Comparative pharmacokinetic study of paclitaxel and docetaxel in streptozotocin-induced diabetic rats

Joo Hyun Leea, Areum Leeb, Ju-Hee Ohb, and Young-Joo Leea, b, *

a Division of Biopharmaceutics, College of Pharmacy, Kyung Hee University, Seoul, Korea
b Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul, Korea

ABSTRACT: The pharmacokinetics of paclitaxel and docetaxel were compared in diabetic rats induced by streptozotocin (DMIS rats) and the impact of altered expression of cytochrome P450 3A (Cyp3A) and P-glycoprotein (P-gp) in the diabetic state. The pharmacokinetics of paclitaxel and docetaxel were determined after intravenous (5 mg/kg) and oral (30 and 40 mg/kg, respectively) administration to both groups and the mRNA expression levels of Cyp3A isozymes and Mdr1a and Mdr1b in the liver and small intestine were determined in control and DMIS rats. After intravenous administration, the AUC and clearance of paclitaxel and docetaxel were not significantly different in DMIS vs control rats. After oral administration, the AUC and Cmax of paclitaxel in DMIS rats were significantly greater than those in the control rats, whereas those of docetaxel was not changed significantly. The mRNA expression levels of hepatic Cyp3A1, Cyp3A9 and Mdr1b were significantly increased in DMIS compared with the control rats. In the intestine, Cyp3A62 expression decreased in the DMIS rats compared with the controls. Thus the pharmacokinetic changes of taxanes observed in the DMIS rats were attributed to changes in P-gp and Cyp3A, predominant factors that control the absorption of paclitaxel and docetaxel, respectively. It seemed that there were different susceptibilities to intestinal P-gp and Cyp3A between the two taxanes. Copyright © 2012 John Wiley & Sons, Ltd.

Key words: taxanes; pharmacokinetics; diabetes mellitus; cytochrome P450 3A; P-glycoprotein

Introduction

It has been reported that drug pharmacokinetics is altered in individuals with diabetes mellitus [1–3]. Although these pharmacokinetic changes may depend upon the disease type, the severity and duration of the disease, impairment of the liver and kidney functions, and the co-administration of other drugs, altered expression of cytochrome P450 3A (Cyp3A) is believed to be the important contributor to these pharmacokinetic alterations [1,4,5]. Recently, it has been suggested that drug efflux transporters, such as P-glycoprotein (P-gp), also play a role in altered drug pharmacokinetics in diabetes mellitus [6,7]. But information on the quantitative impact is missing.

Paclitaxel (Taxol®, Figure 1A) and docetaxel (Taxotere®, Figure 1B) are members of the taxane class of cytotoxic treatments and have been used widely against a variety of cancer types, including breast and ovarian cancers. An important limitation associated with the use of taxanes is a high inter- and intra-patient variability in exposure, efficacy and especially toxicity [8,9]. The major determinants of this variability are Cyp3A and P-gp. Paclitaxel and docetaxel are exclusively metabolized via Cyp3A to several metabolites that are all considered to be therapeutically less effective [10–12]. Paclitaxel and docetaxel also
have high affinities for P-gp, which result in the
limited absorption of the orally administered drug
in the mucosa of the gastrointestinal tract [13,14].

However, at least in terms of absorption, the
predominant factor may be different for paclitaxel
and docetaxel. Intestinal Cyp3A is considered to
contribute to the absorption barrier of docetaxel
more than P-gp, whereas intestinal P-gp is consid-
ered as a major deterrent in the absorption of
paclitaxel [15,16]. As changes of CYP3A and P-gp
are altered simultaneously in many cases [17,18],
it is hard to distinguish the factor causing the
pharmacokinetic changes.

The purpose of this study was to evaluate the
effects of diabetes mellitus induced by treatment
with streptozotocin (DMIS) on the pharmacokinetics
of paclitaxel and docetaxel after intravenous and
oral administration. The mRNA expression levels
of Cyp3A (Cyp3A1, 3A2, 3A9, 3A18 and 3A62)
and Mdr1a and Mdr1b in the liver and intestine of
DMIS and control rats were also examined using a
quantitative polymerase chain reaction to support
the explanation on pharmacokinetic changes of
paclitaxel and docetaxel.

Materials and Methods

Materials
Paclitaxel (Paxel® injectable solution) and docetaxel
trihydrate were supplied from Hanmi Pharmaceutical
Co. Ltd (Seoul, Korea) and Shin Poong Pharmaceutical
Co. Ltd (Ansan, Korea), respectively. Streptozotocin was purchased from Sigma–Aldrich
Corporation (St Louis, MO). Other chemicals were
of reagent or HPLC grade.

Animals
Male Sprague-Dawley rats (210–260 g, Orient, Seoul,
Korea) were used for the experiment. The rats were
maintained at room temperature of 25°C and a
12 h: 12 h light/dark cycle with free access to water
and feed in a housing facility that has been fully
accredited by the Association for Assessment and
Accreditation of Laboratory Animal Care Interna-
tional (Animal Center for Pharmaceutical Research,
College of Pharmacy, Kyung Hee University, Seoul,
Korea). All experiments were conducted according
to the guidelines of the Committee on the Care
and Use of Laboratory Animals of the Kyung Hee
University.

Induction of diabetes mellitus in rats by
streptozotocin injection
Rats with DMIS were used as an animal model
of diabetes mellitus. The rats were randomly
divided into a DMIS group and a control group.
Freshly prepared streptozotocin, 45 mg/kg (approx-
imately 0.3 ml), was injected once to overnight-
fasted rats via the tail vein [19]. An equal volume
of citrate buffer (pH 4.5) was injected into the control
rats. On day 7 after intravenous administration of
streptozotocin (DMIS rats) or citrate buffer, pH 4.5
(control rats), blood glucose levels were measured
using the Medisense Optium kit (Abbott Laborato-
ries, Bedford, MA), and rats with blood glucose
levels higher than 250 mg/dl were selected as being
diabetic. The single injection is able to induce
diabetes 7 days later, confirmed by elevated blood
glucose levels in the streptozotocin injected rats.

Pretreatment of rats for intravenous and oral studies
The procedures used for the pretreatment of rats,
including cannulation of the jugular vein (for drug

Figure 1. Structures of paclitaxel (A) and docetaxel (B)
administration in the intravenous study) and carotid artery (for blood sampling), were described previously [19]. Early in the morning on day 7 after treatment with streptozotocin or citrate buffer (pH 4.5), the carotid artery and the jugular vein (for intravenous study only) of both rat groups were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ), with the rat being maintained under light ether anaesthesia. Both cannulae were exteriorized to the dorsal side of the neck, where each cannula terminated in a long silastic tube (Dow Corning, Midland, MI). Both silastic tubes were covered with a wire sheath to allow free movement of the rats. A 0.3 ml volume of heparinized 0.9% NaCl-injectable solution (15 units/ml) was used to flush the cannula to prevent blood clotting. Each rat was housed individually in a metabolic cage (Daejong Scientific Company, Seoul, Korea) and allowed to recover from anaesthesia for 4–5 h before the initiation of the experiment. Thus, no rat was restrained in the present study.

Intravenous and oral studies of paclitaxel and docetaxel

For the intravenous study, paclitaxel (diluted Paxel® injectable solution with normal saline in a 1:1.4 ratio by volume) and docetaxel (docetaxel trihydrate dissolved in a 1:1:1.3 (v/v/v) solution of Tween 80 and ethanol) at a dose of 5 mg/kg (2 ml/kg) were prepared, respectively. They were infused for 1 min to rats in the control group (n = 3 and 8 for paclitaxel and docetaxel studies, respectively) and DMIS group (n = 5 and 6 for paclitaxel and docetaxel studies, respectively). For paclitaxel, blood samples of approximately 220 μl were collected from the carotid artery at intervals of 0, 15, 30, 60, 90, 120, 180, 240, 360, 480 and 600 min after oral administration. For docetaxel, blood samples (each, approximately 420 μl) were collected at 0, 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 480 and 600 min after oral administration. Other procedures were similar to those used in the intravenous study.

Quantitative polymerase chain reaction (PCR)

Total RNA was isolated from approximately 100–200 mg of rat liver and small intestine. Since regional differences in Cyp3A and Mdr1 mRNA expression in the rat small intestine have been reported [20], the small intestine was divided into two segments; the upper (duodenum; 8 cm loop originated at ~5 cm from the pylorus) and lower (ileum; 8 cm loop to 5 cm from the ileocaecal end) segments. Immediately after the tissues were collected, they were washed in DEPC-treated water, chopped on the ice, and stored at −80 °C in the ice-cold RNAlater® (Ambion, Austin, TX) until RNA extraction. Frozen samples were homogenized in RNAiso (Takara Bio Inc., Otsu, Japan) using a Dounce tissue grinder (Wheaton, Milville, NJ) and the total RNA was isolated according to the manufacturer’s protocol. The amount of purified RNA was estimated by a UV/VIS spectrophotometer and its purity was assessed by the absorbance ratio 260/280 nm. Subsequently, the mRNA was reverse-transcribed to cDNA using Takara RNA PCR™ kit (AMV ver. 3.0) and Takara PCR Thermal Biopharm. Drug Dispos. 33: 474–486 (2012) DOI: 10.1002/bdd
Cycler Dice TP65. For conversion of total RNA to cDNA, a 10 µl reaction mixture was prepared containing 25 mM MgCl2, 10 × RT buffer, RNase free buffer, 2.5 mM dNTP mixture, 50 pmol/µl Random 9mer, 40 units/µl RNase inhibitor, 5 units/µl AMV reverse transcriptase XL and 1 µg/µl experimental sample. The reaction was performed for 10 min at 30°C, 30 min at 42°C, 15 min at 50°C, 5 min at 99°C and 5 min at 5°C and the reverse-transcribed cDNA was used as a template for quantitative PCR. Amplification was performed in the LightCycler 1.5 (Roche Diagnostics, Germany) using a 20 µl reaction mixture containing 2 × SYBR Premix Ex Taq (Takara Bio Inc., Otsu, Japan), 1 µM forward primer, 1 µM reverse primer and template cDNA.

The mRNA expressions of Cyp3A (Cyp3A1, 3A2, 3A9, 3A18 and 3A62) and Mdr1 (Mdr1a and Mdr1b) were measured. Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) was used as a housekeeping gene. Primer sets for Gapdh, Mdr1 and Cyp3A subfamilies were checked against the NCBI BLAST database to ensure specificity for the selected genes. Primer sequences are listed in Table 1.

LC-MS/MS analysis

Concentrations of paclitaxel were determined by LC-MS/MS (Agilent Technologies 1200 series, Agilent, Santa Clara, CA) coupled to a Waters Quattro micro™ API mass spectrometer (Waters, Milford, MA) equipped with an electrospray ionization interface used to generate positive ions [M + H]+. Aliquots of 50 µl of 50% methanol containing 500 ng/ml docetaxel (internal standard) and 1 ml of tert-butyl methyl ether were added to 100 µl of plasma sample. The mixture was then vortex-mixed for 5 min, centrifuged at 15000 rpm for 10 min, and a 1 ml aliquot of the supernatant was evaporated to dryness using a Speed-Vac concentrator (Centra Vac; Vision Scientific Co., Bucheon, Korea). The residue was reconstituted with 50 µl of mobile phase (see below), and a 30 µl aliquot was directly injected onto a Thermohypersil-Keystone C18 column (50 × 2.1 mm internal diameter (i.d.); particle size, 5 µm; Thermo Fisher Scientific Inc. Bellefonte, PA). The mobile phase consisted of methanol (A) and water containing 0.1% v/v formic acid (B). A gradient programme was used as follows: 0 min, 40:60; 3 min, 90:10; 8 min, 90:10; 9 min, 40:60; 12 min, 40:60 (A:B, v/v). The flow rate was 0.3 ml/min. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 854.3 → 104.9 for paclitaxel and m/z 808.3 → 527.2 for docetaxel, respectively. The optimal mass parameters obtained were as follows: capillary voltage, 3.5 kV; cone voltage, 25 and 15 V for paclitaxel and docetaxel, respectively; source temperature, 120°C; and desolvation temperature, 400°C. Nitrogen was used as the desolvation gas and cone gas with flow rates of 800 and 501/h, respectively. The collision energies were 50 eV and 12 eV for paclitaxel and docetaxel, respectively. The analytical data were processed by MassLynx V 4.1 software (Waters, Milford, MA). A calibration study indicated that the detector response was linear over the paclitaxel concentration range 0.005–50 µg/ml.

The analysis method for docetaxel was similar to that for paclitaxel. Aliquots of 50 µl (for intravenous samples) and 20 µl (for oral samples) of acetonitrile containing 500 ng/ml paclitaxel (internal standard) and 1 ml of tert-butyl methyl ether were added to

<table>
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<th>Table 1. Nucleotide sequences of the primers used in quantitative PCR</th>
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<td>Gene</td>
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200μl of plasma sample. The mixture was then vortex-mixed for 5 min, centrifuged at 15000 rpm for 10 min, and a 1 μl aliquot of the supernatant was evaporated to dryness using a Speed-Vac concentrator (Centra Vac; Vision Scientific Co., Bucheon, Korea). The residue was reconstituted with 200 μl (for intravenous samples) or 50 μl (for oral samples) of mobile phase (see below), and a 30 μl aliquot was directly injected onto a YMC-UltraHT Pro C18 column (50 × 2.1 mm internal diameter (i.d.); particle size, 2 μm; YMC Co. Ltd, Kyoto, Japan). The mobile phase, 0.1% formic acid: acetonitrile (35:65, v/v), was run with a flow rate of 0.3 ml/min. Other procedures were similar to those used in the analysis of paclitaxel. A calibration study indicated that the detector response was linear over the docetaxel concentration range 0.005–100 μg/ml.

Data analysis

Standard methods [21] were used to calculate the following pharmacokinetic parameters using non-compartmental analysis (WinNonlin®; Pharsight, Mountain View, CA): the total area under the plasma concentration–time curve from time zero to infinity (AUC) [22], total body clearance (CL), terminal half-life and apparent volume of distribution at steady state (Vss). The peak plasma concentration (Cmax) and time to reach Cmax (Tmax) were directly read from the experimental data. A value of p less than 0.05 was considered to be statistically significant using an unpaired Student’s t-test. All data are expressed as mean ± standard deviation (SD).

Results

Pharmacokinetics of paclitaxel

The mean arterial plasma concentration–time curves of paclitaxel after intravenous (A) and oral (B) administration to control rats and DMIS rats are shown in Figure 2. The relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration of paclitaxel to DMIS rats, the pharmacokinetic parameters were comparable to those of the control rats. After oral administration, the AUC and Cmax of paclitaxel were significantly higher (1.57- and 1.97-fold, respectively) in DMIS rats compared with controls. The terminal half-life of paclitaxel was significantly shorter (31.3% decrease) in DMIS rats than in the controls.

Pharmacokinetics of docetaxel

The mean arterial plasma concentration–time curves of docetaxel after intravenous (A) and oral (B) administration to control rats and DMIS rats are shown in Figure 3. The relevant pharmacokinetic parameters are listed in Table 3. After intravenous administration of docetaxel to DMIS rats, the major pharmacokinetic parameters were comparable to those of the control rats. However, the terminal half-life (23.7% decrease) and Vss (56.0% decrease) were both decreased significantly in DMIS rats. However, after oral administration, the pharmacokinetic
parameters, including AUC, C\textsubscript{max}, T\textsubscript{max} and the terminal half-life of docetaxel were found similar between DMIS and control rats.

**mRNA expression of Cyp3A subfamilies in liver and small intestine**

The hepatic and intestinal mRNA expression levels of rat Cyp3A examined using a quantitative PCR are shown in Figure 4. In the livers of DMIS rats, among the Cyp3A subfamilies, Cyp3A1 and Cyp3A9 mRNA expressions were significantly greater (2.30- and 1.97-fold, respectively) than those of the controls (Figures 4A, E). But the mRNA expression of hepatic Cyp3A2, 3A18 and 3A62 were unchanged in DMIS rats (Figure 4C, G, I). In the duodenum and ileum, Cyp3A1 and 3A2 mRNA expression was not detected in both groups of rats (Figures 4B, D). In the DMIS rats, the mRNA expression of duodenal and not ileal Cyp3A62 was significantly lower (35.2%) (Figure 4J), though Cyp3A9 and 3A18 were comparable to those of the control rats (Figures 4F, H).

**mRNA expression of Mdr1 subfamilies in liver and small intestine**

Figure 5 represents the mRNA expression of Mdr1a and Mdr1b in the liver and intestine of the DMIS and control rats. In the DMIS rats, the mRNA expression levels of hepatic Mdr1b and duodenal Mdr1a were significantly greater (6.48-fold, Figure 5C) and considerably lower.
The hepatic mRNA expression of Mdr1a was unchanged by diabetes mellitus (Figure 5A) and intestinal Mdr1b (Figure 5D) was not detected in either DMIS or control rats.

Discussion

Induction of diabetes mellitus in rats by streptozotocin was evident, based on the significantly higher blood glucose level and the decrease in body weight gain (Tables 2 and 3). The pharmacokinetics of paclitaxel and docetaxel after intravenous administration in DMIS rats seemed not to be greatly different from the pharmacokinetics in the control rats. The AUC and CL values of both drugs were not significantly changed in DMIS rats compared with the controls (Tables 2 and 3). For drugs that are metabolized by the liver and intestine, metabolism is dependent on the route of administration [23]. When a drug is administered intravenously, the majority of metabolism occurred in the liver [23,24]. Thus the comparable pharmacokinetics of intravenously administered taxanes in DMIS rats compared with the control rats could be attributable to the comparable hepatic clearance.

However, in our study, although the major form of Cyp3A, i.e. Cyp3A2 [25], was not significantly changed, Cyp3A1 and Cyp3A9 were significantly increased in the DMIS rats (Figure 4). This was consistent with previous results. Kim et al. reported that in DMIS rats, the protein expression of hepatic Cyp3A1 using immunoblot analysis increased 1.9-fold, and the mRNA level of the same gene using northern blot analysis increased 2.2-fold, relative to the control rats [26]. Increases in hepatic Cyp3A1 [27,28] and Cyp3A2 [29,30] in DMIS rats based on western blot analysis and/or various enzyme activity tests have also been reported.

Our results on comparable hepatic mRNA expression of Mdr1a and the increased expression of Mdr1b (Figure 5) were also supported by others. Kameyama et al. reported that in livers of DMIS rats, only Mdr1b mRNA expression was up-regulated (2-fold) and this increase was responsible for raising the liver concentration of P-gp, as confirmed by western blot analysis [30]. Pretreatment with acetylaminoflourene, a P-gp inducer, was reported significantly to increase the biliary excretion of [3H]cyclosporine A in isolated perfused rat liver (0.61 vs 0.33, p < 0.05) [31].

The inconsistency between the comparable AUC values of taxanes and increased mRNA expression of hepatic Cyp3A and Mdr1 could be due to the dose of taxanes. The metabolic capacity of the liver may not be saturated with the intravenous dose of 5 mg/kg, thus all the taxanes in the body could be metabolized fully. In other words, the dose seemed to be not sufficient for detecting the metabolic changes. A decrease in intestinal metabolism and efflux due to the decreased mRNA
Figure 4. The relative mRNA expression levels of Cyp3A1 (A, B), 3A2 (C, D), 3A9 (E, F), 3A18 (G, H), and 3A62 (I, J) in livers (A, C, E, G, I) and intestine (duodenum and ileum) (B, D, F, H, J) of control rats (white column) and DMIS rats (black column). The mRNA expression levels of target genes were normalized to that of Gapdh. *p < 0.05 and **p < 0.01 vs control rats (Student's t-test). Bars represent SD (n = 5, each). N.D., not detected.
expression levels of intestinal Cyp3A and Mdr1 in DMIS rats (Figures 4 and 5) may contribute somewhat to the comparable AUC and CL values of both drugs. A similar result was also reported with cyclosporine A. Although cyclosporine A is a dual substrate for Cyp3A and P-gp as are taxanes, there were no significant changes in the systemic and lymphatic availabilities after intravenous administration in diabetic rats compared with those of the controls [32].

After the oral administration of paclitaxel to DMIS rats, the AUC and C_{max} were significantly greater compared with the control rats (Table 2). However, the oral AUC and C_{max} of docetaxel in DMIS rats were not significantly changed compared with the controls (Table 3). Only the slightly greater plasma concentrations of docetaxel in the early phase of DMIS rats implied the decrease in intestinal P-gp expression (p = 0.008, ANOVA).

In the intestine of DMIS rats, Borbás et al. reported that there is a significant decrease (50.7% decrease) in the activity of testosterone 6β-hydroxylase (a Cyp3A1/2 marker) relative to the control rats [28]. When the mRNA expression levels of Cyp3A isoform in the intestine of DMIS and control rats were examined, the mRNA of Cyp3A1 and 3A2 in the small intestine was not detected (Figure 4), suggesting that drug metabolism in the rat intestine is predominantly mediated by other Cyp3A isoforms. The expression of Cyp3A62 was found decreased significantly in the duodenum of DMIS rats (Figure 4). As the relative amounts (molecular number of Cyp3A mRNA to total RNA amount (attomole of Cyp3A mRNA/μg total RNA) of each Cyp3A isoform in the male Sprague-Dawley rat total intestine (duodenum, jejunum and ileum) are reported as follows: Cyp3A62 (23.0) > Cyp3A9 (22.0) > Cyp3A18 (1.19) > Cyp3A1 and 3A2
(not detected) [25], thus, the decrease of intestinal Cyp3A62 would affect the change in the intestinal metabolism of taxanes. With respect to transporters, Nawa et al. reported the decreased expression level of intestinal Mdr1a under streptozotocin-induced diabetic conditions [33]. In our studies, the mRNA of Mdr1a of duodenum considerably decreased in the DMIS rats (Figure 5). Down-regulation of intestinal P-gp may increase absorption of taxanes by blocking efflux transport.

The negligible changes in the pharmacokinetics of docetaxel compared with paclitaxel after the oral administration to DMIS rats could be due to the difference in the contribution of P-gp inhibition to drug absorption. Bardelmeijer et al. identified that docetaxel is a weaker substrate of P-gp than paclitaxel using in vitro transport studies [34]. Furthermore, in the Mdr1a/1b knockout mice, the fold increase of AUC after oral administration was 6.4 and 2.83 for the paclitaxel [14] and docetaxel [16], respectively. For docetaxel, intestinal Cyp3A was a predominant factor for the absorption. Knockout of Cyp3A gave rise to a dramatic increase of oral AUC, 11.5-fold, whereas knockout of Mdr1a/1b increased the AUC of docetaxel by only about 2.8-fold [16].

In addition, Vaclavikova et al. conducted enzymatic kinetic studies for Cyp3A1/2 with paclitaxel and docetaxel in microsomes of various species [35]. In the rat liver microsomes, the affinity and maximum velocity of docetaxel were higher and greater, respectively, than paclitaxel. Thus the metabolism via intestinal Cyp3A of docetaxel could be more sensitively changed than paclitaxel in DMIS rats. Thus, if we consider the significantly higher absorption of paclitaxel in DMIS rats, the unchanged absorption of docetaxel suggests that the alteration of intestinal Cyp3A in DMIS rats may not be marked enough to exert significant influence on the absorption of Cyp3A substrates that are mostly cleared heptatically.

Paclitaxel seemed to be more susceptible to the regulation of P-gp, rather than Cyp3A in intestinal absorption. While the oral AUC of paclitaxel administered with ketoconazole, a Cyp3A inhibitor, increased 1.6-fold, that of paclitaxel administered with KR-30031, a P-gp inhibitor, increased 7.5-fold above that of the drug administered alone [15]. Considering the significant change of oral pharmacokinetics of paclitaxel in DMIS rats and the different sensitivities of paclitaxel and docetaxel to P-gp and Cyp3A, decreased Mdr1a rather than Cyp3A in DMIS rats seemed to have a greater influence on the absorption of these taxanes.

Interestingly, a smaller terminal half-life and $V_{ss}$ of docetaxel after intravenous administration were observed in the DMIS rats. As mentioned above, the decreased tissue distribution by modulation of P-gp function may be one reason for the decreased $V_{ss}$. A decreased distribution of P-gp substrate in the brain was reported in DMIS rats with functional induction of P-gp across the blood–brain barrier [7]. However, this explanation requires further investigation, because controversial results have also been reported by other researchers [6]. Multidrug resistance-associated protein (Mrp) 2 could have a role in biliary excretion of paclitaxel and docetaxel because taxanes are substrates for Mrp2 in vitro [36]. But the in vivo significance of Mrp2 for the pharmacokinetic changes seems to be limited [37]. There are also controversial results for the alteration of hepatic Mrp2 induced by streptozotocin [38–40].

A decreased uptake into hepatocytes could also contribute to the decreased $V_{ss}$. In addition to P-gp, the organic anion-transporting polypeptide-1B3 (OATP1B3) is known as a key regulator of hepatic uptake of taxanes in humans [41] and a decreased expression of Oatps has been observed in the livers of ob/ob mice, an animal model of obesity and type II diabetes [42]. A decreased mRNA expression of Oatp1b2, of which the amino acid identity with OATP1B3 is 66% [43], was also confirmed in our laboratory (100 vs 71.5% for the control and DMIS rats, respectively; $p < 0.089$).

Osicka et al. examined the $\alpha$-tubulin protein content by immunohistochemistry in the Wistar-Kyoto control and DMIS rats [44]. There was a significant decrease of approximately one-third in diabetic rats (20.6 ± 1.2) compared with the control rats (30.3 ± 1.5). A decreased expression of $\beta$-tubulin in DMIS rats was also reported in other studies [45, 46]. Therefore the $V_{ss}$ of docetaxel, a tubulin-binding drug, could be smaller in DMIS rats than in the control rats. A decreased intracellular trapping by tubulin may contribute to the decreased terminal half-life of docetaxel also.

On the contrary, the $V_{ss}$ and terminal half-life of paclitaxel after intravenous administration were unchanged in the DMIS rats, possibly due
to the difference of affinity. The affinity for the binding site of microtubules, determined from the displacement of the fluorescent taxoid flutax-2, of docetaxel, was reported to be much higher than that of paclitaxel [47]. Although the mechanism of change in $V_{\text{ss}}$ and the terminal half-life of docetaxel is unknown, this is the first report in DMIS rats and remains a matter to be discussed in further studies.

In comparison with the similar terminal half-life values of docetaxel after intravenous and oral administration, the terminal half-life of paclitaxel after oral administration was longer compared with the intravenous administration in the control rats (Table 2). This could be due to the slower absorption and multi-exponential elimination of paclitaxel as seen in Figure 2B. The sampling time (10 h) also seemed to be insufficient for the detection of terminal half-life. After oral administration, the absorption may affect the elimination phase of the drug and thus terminal half-life values after oral administration are ‘apparent values’ [48]. Thus the decreased terminal half-life of paclitaxel after oral administration in DMIS rats seems not to have significant meaning.

In conclusion, the effects of diabetes mellitus on the pharmacokinetics of taxanes were discriminative between paclitaxel and docetaxel and depended on the administration route. After intravenous administration, systemic exposures of paclitaxel and docetaxel were not affected by diabetes mellitus. After oral administration to the DMIS rats, the $AUC$ and $C_{\text{max}}$ of paclitaxel were increased significantly, whereas those of docetaxel were not changed significantly. This was most likely due to the impact of decreased intestinal P-gp rather than Cyp3A, because paclitaxel and docetaxel are more susceptible to the intestinal P-gp and Cyp3A, respectively. Thus, the alteration of P-gp in DMIS rats should be focused on and the quantitative contribution should be examined further. Although these up- and down- regulations in DMIS rats have not yet been confirmed in diabetic patients, the findings that the diabetes affects the pharmacokinetics of taxanes and that the tendency is dissimilar for paclitaxel and docetaxel are considered to be noteworthy in the context of pharmacokinetics in diabetic patients.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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