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ORIGINAL PAPER

Effects of polymorphisms in CYP2D6 and ABC transporters and side effects induced by gefitinib on the pharmacokinetics of the gefitinib metabolite, *O*-desmethyl gefitinib

Hiroyuki Kobayashi[1] · Kazuhiro Sato[2] · Takenori Niioka[1] · Masahide Takeda[2] · Yuji Okuda[2] · Mariko Asano[2] · Hiroshi Ito[2] · Masatomo Miura[1]

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Abstract We investigated the effects of polymorphisms in *CYP2D6*, *ABCB1*, and *ABCG2* and the side effects induced by gefitinib on the pharmacokinetics of *O*-desmethyl gefitinib, the active metabolite of gefitinib. On day 14 after beginning therapy with gefitinib, plasma concentrations of gefitinib and *O*-desmethyl gefitinib were measured. Patients were grouped into three groups according to their combination of *CYP2D6* alleles: homozygous extensive metabolisers (EMs; **1/*1*, **1/*2*, and **2/*2*; $n = 13$), heterozygous EMs (**1/*5*, **2/*5*, **1/*10*, and **2/*10*; $n = 18$), and intermediate metabolisers (IMs; **5/*10* and **10/*10*; $n = 5$). The median AUC_{0-24} of *O*-desmethyl gefitinib in *CYP2D6* IMs was 1460 ng h/mL, whereas that in homozygous EMs was 12,523 ng h/mL ($P = 0.021$ in univariate analysis). The median AUC ratio of *O*-desmethyl gefitinib to gefitinib differed among homozygous EMs, heterozygous EMs, and IMs at a ratio of 1.41:0.86:0.24 ($P = 0.030$). On the other hand, there were no significant differences in the AUC_{0-24} of *O*-desmethyl gefitinib between *ABCB1* and *ABCG2* genotypes. In a multivariate analysis, *CYP2D6* homozygous EMs ($P = 0.012$) were predictive for a higher AUC_{0-24} of *O*-desmethyl gefitinib. The side effects of diarrhoea, skin rash, and hepatotoxicity induced by gefitinib were unrelated to the AUC_{0-24} of *O*-desmethyl gefitinib. *CYP2D6* polymorphisms were associated with the formation of *O*-desmethyl gefitinib from gefitinib. In *CYP2D6* homozygous EMs, the plasma concentrations of *O*-desmethyl gefitinib were higher over 24 h after taking gefitinib than those of the parent compound; however, side effects induced by gefitinib were unrelated to *O*-desmethyl gefitinib exposure.

Keywords Gefitinib · *O*-Desmethyl gefitinib · CYP2D6 · ABC transporter

Abbreviations

ABC	ATP-binding cassette
AUC	Area under the observed plasma concentration–time curve
BCRP	Breast cancer resistance protein
CYP	Cytochrome P450
EGFR	Epidermal growth factor receptor
EMs	Extensive metabolisers
HPLC	High-performance liquid chromatography
IMs	Intermediate metabolisers
NSCLC	Non-small cell lung cancer
SNP	Single nucleotide polymorphism

Background

Gefitinib is a selective inhibitor of the epidermal growth factor receptor (EGFR) and is used for the treatment of patients with non-small cell lung cancer (NSCLC) [1]. Gefitinib is metabolised by cytochrome P450 (CYP) 2D6 to the *O*-desmethyl metabolite [2–5], which inhibits subcellular EGFR tyrosine kinase through a mechanism similar to that of gefitinib (half-maximal inhibitory concentration [IC_{50}] = 0.036 versus 0.022 μM, respectively). However, because the transition to tissue is low for this metabolite,

✉ Masatomo Miura
m-miura@hos.akita-u.ac.jp

[1] Department of Pharmacy, Akita University Hospital, 1-1-1 Hondo, Akita 010-8543, Japan

[2] Department of Cardiovascular and Respiratory Medicine, Akita University Graduate School of Medicine, Akita, Japan

O-desmethyl gefitinib has minor effects on tumour growth [6]. In an alternative pathway, gefitinib is metabolised to its morpholine ring-opened metabolite by CYP3A4 and CYP3A5 [2]. Among gefitinib metabolites, only *O*-desmethyl gefitinib can be identified in human plasma at concentrations similar to those of the parent compound; the plasma concentrations of other gefitinib metabolites are very low or even undetectable [2, 4, 7].

The formation of *O*-desmethyl gefitinib seems to be dependent on CYP2D6 activity [8–10]. Therefore, polymorphisms in the *CYP2D6* gene influence the rate of elimination of gefitinib. Single administration of gefitinib has been reported to yield concentrations that are about twofold higher in poor metabolisers (PMs) of CYP2D6 (e.g. **4/*4*, **4/*5*, and **3/*4*) compared with extensive metabolisers of CYP2D6 (patients having the **1* or **2* allele) [9]. In Asian populations, however, the non-functional *CYP2D6*3*, **4*, and **6* allelic variants have not been observed, and the frequency of the non-functional *CYP2D6*5* allele is very low [11]. The *CYP2D6*10* allele, the most common allele in Asians at frequencies of 33–50 %, is a reduced-function allele [12, 13]. However, in our previous study, Japanese patients with NSCLC having the *CYP2D6*5* or **10* allele did not exhibit abnormalities in gefitinib exposure [14].

The effects of *CYP2D6* polymorphisms on gefitinib exposure have been studied [9, 14]; however, the effects of *CYP2D6*5* and **10* polymorphisms on the formation of *O*-desmethyl gefitinib from gefitinib have not been clarified. Because in vitro studies have reported that the formation of *O*-desmethyl gefitinib from gefitinib is mediated by CYP2D6 alone [3–5], individual variability in CYP2D6 metabolism could be more relevant for *O*-desmethyl gefitinib than for the parent compound. Accordingly, in patients with NSCLC, we may be able to estimate the involvement of *CYP2D6* polymorphisms in gefitinib exposure by using the individual pharmacokinetics of gefitinib and *O*-desmethyl gefitinib. In addition, the relationship between *O*-desmethyl gefitinib exposure and side effects, such as diarrhoea, skin rash, and hepatotoxicity, has not been clarified, although this metabolite is not expected to affect the tumour [6]. *O*-Desmethyl gefitinib is excreted predominantly in faeces [2, 15]. ATP-binding cassette (ABC) transporters, such as P-glycoprotein (encoded by the *ABCB1* gene) and breast cancer resistance protein (BCRP, encoded by the *ABCG2* gene), may be involved in the biliary excretion of *O*-desmethyl gefitinib because gefitinib is a substrate of P-glycoprotein and BCRP [7, 16]. However, no studies have reported the effects of *ABCB1* and *ABCG2* polymorphisms on *O*-desmethyl gefitinib exposure.

In the present study, we investigated the effects of polymorphisms in *CYP2D6*, *ABCB1*, and *ABCG2* and the side effects induced by gefitinib on the pharmacokinetics of *O*-desmethyl gefitinib.

Methods

Patients and protocols

Thirty-six Japanese patients with NSCLC (24 women and 12 men) taking gefitinib (Iressa®; AstraZeneca, Osaka, Japan), who were treated at the Akita University Hospital from January 2011 through April 2015, were prospectively enrolled in the study. All 28 patients with NSCLC who had undergone analysis of the *CYP2D6* genotype in our previous study [14] were included in the current study. The demographic and clinical characteristics of the patients on day 14 after gefitinib therapy are listed in Table 1. The study protocol was approved by the Ethics Committee of Akita University School of Medicine (number 1140), and all patients provided written informed consent for participation in the study. The toxicity grades for diarrhoea, skin rash, and hepatotoxicity were categorised as described in the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Gefitinib 250 mg was orally administered once daily at 08:00 (morning time). During the study period, the patients were not permitted to consume or ingest drugs or foods that were known to affect CYP3A and P-glycoprotein function. On day 14 after beginning gefitinib therapy, whole blood samples were collected just prior to and at 1, 2, 4, 6, 8, 12, and 24 h after oral gefitinib administration. Plasma was isolated by centrifugation at 1900×*g* for 15 min and was stored at −40 °C until analysis.

Analytical methods

O-Desmethyl gefitinib was purchased from Toronto Research Chemicals Inc. (Ontario, Canada). Plasma concentrations of gefitinib and *O*-desmethyl gefitinib were measured by high-performance liquid chromatography (HPLC) [17]. Namely, following the addition of erlotinib (25 ng/10 μL methanol) as an internal standard to a 100-μL plasma sample, the plasma sample was diluted with 900 μL water and vortexed for 30 s. This mixture was applied to an Oasis HLB extraction cartridge that had been activated previously with methanol and water (1.0 mL each). The cartridge was then washed with 1.0 mL water and 1.0 mL of 40 % methanol in water and eluted with 1.0 mL of 100 % methanol. Eluates were evaporated to dryness in a vacuum at 40 °C using a rotary evaporator (Iwaki, Tokyo, Japan). The resulting residue was then dissolved in 20 μL methanol and vortexed for 30 s; 20 μL of the mobile phase was added to the sample, and the

Table 1 Clinical characteristics of patients receiving gefitinib

Characteristics	Frequency
Total number	36
Female/male	24: 12
Age (year)	68.4 (65.3–71.5)
Body weight (kg)	53.1 (49.9–56.4)
Body surface area (m^2)	1.51 (1.46–1.57)
Laboratory test values	
White blood cells ($\times 10^3$ mm^{-3})	6.5 (5.5–7.4)
Red blood cells ($\times 10^4$ mm^{-3})	427 (409–445)
Platelets ($\times 10^4$ mm^{-3})	203 (184–222)
Aspartate transaminase (IU/L)	34.2 (5.7–62.8)
Alanine transaminase (IU/L)	43.7 (3.3–84.0)
Serum albumin (g/dL)	3.8 (3.6–4.0)
Total bilirubin (mg/dL)	0.6 (0.5–0.6)
Serum creatinine (mg/dL)	0.58 (0.51–0.64)
EGFR mutation status	Exon19/exon21 = 18:18
CYP2D6	Homozygous EMs (**1/*1*: **1/*2*: **2/*2* = 6:6:1)
	Heterozygous EMs (**1/*5*: **1/*10*: **2/*5*: **2/*10* = 1:12:3:2)
	IMs (**5/*10*: **10/*10* = 2: 3)
ABCB1 1236C>T	C/C: C/T: T/T = 4:11:21
ABCB1 2677G>T/A	G/G: G/T: G/A: T/T: T/A: A/A = 6:18:1:7:3:1
ABCB1 3435C>T	C/C: C/T: T/T = 13:15:8
ABCG2 421C>A	C/C: C/A: A/A = 18:15:3

Data are presented as number or mean (two-sided 95 % confidence interval)

EMs extensive metabolisers, *IMs* intermediate metabolisers

sample was vortexed for another 30 s. A 20-μL aliquot of the sample was then processed by HPLC. The HPLC system was comprised of a PU-2080 plus chromatography pump (JASCO, Tokyo, Japan) equipped with a CAPCELL PAK C18 MG ∏ HPLC column (250 mm × 4.6 mm I.D.; Shiseido, Tokyo, Japan), a UV-2075 light source, and an ultraviolet detector (JASCO). The mobile phase was 0.5 % KH_2PO_4 (pH 3.5)–acetonitrile–methanol (55:25:20, v/v/v) and was degassed in an ultrasonic bath prior to use. The flow rate was 0.5 mL/min at ambient temperature, and sample detection was carried out at 250 nm. The coefficients of variation for intra- and interday assays were less than 8.4 %. Accuracies for intra- and interday assays were within 3.1 %. The limits of quantification for gefitinib and *O*-desmethyl gefitinib were each 10 ng/mL.

Identification of genotypes

DNA was extracted from peripheral blood samples using a QIAamp Blood Mini Kit (Qiagen, Tokyo, Japan) and was stored at −80 °C until analysis. The *CYP2D6*5* (deleted) allele and *CYP2D6*10* (reduced) allele were identified using long polymerase chain reaction (PCR) analysis and the PCR–restriction fragment length polymorphism (RFLP) method, respectively, as described by Naveen et al. [18]. The patients were grouped into three groups according to the combination of alleles: extensive metabolisers (EMs; *CYP2D6*1/*1*, **1/*2*, and **2/*2*; $n = 13$), heterozygous EMs (*CYP2D6*1/*5*, **2/*5*, **1/*10*, and **2/*10*; $n = 18$), and intermediate metabolisers (IMs; *CYP2D6*5/*10* and **10/*10*; $n = 5$; Table 1). Genotyping procedures identifying the C and T alleles in exon 26 (3435C > T) of the *ABCB1* gene were performed using the PCR–RFLP method described by Cascorbi et al. [19]. The *ABCG2* 421C > A polymorphism was genotyped by the PCR–RFLP method described by Kobayashi et al. [20]. The analytic results obtained from PCR–RFLP were confirmed using a fully automated single nucleotide polymorphism (SNP) detection system (prototype i-densy™, ARKRAY Inc., Kyoto, Japan). All frequencies for the different analysed loci were at Hardy–Weinberg equilibrium.

Pharmacokinetic analysis

The pharmacokinetic analyses of gefitinib and *O*-desmethyl gefitinib were carried out using standard non-compartmental methods with Phoenix WinNonlin Version 6.4 (Pharsight Co., Mountain View, CA, USA). The area under the observed plasma concentration–time curve

(AUC) from 0 to 24 h was calculated using the linear trapezoidal rule.

Statistical analyses

The Shapiro–Wilk test was used to assess the data distribution. The clinical characteristics of patients taking gefitinib were expressed as the number or mean value (two-sided 95 % confidence interval) on day 14 after beginning gefitinib therapy. The AUC_{0-24} of *O*-desmethyl gefitinib, gefitinib, total AUC_{0-24} of gefitinib plus *O*-desmethyl gefitinib, and AUC ratio of *O*-desmethyl gefitinib to gefitinib were expressed as medians and first to third quartiles. Kruskal–Wallis tests or Mann–Whitney *U* tests were used to determine the differences in continuous values between groups. Spearman's rank correlation coefficient tests were applied to assess correlations between the AUC value and patient clinical characteristics, and all results were expressed as the correlation coefficient of determinant (*r*). A stepwise multiple linear regression analysis for AUC value was performed to determine the effects of factors with *P* values of greater than 0.2 as examined in univariate analysis. For each patient, the genotypes of the CYP2D6 or drug transporters were replaced with dummy variables (1 and 0, 0 and 1, or 0 and 0). Differences or correlations with *P* values of less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS 20.0 for Windows (SPSS IBM Japan Inc., Tokyo, Japan).

Results

There were no significant correlations between the AUC_{0-24} of *O*-desmethyl gefitinib or the AUC ratio of *O*-desmethyl gefitinib to gefitinib and gender, age, body weight, body surface area, or laboratory test values (Table 2).

The genotype frequency for the *CYP2D6* polymorphism in 36 Japanese patients with NSCLC is shown in Table 1. The mean plasma concentrations of *O*-desmethyl gefitinib were lowest in patients having *CYP2D6*5/*10* or **10/*10*, intermediate in the those having heterozygous *CYP2D6*1* or **2* alleles, and highest in those having *CYP2D6*1/*1*, **1/*2*, or **2/*2* (Fig. 1). The median AUC_{0-24} of *O*-desmethyl gefitinib in patients with *CYP2D6*5/*10* or **10/*10* was 1460 ng h/mL, whereas that in patients having *CYP2D6*1/*1*, **1/*2*, or **2/*2* was 12,523 ng h/mL ($P = 0.021$, Table 3). The ratio of the AUC of *O*-desmethyl gefitinib to that of gefitinib differed significantly among the three groups, with the median ratio for homozygous EMs to heterozygous EMs to IMs being 1.41:0.86:0.24 ($P = 0.030$, Table 3). However, there was no significant difference in the AUC_{0-24} of gefitinib among the *CYP2D6* genotypes ($P = 0.467$). On the other hand, there were no significant differences in the AUC_{0-24} values of *O*-desmethyl gefitinib in patients with the *ABCB1* or *ABCG2* transporter genotypes (Table 3).

The results of multivariate analysis including covariates are listed in Table 4. *CYP2D6* EMs, such as those having **1/*1*, **1/*2*, and **2/*2* ($P = 0.012$), were independently predictive for higher AUC_{0-24} of *O*-desmethyl gefitinib. In addition, for the AUC ratio of *O*-desmethyl gefitinib to gefitinib, *CYP2D6*1/*1*, **1/*2*, and **2/*2* ($P = 0.001$) and younger age ($P = 0.032$) were independently predictive.

The AUC_{0-24} values of *O*-desmethyl gefitinib in patients with diarrhoea ($n = 17$) were significantly lower than those in patients without diarrhoea ($n = 19$), suggesting that exposure to gefitinib, but not its *O*-desmethyl metabolite, contributed to the incidence of diarrhoea (Table 5). In addition, there was no significant difference in *O*-desmethyl gefitinib exposure between patients with ($n = 22$) and without ($n = 14$) a skin rash and between patients with ($n = 20$) and without ($n = 16$) hepatotoxicity (Table 5).

Discussion

Side effects of diarrhoea, skin rash, and hepatotoxicity induced by gefitinib were unrelated to the plasma exposure of the active *O*-desmethyl metabolite and the total exposure of gefitinib and *O*-desmethyl gefitinib. The patients with higher exposure to the parent compound gefitinib exhibit initial onset of diarrhoea, followed later by hepatotoxicity with continuous administration [14, 17]. Higher gefitinib exposure, but not *O*-desmethyl metabolite exposure, seemed to be associated with the incidence of diarrhoea and hepatotoxicity. On the other hand, in patients showing lower plasma exposure of *O*-desmethyl gefitinib, it is possible that the concentration of the metabolite may be high in the gastrointestinal tract, potentially stimulating the gut and resulting in induction of diarrhoea, because *O*-desmethyl gefitinib is excreted predominantly in faeces after metabolism in the liver [2, 15]. However, three of five patients with the *CYP2D6*5/*10* or **10/*10* genotype, showing low metabolite formation, also developed diarrhoea. Therefore, the analysis of the plasma concentrations of gefitinib, but not *O*-desmethyl gefitinib, may be necessary because of its direct relationship with the occurrence of diarrhoea and hepatotoxicity. On the other hand, gefitinib-induced skin rash did not appear to be related to exposure of either gefitinib or *O*-desmethyl gefitinib.

*CYP2D6*5* and **10* polymorphisms were associated with the formation of *O*-desmethyl gefitinib from gefitinib. By analysis of the *O*-desmethyl metabolite of gefitinib, we were able to confirm the effects of the *CYP2D6* polymorphism on the formation of *O*-desmethyl gefitinib. In

Table 2 Comparisons and correlations of clinical characteristics and AUC_{0-24} of *O*-desmethyl gefitinib or AUC ratio of *O*-desmethyl gefitinib to gefitinib

Clinical characteristics	*O*-Desmethyl gefitinib AUC_{0-24}		AUC ratio of *O*-desmethyl gefitinib to gefitinib	
	Median (quartile 1–quartile 3) (ng h/mL)	*P* value	Median (quartile 1–quartile 3)	*P* value
Gender		0.562		0.882
Male	6203 (2989–12,347)		0.66 (0.27–2.06)	
Female	7498 (4201–13,731)		0.91 (0.25–1.16)	
	Correlation coefficient (*r*)	*P* value	Correlation coefficient (*r*)	*P* value
Age	−0.113	0.511	−0.224	0.190
Body weight	−0.165	0.337	−0.095	0.581
Body surface area	−0.087	0.612	−0.037	0.831
Laboratory test values				
White blood cells	−0.021	0.903	−0.051	0.766
Red blood cells	−0.068	0.694	0.033	0.848
Platelets	−0.011	0.949	0.052	0.765
Aspartate transaminase	0.080	0.643	0.080	0.641
Alanine transaminase	−0.168	0.326	−0.145	0.399
Serum albumin	−0.130	0.451	−0.078	0.652
Total bilirubin	0.112	0.514	−0.096	0.577
Serum creatinine	−0.083	0.629	−0.204	0.233

The values are expressed as the median and first to third quartile or correlation coefficient

AUC_{0-24}, area under the plasma concentration–time curve from 0 to 24 h

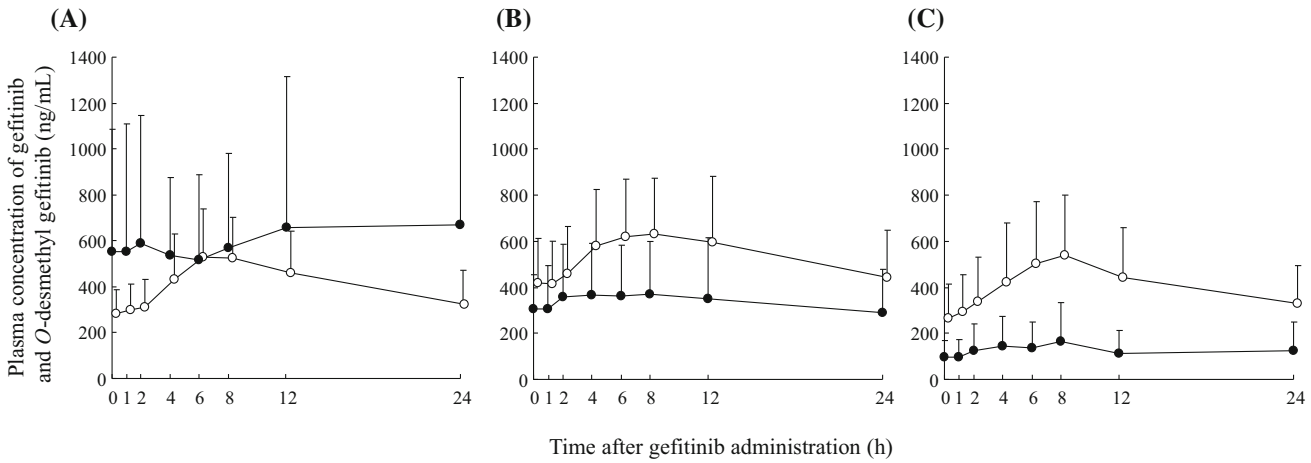

Fig. 1 Mean ± SD plasma concentration–time profiles of gefitinib (*open circles*) and *O*-desmethyl gefitinib (*solid circles*). **a** Homozygous extensive metabolisers (*CYP2D6*1/*1*, **1/*2*, and **2/*2*, $n = 13$), **b** heterozygous extensive metabolisers (*CYP2D6*1/*5*, **2/*5*, **1/*10*, and **2/*10*, $n = 18$), and **c** intermediate metabolisers (IMs; *CYP2D6*5/*10*, and **10/*10*, $n = 5$)

one clinical study, single administration of gefitinib in healthy volunteers resulted in a mean $AUC_{0-\infty}$ of *O*-desmethyl gefitinib of 1640 ng h/mL in CYP2D6 EMs; however, no such value was found in CYP2D6 PMs, i.e. those with genotypes **4/*4*, **4/*5*, and **3/*4* [9]. This previous result [9] was similar to the results obtained from our present study. However, the mean $AUC_{0-\infty}$ of gefitinib in CYP2D6 PMs was about twofold higher than that in CYP2D6 EMs with the **1* or **2* allele (3060 vs. 1430 ng h/mL) [9]. In the present study, there were no significant differences in the AUC_{0-24} of gefitinib between CYP2D6 EMs and IMs. We cannot completely explain

Table 3 Comparison of the AUC_{0-24} of *O*-desmethyl gefitinib and AUC ratio of *O*-desmethyl gefitinib to gefitinib between CYP or drug-transporter genotype groups

Study group	*N*	*O*-Desmethyl gefitinib AUC_{0-24} (ng h/mL)	*P* values	Gefitinib AUC_{0-24} (ng h/mL)	*P* values	AUC ratio of *O*-desmethyl gefitinib to gefitinib	*P* values
CYP2D6			0.021		0.467		0.030
**1/*1 + *1/*2 + *2/*2*	13	12,523 (4592–19,969)		8850 (8230–11,237)		1.41 (0.65–2.13)	
**1/*5 + *2/*5 + *1/*10 + *2/*10*	18	7434 (4387–11,721)		11,741 (8542–15,676)		0.86 (0.44–0.96)	
**5/*10 + *10/*10*	5	1460 (1437–4048)		10,734 (5974–13,016)		0.24 (0.19–0.26)	
ABCB1 1236C>T			0.496		0.337		0.805
C/C	4	5532 (1040–11,136)		7413 (3628–11,741)		0.55 (0.15–2.38)	
C/T	11	7272 (4376–17,997)		10,738 (8775–13,103)		0.82 (0.39–1.25)	
T/T	21	7480 (4048–12,170)		10,734 (7564–15,676)		0.81 (0.26–1.22)	
ABCB1 2677G>T/A			0.456		0.218		0.434
G/G	6	6615 (1315–12,523)		8223 (4009–12,665)		0.68 (0.19–1.99)	
G/T + G/A	19	7417 (4398–15,161)		10,386 (8542–15,676)		0.82 (0.51–1.41)	
T/T + T/A + A/A	11	6433 (1449–12,011)		11,964 (9191–14,782)		0.57 (0.14–1.01)	
ABCB1 3435C>T			0.798		0.180		0.376
C/C	13	7515 (4355–12,170)		10,734 (8230–12,845)		0.80 (0.25–1.22)	
C/T	15	7272 (4495–13,220)		8700 (7297–12,299)		0.82 (0.51–1.75)	
T/T	8	7419 (1449–13,731)		12,918 (10,574–17,356)		0.58 (0.14–1.04)	
ABCG2 421C>A			0.521		0.323		0.308
C/C	18	7647 (3117–12,170)		10,963 (8850–15,152)		0.66 (0.24–0.99)	
C/A + A/A	18	7417 (4592–15,802)		9192 (7030–13,362)		0.93 (0.44–1.99)	

Data are presented as median and first to third quartile range

AUC_{0-24}, area under the plasma concentration–time curve from 0 to 24 h

Table 4 Stepwise selection multiple linear regression analysis of explanatory variables for the AUC_{0-24} of *O*-desmethyl gefitinib and *O*-desmethyl gefitinib to gefitinib

Pharmacokinetic parameters	Slope	SE	SRC	*P* value	R^2
O-Desmethyl gefitinib AUC_{0-24} (ng h/mL)					0.173
CYP2D6 homozygous EMs (= 1)	7787	2920	0.416	0.012	
			Intercept = 6994 (SE = 1755)		
AUC ratio of *O*-desmethyl gefitinib to gefitinib					0.318
CYP2D6 homozygous EMs (=1)	1.185	0.341	0.503	0.001	
Age (year)	−0.041	0.018	−0.324	0.032	
			Intercept = 3.454 (SE = 1.252)		

AUC_{0-24}, area under the plasma concentration–time curve from 0 to 24 h

EMs extensive metabolisers, *SE* standard error, *SRC* standardised regression coefficient, R^2 determination coefficient

this discrepancy. CYP3A4 and CYP3A5 are the main enzymes involved in other gefitinib metabolic pathways [4, 5]. Repeated administration of gefitinib in patients with reduced CYP2D6 activity may result in metabolism of gefitinib mainly through the CYP3A4 and CYP3A5 pathways. van Waterschoot et al. [21] reported that gefitinib is a potent stimulator of triazolam metabolism by CYP3A4 both in vitro and in vivo. The higher gefitinib exposure after single administration of gefitinib in CYP2D6 IMs or PMs may enhance CYP3A4 activity to a greater extent than that in CYP2D6 EMs by repetitive administration of gefitinib. Consequently, no significant differences in the AUC_{0-24} of gefitinib at steady state may be observed between CYP2D6 EMs and IMs.

To date, more than 100 allelic variants have been reported for CYP2D6 [11]. However, in our present study, only *CYP2D6*5* and **10* were analysed. Therefore, the *CYP2D6* allele having no or reduced enzyme activity may

Table 5 Comparisons of side effects and AUC_{0-24} values of *O*-desmethyl gefitinib

Side effects	*N*	*O*-Desmethyl gefitinib AUC_{0-24} (ng h/mL)	*P* values	Total AUC_{0-24} of gefitinib plus *O*-desmethyl gefitinib (ng h/mL)	*P* values
Diarrhoea			0.033		1.000
No	19	9749 (6493–13,842)		20,566 (13,696–24,171)	
Yes	17	4048 (2129–10,638)		19,835 (15,086–22,352)	
Skin rash			0.597		0.911
No	14	9671 (4355–15,161)		19,633 (15,731–23,409)	
Yes	22	6534 (3849–11,721)		20,200 (14,476–24,541)	
Hepatotoxicity			0.962		0.519
No	16	7688 (3558–13,842)		19,563 (11,126–24,171)	
Yes	20	7376 (3949–12,063)		20,558 (15,408–23,975)	

Data are presented as the median and first to third quartile range

AUC_{0-24}, area under the plasma concentration–time curve from 0 to 24 h

Follow-up period: diarrhoea and skin rash, 2 weeks; hepatotoxicity, 1 year

be included in the CYP2D6 EMs classified in the present study. In fact, the AUC_{0-24} of *O*-desmethyl gefitinib and the AUC ratio of *O*-desmethyl gefitinib to gefitinib in patients with *CYP2D6*1/*1*, **1/*2*, or **2/*2* in the present study exhibited large ranges between 1208 and 46,320 ng h/mL and between 0.07 and 5.50, respectively. There was about a 38-fold variation in patients with *CYP2D6*1/*1*, **1/*2*, or **2/*2* between the lowest and highest measurements of *O*-desmethyl gefitinib formation from gefitinib. In the Asia population, by analysing other *CYP2D6* variants, such as **41* and **49* [11], our results may be more clear. In the previous study by Swaisland et al. [9], there was about a 39-fold variation between the lowest and highest gefitinib $AUC_{0-\infty}$ values in *CYP2D6* PMs, showing that there was considerable overlap in the ranges of individual values. Therefore, they concluded that *CYP2D6* genotyping prior to initiation of gefitinib therapy is not necessary [9]. We agree with this conclusion and recommend that plasma gefitinib concentrations should be analysed after beginning gefitinib therapy rather than analysing *CYP2D6* polymorphisms before initiating therapy because of the direct relationship of plasma gefitinib concentrations with side effects [14, 17]. On the other hand, however, Xin et al. [22] reported that the trough plasma concentration of gefitinib is not significantly associated with diarrhoea and hepatotoxicity. Hence, additionally studies having larger sample sizes are necessary, and these results might be interpreted within the context of the study limitations.

In the present study, neither *ABCB1* nor *ABCG2* allelic variants had a large influence on the AUC_{0-24} of *O*-desmethyl gefitinib in Japanese patients with NSCLC. P-glycoprotein and BCRP have been reported to contribute to the absorption and disposition of gefitinib [7, 16]; however, in a previous study and the present study [10, 14, 23], no associations between gefitinib exposure and *ABCB1* or *ABCG2* polymorphisms were found. Because *O*-desmethyl gefitinib has a higher solution than gefitinib, the transport of this metabolite may not be affected by ABC transporters. Consequently, these transporters do not seem to cause direct interindividual variability in *O*-desmethyl gefitinib pharmacokinetics. Our findings showed that the AUC_{0-24} of *O*-desmethyl gefitinib was significantly influenced only by *CYP2D6* polymorphisms.

Conclusion

*CYP2D6*5* and **10* polymorphisms are associated with the formation of *O*-desmethyl gefitinib from gefitinib. In *CYP2D6* homozygous EMs, the plasma concentrations of *O*-desmethyl gefitinib were higher over 24 h after taking gefitinib than those of the parent compound; however, the side effects of diarrhoea, skin rash, and hepatotoxicity induced by gefitinib were unrelated to plasma exposure to *O*-desmethyl gefitinib.

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Authors' contributions HK, KS, HI, and MM participated in the design of the study and reviewed the results. KS, MT, YO, MA, and HI were responsible for the patient collection and involved in acquisition of data. HK carried out genotyping. MM analysed plasma concentrations. HK, TN, and MM were responsible for the statistical analysis. HK and MM drafted the manuscript. KS, KS, MT, YO, MA, and HI helped to draft the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest.

Disclosure All authors report that they have no relevant relationships to disclose.

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