)
Major	0.8	402	*1/*1	elevated lipase levels (grade 3)

[a]: The normal range is 0.3 to 1.3 mg/dl.

[b]: Severe toxicity included any grade 3 or higher toxicity and grade 2 toxicity requiring drug interruption.