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Impact of CYP3A5 and ABCB1 Gene Polymorphisms on Fentanyl Pharmacokinetics and Clinical Responses in Cancer Patients Undergoing Conversion to a Transdermal System

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Summary: The aim of this study was to evaluate the influence of CYP3A5 and ABCB1 gene polymorphisms on fentanyl pharmacokinetics and clinical responses in cancer patients undergoing conversion to a transdermal system. Sixty Japanese cancer patients being treated with a fentanyl transdermal reservoir system according to the current Japanese guidelines were enrolled. Blood samples were obtained 192 h after conversion to the fentanyl transdermal system. Clinical responses after fentanyl application were evaluated by determining the incidences of adverse effects and rescue medication. The plasma concentration of fentanyl normalized with the measured absorption rate was significantly higher in the CYP3A5*3/*3 group than in the *1/*1 and *1/*3 groups (p = 0.048 and 0.021, respectively). Greater incidences of central adverse effects were observed in CYP3A5*3/*3 patients than in *1/*1+*1/*3 patients (odds ratio [OR], 3.49; 95% confidence interval [95% CI], 1.13–10.75; p = 0.029). Fewer patients with the ABCB1 1236TT allele than the 1236C allele needed rescue medication (OR, 0.17; 95% CI, 0.03–0.89; p = 0.036). CYP3A5*3 affected the pharmacokinetics of fentanyl and increased the incidence of central adverse effects. ABCB1 1236TT was associated with decreased administration of rescue medication after switching to the transdermal fentanyl system. In conclusion, these gene polymorphisms may predict clinical responses to fentanyl in cancer patients being converted to the transdermal system.

Keywords: fentanyl; CYP3A5; ABCB1; pharmacokinetics; opioid switching

Introduction

Fentanyl is a synthetic opioid analgesic which interacts primarily with μ opioid receptors. Fentanyl is readily absorbed through the skin because of its low molecular weight, high lipophilicity, and optimal skin flux. Fentanyl has been formulated in a transdermal system which is applied to patients for continuous relief from severe cancer pain.[1)] This transdermal system is suitable for patients with dysphagia caused by esophageal and neck cancer.[2,3)] In a clinical setting, the fentanyl transdermal system is available as the baseline opioid for severe cancer pain because it releases the fentanyl constantly over a 72-h period. Fentanyl has less adverse effects such as dizziness, vomiting, and nausea than morphine.[4,5)]

Opioid doses are difficult to optimize in patients who experience poor analgesic response titration or adverse effects before achieving sufficient analgesia. Opioid switching improves the balance between analgesia and adverse effects.[4,6–8)] Conversion ratios for oral morphine to transdermal fentanyl of 100:1 or 70:1 (mg of daily oral morphine: mg of daily transdermal fentanyl) have also been recommended in patients with pain uncontrolled with a conversion ratio of 150:1.[6)] Opioid switching to the fentanyl transdermal system often leads to the recurrence of pain or drowsiness, delirium, sedation, and respiratory depression.[6,9,10)]

Fentanyl pharmacokinetics shows large interindividual variation.[11,12)] Fentanyl is metabolized by CYP3A, and its metabolism varies markedly between patients.[13,14)] CYP3A5

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is the most important genetic contributor to interindividual differences in CYP3A-dependent drug clearance in humans.[15] The CYP3A5 gene polymorphisms cause fentanyl adverse effects such as delirium, respiratory depression, and gastrointestinal dysfunction.[14] P-Glycoprotein (ABCB1) limits the passage of fentanyl across the blood-brain barrier.[16] ABCB1 gene polymorphisms have been shown to cause respiratory suppression in Korean patients receiving intravenous fentanyl.[9] Patients with the ABCB1 C3435T homozygous mutation are good responders to morphine.[17] It remains to be clarified whether or not polymorphisms of CYP3A5 and ABCB1 genes contribute to the pharmacokinetic variability of fentanyl in patients treated using the transdermal system. In addition, the influences of these gene polymorphisms on the incidence of adverse effects and clinical responses to fentanyl have not yet been fully examined in cancer patients who are switched over to the transdermal system.

The clinical implications of CYP3A5 and ABCB1 gene polymorphisms on transdermal fentanyl administration have not been fully clarified in cancer patients. Prediction of the clinical responses to fentanyl based on these gene polymorphisms can be useful for cancer pain management. The aim of this study was to evaluate the impact of CYP3A5 and ABCB1 gene polymorphisms on the pharmacokinetics of fentanyl and the clinical responses in cancer patients who are switched over to the transdermal system.

Methods

Ethics: This study was performed in accordance with The Declaration of Helsinki and its amendments. The protocol was approved by the Ethics Committee of Hamamatsu University Hospital. Each patient received information about the scientific aim of this study and provided written informed consent.

Patients and study schedule: Sixty Japanese cancer patients treated with a fentanyl transdermal reservoir system (Durotep® Patch, Janssen Pharmaceutical K.K., Tokyo, Japan) at Hamamatsu University Hospital between April 1, 2007 and September 1, 2008 were enrolled. Each patient had been treated with oral morphine or oxycodone but was then switched over to the fentanyl transdermal reservoir system because of either insufficient pain control, difficulty in swallowing, or increased adverse effects such as drowsiness and nausea associated with the previous opioids. According to the current guidelines for the management of cancer pain published by the Japanese Society for Palliative Medicine, daily doses of oral morphine of 45–134 mg, 135–224 mg, and 225–314 mg were converted to fentanyl delivery rates of 25, 50, and 75 μg/h, respectively.[18] Daily doses of oral oxycodone of 30–89 mg, 90–149 mg, and 150–209 mg were converted to fentanyl delivery rates of 25, 50, and 75 μg/h, respectively. The initial dose of fentanyl corresponded to the dose of the opioid administered to that point, and oral liquid morphine or powdered oxycodone was administered as a rescue medication according to the current Japanese guidelines. The dose given *via* the fentanyl transdermal reservoir system was 25 or 50 μg/h, depending on the conversion dose. Fentanyl delivered at a rate of 25 and 50 μg/h was replaced every 72 h. Blood was sampled 192 h after switching to the transdermal reservoir system in consideration of the time-to-steady state of fentanyl. Blood specimens were taken from a forearm vein into tubes containing EDTA. Used transdermal reservoir systems were recovered from the enrolled patients. The adverse effects and efficacy of fentanyl were evaluated by determining the incidence of adverse effects and rescue medication for breakthrough pain during the 192 h after the switchover from other opioids.

Determination of fentanyl in human plasma and the transdermal reservoir system: Fentanyl citrate was purchased from Mallinckrodt Inc. (St. Louis, MO, USA). Papaverine hydrochloride used as an internal standard was obtained from Wako Pure Chemicals (Osaka, Japan). Determination of fentanyl in human plasma and the transdermal reservoir system was performed as previously described.[19] The intra- and interassay accuracies of quality control for fentanyl in human plasma were 97.3–101.2% and 97.9–100.4%, respectively. The intra- and interassay precisions of quality control for fentanyl in human plasma were 1.2–2.8% and 0.3–6.5%, respectively. The mean extraction recoveries from 25 and 50 μg/h fentanyl delivery were 88.3% and 90.9%, respectively. The precisions of extraction were 1.5% and 4.8%, respectively. The lower limit of quantification for fentanyl was 0.05 ng/mL in the human plasma.

Pharmacokinetic parameters of fentanyl: The theoretical delivery amount of fentanyl employed the product of the delivery rate described in the package insert and 72 h. The theoretical delivery rate of fentanyl in the transdermal reservoir system was calculated by dividing the theoretical delivery amount of fentanyl per hour (dose of fentanyl per hour) by the patient's body weight. The measured absorption rate of fentanyl was estimated using the following equation (Eq. (1)).

Measured absorption rate of fentanyl in transdermal reservoir system from cancer patient (μg/h/kg)
= [theoretical amount of fentanyl in non-applied transdermal reservoir system (μg)
−measured residual amount of fentanyl in applied transdermal reservoir system (μg)]
/72(h)/body weight of cancer patient (kg) (1)

The plasma concentration of fentanyl was normalized with its theoretical delivery rate or measured absorption rate in the pharmacokinetic analysis. Total clearance of fentanyl (CL/F) was calculated using its plasma concentration 192 h after starting the transdermal reservoir system and the measured absorption rate.

Genotyping of CYP3A5 and ABCB1: Genomic DNA from peripheral leukocytes was isolated using a DNA Extractor WB Kit (Wako Pure Chemicals). CYP3A5 and ABCB1 gene variants (C1236T in exon 12, G2677A/T in exon 21, and C3435T in exon 26) were determined using modified polymerase chain reaction (PCR) and restriction fragment length polymorphism amplification techniques previously described by Fukuen *et al.*[20] and Park *et al.*,[9] respectively. In brief, PCR reactions were carried out in 50 μL of solution consisting of 90 ng of genomic DNA template, 0.4 μM of a primer, 0.2 mM of dNTPs (Takara Bio Inc., Shiga, Japan), and 1 unit of TaKaRa Ex Taq™ (Takara). Amplification conditions consisted of an initial denaturation for 10 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C for CYP3A5 with a final extension of 5 min at 72°C. Amplification conditions consisted of an initial denaturation for 10 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C for ABCB1 with a final extension of 10 min at 72°C. Next, the amplicons were digested by restriction enzymes. The restriction enzymes used were 5 units of *Dde* I (Roche Diagnostics, Mannheim, Germany) for CYP3A5, 1 unit of *Dra* II (Roche Diagnostics) for C1236T, 2 units of *Rsa* I (Roche Diagnostics) for G2677A, 2 units of *Ban* I (New England Biolabs, Ipswich, MA, USA) for G2677T, and 1 unit of *Nde* I (Roche Diagnostics) for C3435T.

Evaluation of adverse effects caused by fentanyl: The adverse effects of fentanyl were investigated by examination of the patient medical records. The degree of severity was graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (CTCAE version 4.0). Drowsiness, delirium, restlessness, sedation, and dyspnea were defined as central nervous system adverse effects. Constipation, nausea, vomiting, and rescue medication were also investigated. Vomiting was distinguished from a central nervous depressant adverse effect in this study. Pain intensity was assessed using the Numerical Rating Scale, which ranges from 0 (no pain) to 10 (worst possible pain). Rescue medication was used as a treatment for breakthrough pain or additional medication until adequate analgesia was obtained. The adverse effects and efficacy of fentanyl were evaluated by determining the incidence of adverse effects and rescue medication for breakthrough pain.

Statistical methods: All statistical analyses were performed using SPSS 15.0J software (SPSS Japan Inc., Tokyo, Japan). All values in tables are expressed as the median and interquartile range unless otherwise noted. The level of statistical significance was set at $p < 0.05$. Allelic frequencies were calculated by direct counting. The influences of CYP3A5 and ABCB1 gene polymorphisms on the pharmacokinetic parameters of fentanyl were analyzed using the nonparametric Kruskal-Wallis test and post hoc Bonferroni comparison between genotypes, because normal distribution and equal variances between groups could not be assumed. Associations between each gene polymorphism and adverse effects were evaluated using Fisher's exact probability test. Logistic regression analysis was used between adverse effects (1: adverse effects, 0: absence) or rescue medication (1: administration, 0: absence) as the dependent variable and gene polymorphism of CYP3A5 or ABCB1 (1: CYP3A5*3/*3, 1236C allele non-carrier, 2677G allele non-carrier and 3435C allele non-carrier, respectively, 0: CYP3A5*1 carrier, 1236C allele carrier, 2677G allele carrier and 3435C allele carrier) as the independent variables. Odds ratios (OR) and their 95% confidence intervals (95% CI) were evaluated.

Results

Patient characteristics: The medians and interquartile ranges of age, gender, body mass index, and dose of fentanyl are presented in **Table 1**. Thirty-one patients (51.7%) had been treated with morphine and the others with oxycodone. The reasons for switching to fentanyl were dysphagia (55.0%), inadequate pain control (28.3%), undesired adverse effects (10%), and others (6.7%). Pharyngeal and lung cancers accounted for nearly one half of the cancers in the patients enrolled in this study (**Table 2**).

Plasma concentration of fentanyl in cancer patients: A large interindividual variation was observed in the plasma concentration of fentanyl (median and interquartile range, 1.37 and 0.75–2.03 ng/mL, respectively). The median and interquartile range of the theoretical and measured delivery rates of the fentanyl transdermal reservoir system per kg of body weight were 1.04 and 0.68–1.74 μg/h/kg and 0.83 and 0.62–1.34 μg/h/kg, respectively. The plasma concentration of fentanyl was significantly correlated with the theoretical delivery rate ($r = 0.617$, $p < 0.01$) and measured absorption rate ($r = 0.628$, $p < 0.01$) of fentanyl in the transdermal reservoir system (**Fig. 1**).

Table 1. Demographic characteristic data of the cancer patients

Age (years)	64 (57–73)
Gender (male/female)	40/20
Body mass index (kg/m^2)	20.0 (18.2–22.3)
Serum albumin (g/L)	29 (24–34)
Initial opioids (morphine/oxycodone)	31/29
Dose of fentanyl (μg/h)	37.5 (25.0–50.0)
Reason for switching to fentanyl	
Dysphagia	33
Inadequate pain control	17
Undesired adverse effects	6
Others	4

Gender did not affect measured absorption rate and CL/F of fentanyl (p = 0.858 and 0.185, respectively). There were no significant differences in measured absorption rate and CL/F of fentanyl between male and female (p = 0.858 and 0.185, respectively) or between patients over 65 years and patients under 65 years (p = 0.530 and 0.114, respectively).

Allele frequency of CYP3A5 and ABCB1: There were 5 (8.3%), 20 (33.3%), and 35 (58.3%) patients with CYP3A5*1/*1, *1/*3, and *3/*3, respectively. The CYP3A5*3 allele frequency was 75.0%. There were 13 (21.7%), 24 (40.0%), and 23 (38.3%) patients with ABCB1 1236CC, CT, and TT genotypes, respectively. The ABCB1 1236C allele frequency was 41.7%. There were 12 (20.0%), 6 (10.0%), 19 (31.7%), 11 (18.3%), 9 (15.0%), and 3 (5.0%) patients with ABCB1 2677GG, GA, GT, TT, TA, and AA genotypes, respectively. The allele frequencies of ABCB1 2677G, T, and A were 40.8%, 41.7%, and 17.5%, respectively. There were 19 (31.7%), 28 (46.7%), and 13 (21.7%) patients with ABCB1 3435CC, CT, and TT genotypes, respectively. The ABCB1 3435C allele frequency was 55.0%.

Influence of CYP3A5 and ABCB1 gene polymorphisms on fentanyl pharmacokinetics: The median of the plasma concentration of fentanyl normalized with the measured absorption rate was significantly higher in patients in the CYP3A5*3/*3 group than in the CYP3A5*1/*1 and CYP3A5*1/*3 groups (p = 0.048 and 0.021, respectively). There were no significant differences in the theoretical delivery rate and measured absorption rate among the CYP3A5*3 genotype groups (**Table 3**). Higher correlation coefficients were observed between the plasma concentration of fentanyl and measured absorption rate in CYP3A5*1/*1+*1/*3 than in CYP3A5*3/*3 patients ($r = 0.85$ and 0.64, respectively). There were no significant differences in fentanyl pharmacokinetic parameters among the ABCB1 genotype groups (**Table 4**).

Influence of CYP3A5 and ABCB1 gene polymorphisms on fentanyl-related adverse effects: The incidence of central adverse effects was slightly higher in the CYP3A5*3/*3 group than in the CYP3A5*1/*1+*1/*3 group (p = 0.07) (**Table 5**), and the incidence of nausea or vomiting was slightly more frequent in the ABCB1 2677G allele carriers than in the ABCB1 2677G allele non-carriers (p = 0.16). The incidence of constipation

Table 2. Diagnosis for the cancer patients

Diagnosis	Number of patients
Pharyngeal cancer	15
Lung cancer	11
Esophageal cancer	6
Multiple myeloma	4
Tongue cancer	4
Brain tumor	2
Paranasal sinus cancer	2
Gastric cancer	2
Pancreatic cancer	2
Malignant lymphoma	2
Prostatic cancer	2
Others	8

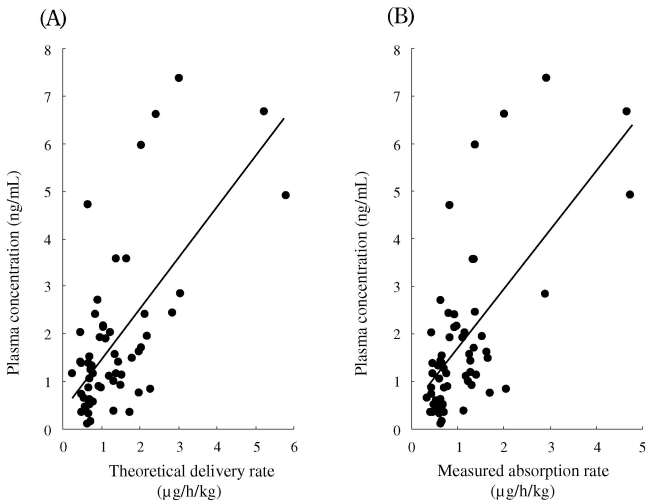

Fig. 1. The relationships between plasma fentanyl concentration at 192 h after reservoir-TTS application and theoretical delivery rate (A) and measured absorption rate (B) in 60 cancer patients

Table 3. Influence of CYP3A5*3 on fentanyl pharmacokinetics in cancer patients

Parameters	CYP3A5*3		
	*1/*1 (n = 5)	*1/*3 (n = 20)	*3/*3 (n = 35)
Theoretical delivery rate (μg/h/kg)	0.99 (0.69–1.72)	1.36 (0.68–2.00)	0.91 (0.67–1.41)
Measured absorption rate (μg/h/kg)	0.69 (0.67–0.69)	1.20 (0.61–1.66)	0.78 (0.62–1.30)
Plasma concentration of fentanyl (ng/mL)	0.52 (0.45–0.87)	1.04 (0.61–1.94)	1.42 (1.09–2.09)
Plasma concentration of fentanyl normalized by theoretical delivery rate (ng/mL per μg/h/kg)	0.78 (0.74–0.87)[a]	0.84 (0.76–1.17)[a]	1.72 (0.91–2.31)
Plasma concentration of fentanyl normalized by measured absorption rate (ng/mL per μg/h/kg)	0.82 (0.77–1.25)[a]	1.03 (0.80–1.74)[a]	2.01 (1.21–2.44)
Total clearance of fentanyl, CL/F (L/h)	67.4 (47.2–78.2)[a]	58.4 (41.3–75.6)[a]	28.0 (20.6–55.1)

Data are expressed as median and interquartile range in parentheses.
Statistical analysis was performed using Kruskal-Wallis and Mann-Whitney U tests.
[a]Statistical significance p < 0.05 *vs.* *3/*3.

Table 4. Influence of ABCB1 (A) C1236T, (B) G2677A/T and (C) C3435T on the plasma disposition of fentanyl in cancer patients

(A)

Parameters	ABCB1 C1236T		
	CC ($n = 13$)	CT ($n = 24$)	TT ($n = 23$)
Theoretical delivery rate (μg/h/kg)	1.05 (0.76–1.97)	1.34 (0.71–1.74)	0.71 (0.65–1.25)
Measured absorption rate (μg/h/kg)	0.96 (0.63–1.53)	1.19 (0.68–1.33)	0.64 (0.60–1.11)
Plasma concentration of fentanyl (ng/mL)	1.62 (1.17–2.16)	1.21 (0.84–1.79)	1.12 (0.45–2.01)
Plasma concentration of fentanyl normalized by theoretical delivery rate (ng/mL per μg/h/kg)	1.31 (0.86–2.24)	1.18 (0.83–1.84)	0.85 (0.70–1.86)
Plasma concentration of fentanyl normalized by measured absorption rate (ng/mL per μg/h/kg)	1.44 (1.10–2.34)	1.43 (0.99–2.11)	1.09 (0.75–2.27)
Total clearance of fentanyl, CL/F (L/h)	35.6 (25.3–59.0)	40.4 (27.0–65.3)	57.2 (25.6–90.0)

(B)

Parameters	ABCB1 G2677A/T					
	GG ($n = 12$)	GA ($n = 6$)	GT ($n = 19$)	TT ($n = 11$)	TA ($n = 9$)	AA ($n = 3$)
Theoretical delivery rate (μg/h/kg)	1.29 (0.81–1.41)	0.84 (0.71–2.50)	1.04 (0.65–1.88)	0.96 (0.64–1.25)	0.78 (0.69–1.32)	1.97 (1.32–2.07)
Measured absorption rate (μg/h/kg)	1.20 (0.75–1.33)	0.64 (0.55–2.32)	0.69 (0.60–1.49)	0.83 (0.61–1.14)	0.74 (0.67–1.22)	1.53 (0.98–1.58)
Plasma concentration of fentanyl (ng/mL)	1.64 (1.03–2.23)	1.79 (1.41–5.01)	1.05 (0.59–1.82)	1.91 (1.20–2.01)	0.91 (0.52–1.17)	1.62 (1.25–1.78)
Plasma concentration of fentanyl normalized by theoretical delivery rate (ng/mL per μg/h/kg)	1.26 (0.75–1.74)	2.10 (1.46–2.24)	0.87 (0.74–1.66)	1.73 (0.86–3.04)	0.76 (0.74–1.51)	0.89 (0.85–1.10)
Plasma concentration of fentanyl normalized by measured absorption rate (ng/mL per μg/h/kg)	1.36 (0.99–1.88)	2.22 (1.60–2.84)	1.12 (0.78–2.20)	2.18 (1.21–3.35)	0.83 (0.77–1.58)	1.27 (1.13–1.64)
Total clearance of fentanyl, CL/F (L/h)	45.7 (33.8–59.6)	24.1 (18.8–26.8)	48.7 (31.6–77.8)	27.2 (17.5–64.2)	67.4 (29.7–76.4)	39.9 (37.8–52.1)

(C)

Parameters	ABCB1 C3435T		
	CC ($n = 19$)	CT ($n = 28$)	TT ($n = 13$)
Theoretical delivery rate (μg/h/kg)	1.05 (0.68–1.79)	1.18 (0.67–1.17)	0.85 (0.69–1.20)
Measured absorption rate (μg/h/kg)	0.96 (0.62–1.38)	0.85 (0.61–1.11)	0.79 (0.63–1.12)
Plasma concentration of fentanyl (ng/mL)	1.57 (1.00–2.10)	1.03 (0.62–1.52)	1.91 (1.12–2.03)
Plasma concentration of fentanyl normalized by theoretical delivery rate (ng/mL per μg/h/kg)	1.36 (0.90–2.02)	0.85 (0.68–2.91)	1.73 (0.78–2.93)
Plasma concentration of fentanyl normalized by measured absorption rate (ng/mL per μg/h/kg)	1.75 (1.27–2.17)	1.06 (0.79–2.89)	2.18 (0.96–3.09)
Total clearance of fentanyl, CL/F (L/h)	35.6 (26.3–51.5)	57.5 (33.9–85.3)	27.2 (17.0–66.4)

Data are expressed as median and interquartile range in parentheses.
Statistical analysis was performed using Kruskal-Wallis test.

Table 5. Influence of CYP3A5 and ABCB1 gene polymorphisms on incidence of adverse effects and rescue medication in cancer patients switching to fentanyl transdermal system

Genotypes		Adverse effects			Rescue medication, *n*
		Central adverse effects, *n*	Constipation, *n*	Nausea or vomiting, *n*	
CYP3A5*3	*1/*1 + *1/*3 ($n = 25$)	10	10	9	11
	*3/*3 ($n = 35$)	23	19	10	14
ABCB1 C1236T	CC + CT ($n = 37$)	22	18	12	19
	TT ($n = 23$)	11	11	7	6
	GG + GA + GT ($n = 37$)	20	16	9	17
	TT + TA + AA ($n = 23$)	13	13	10	8
	CC + CT ($n = 47$)	26	23	14	20
	TT ($n = 13$)	7	6	5	5

was slightly higher in the ABCB1 3435C allele carriers than in the ABCB1 3435C allele non-carriers (53.5% and 35.3%, respectively; $p = 0.26$). No significant differences in the incidence of adverse effects or administration of rescue medication were observed in the ABCB1 G2677A/T or C3435T carriers. The number of patients with rescue medication was slightly higher in the ABCB1 1236C allele carriers than in the ABCB1 1236C allele non-carriers (51.4% and 26.1%, respectively; $p = 0.07$).

Risk of adverse effects and administration of rescue medication: Central adverse effects of grades 1, 2, and 3 were observed in 19 patients (31.7%), 12 patients (20.0%), and 2 patients (3.3%), respectively. Grade 1, 2, and 3 constipation was observed in 19 patients (31.7%), 7 patients (11.7%), and 3 patients (5.0%), respectively. Grade 1 and 2 nausea and vomiting were observed in 13 patients (21.7%) and 6 patients (10.0%), respectively. CYP3A5*3/*3 patients had a 3.49-fold higher risk of central adverse effects than CYP3A5*1/*1 and CYP3A5*1/*3 patients (OR, 3.49; 95% CI, 1.13–10.75; and $p = 0.029$). The incidence of nausea and vomiting tended to be 3.14-fold higher in the ABCB1 2677G allele non-carriers than in the ABCB1 2677G allele carriers (OR, 3.14; 95% CI, 0.76–12.89; and $p = 0.11$). Constipation tended to be 2.30-fold higher in the ABCB1 2677G allele non-carriers than in the ABCB1 2677G allele carriers (OR, 2.30; 95% CI, 0.58–9.08; and $p = 0.24$). The odds ratio for rescue medication was significantly lower in the ABCB1 1236C allele non-carriers than in the ABCB1 1236C allele carriers (OR, 0.17; 95% CI, 0.03–0.89; and $p = 0.036$). Rescue medication was administered to slightly fewer patients with the ABCB1 3435TT allele than the ABCB1 3435C allele (OR, 5.21; 95% CI, 0.58–46.99; and $p = 0.14$).

Discussion

This study evaluated the influences of CYP3A5 and ABCB1 gene polymorphisms on fentanyl pharmacokinetics and clinical responses after switching to a transdermal system. Recently, CYP3A5 and ABCB1 gene polymorphisms have been reported to be factors involved in the pharmacokinetics and adverse effects of fentanyl.[9,14,17] However, the clinical implications of these gene polymorphisms on transdermal fentanyl therapy had not been fully clarified. In our findings, higher plasma concentrations of fentanyl and higher incidences of central adverse effects were observed in cancer patients with CYP3A5*3/*3. In addition, ABCB1 1236TT was associated with decreased rescue medication after application of the fentanyl transdermal system. CYP3A5*3 and ABCB1 C1236T can contribute to interindividual differences in the clinical responses to the fentanyl transdermal system in cancer patients. To the best of our knowledge, this is the first report revealing the influence of CYP3A5 and ABCB1 gene polymorphisms on the pharmacokinetics of fentanyl and clinical responses to the drug in cancer patients switched over to the transdermal system.

In the present study, the plasma concentrations of fentanyl were higher in patients with CYP3A5*3/*3 than CYP3A5*1/*1 and *1/*3. The median of the plasma concentration of fentanyl normalized with the measured absorption rate was also twice as high in CYP3A5*3/*3 as in CYP3A5*1/*1+*1/*3. These results indicated that CYP3A5*3 decreases the metabolic clearance of fentanyl. In addition, a higher correlation coefficient was observed between the plasma concentration of fentanyl and the measured absorption rate in patients with CYP3A5*1/*3 than with CYP3A5*3/*3. This result suggests that CYP3A5*3 can affect the pharmacokinetics of fentanyl. CYP3A5 appears to account for up to 50% of total hepatic CYP3A protein in individuals carrying at least one CYP3A5*1 allele. CYP3A5 was reported to show approximately 85% amino acid sequence identity and large overlapping substrates with CYP3A4.[21] The Japanese allele frequency of CYP3A5*3 is 0.7675,[20] which is similar to that found in the present study (0.750). CYP3A5 is polymorphically expressed in individuals carrying the wild-type CYP3A5*1 allele, and homozygous subjects with CYP3A5*3 are considered to lack CYP3A5 activity.[15] Thus, higher plasma concentrations of fentanyl in patients with CYP3A5*3 in this study was caused most likely due to less CYP3A activity.

Gene polymorphisms of ABCB1 C1236T, G2677A/T, and C3435T caused no significant difference in the plasma concentration of fentanyl, indicating that ABCB1 gene polymorphisms did not affect the variability of plasma concentrations of fentanyl. However, the influence of ABCB1 gene polymorphisms on the pharmacokinetic variability of fentanyl in the central nervous system remains to be clarified. ABCB1 gene polymorphisms have been reported to be associated with increased incidences of central adverse effects of fentanyl in Koreans.[9] The variability of fentanyl pharmacokinetics in the central nervous system would be affected by ABCB1 gene polymorphisms.

Univariate analysis was not able to ascertain the influences of CYP3A5 and ABCB1 gene polymorphisms on the incidences of adverse effects and rescue administration in this study. However, some previous reports examined the effects of the gene polymorphisms mentioned above on the response to fentanyl administered for pain treatment. Jin *et al.* reported that CYP3A5*3 caused fentanyl toxicity in Caucasian subjects.[22] Park *et al.* reported that ABCB1 gene polymorphisms caused central adverse effects in Koreans, such as suppression of the respiratory rate.[9] Our results also demonstrated that the clinical responses to fentanyl were affected by both CYP3A5 and ABCB1 gene polymorphisms, and each appears to be masked by the other. Multivariate analysis was also used to evaluate the influence of CYP3A5 and ABCB1 gene polymorphisms on the incidence of adverse effects and rescue medication.

Patients with CYP3A5*3/*3 have a higher risk of central adverse effects than those with CYP3A5*1/*1+*1/*3 because higher plasma concentrations of fentanyl and higher

incidences of adverse effects were observed in patients with CYP3A5*3/*3 than in those with CYP3A5*1/*1+*1/*3. Our data indicated that CYP3A5 was involved in the metabolic processing of fentanyl since CYP3A5*3 was associated with a greater number of fentanyl adverse effects.[13,15] Jin *et al.*[22] and Lamba *et al.*[23] reported that CYP3A5*3/*3 caused higher plasma concentrations of fentanyl and higher incidences of central adverse effects. With respect to usage in genotyping of CYP3A5*3, CYP3A5*3/*3 patients with borderline high fentanyl conversion values may require lower doses in order to avoid central adverse effects, based on our clinical data.

ABCB1 gene polymorphisms (C1236T, G2677A/T, and C3435T) are associated with functional impairment of P-glycoprotein and cause poor excretion function.[24] Fewer patients with the ABCB1 1236TT allele needed the rescue medication compared to the ABCB1 1236C allele in the present study. Previously, the ABCB1 C1236T variant was shown to cause significantly higher peak blood concentrations of oral cyclosporine, a substrate for P-glycoprotein.[25] Homozygous ABCB1 C1236T was significantly associated with an increased level of morphine related central adverse effects such as drowsiness and confusion or hallucinations.[26] The amount of fentanyl in the central nervous system could be significantly higher and result in a more intensive effect in ABCB1 1236TT carriers due to their poor excretory function. In the present study, ABCB1 G2677A/T and C3435T gene polymorphisms had no effects on the incidence of adverse effects or rescue administration. Lötsch *et al.* reported that the ABCB1 C3435T variant was associated with decreased opioid dosage requirements in chronic pain patients.[27] Park *et al.* reported that the ABCB1 G2677A/T and C3435T variants caused respiratory depression in postoperative patients.[9] ABCB1 gene variants caused the differences in clinical responses between the opioids, intensities and types of pain.[17,28,29] The discrepancies can be explained based on the differences in fentanyl sensitivity and distribution. The three ABCB1 gene polymorphisms show differences in the allele frequencies between races. ABCB1 1236CC + CT patients with borderline high fentanyl conversion values may require higher doses to achieve stable pain control after switching over to the fentanyl transdermal system.

Despite higher plasma concentrations of fentanyl, the incidences of constipation, and nausea and vomiting were not significantly higher in the CYP3A5*3/*3 group patients than in the CYP3A5*1/*1+*1/*3 group patients. The difference may be based on the lower threshold concentrations for constipation and for nausea and vomiting than that for analgesia. In addition, gene polymorphisms of opioid μ receptor (OPRM1) would affect the clinical response to fentanyl. Klepstad *et al.*[30] and Chou *et al.*[31] reported that OPRM1 G118 homozygotes have a poorer response to morphine administered for postoperative pain control than A118 homozygotes or heterozygotes.

Gender and age caused no significant difference in the CL/F or measured absorption rate of fentanyl in Japanese. There are several reports suggesting other factors including cancer types[32,33] and cachexia[34] affect CL/F and transdermal absorption of fentanyl. In the present study, half of the patients had pharyngeal, lung or esophageal cancer. There was no difference in the CL/F or measured absorption rate of fentanyl among pharyngeal, lung and esophageal cancer. In addition, most enrolled patients tended to have the cachexia based on the level of serum albumin. Our data indicate that the difference in gender and age do not strongly affect the fentanyl pharmacokinetics.

CYP3A5*3 and ABCB1 C1236T affected the pharmacokinetics of and clinical responses to fentanyl in cancer patients receiving the fentanyl transdermal system in the present study. We performed the statistical analysis using univariate and multivariate analyses to support our clinical results. As a limitation of the present study, our sample size could not identify the specific factors including age, concomitant drug, cachexia and cancer types. These factors also may affect the clinical responses to fentanyl together with gene polymorphisms of CYP3A5 and ABCB1. The more solid conclusion on the clinical implications of gene polymorphisms of CYP3A5 and ABCB1 would be obtained with the stratified analyses and haplotype analyses, which need a larger number of cancer patients.

In conclusion, CYP3A5*3 and ABCB1 C1236T gene polymorphisms may predict the incidence of adverse effects and permit the individualization of fentanyl dosages in cancer patients undergoing a switchover to the fentanyl transdermal system.

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