Alteration in the Plasma Concentrations of Endogenous Organic Anion–Transporting Polypeptide 1B Biomarkers in Patients with Non–Small Cell Lung Cancer Treated with Paclitaxel

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ABSTRACT

Paclitaxel has been considered to cause OATP1B–mediated drug–drug interactions at therapeutic doses; however, its clinical relevance has not been demonstrated. This study aimed to elucidate in vivo inhibition potency of paclitaxel against OATP1B1 and OATP1B3 using endogenous OATP1B biomarkers. Paclitaxel is an inhibitor of OATP1B1 and OATP1B3, with Kᵢ of 0.579 ± 0.107 and 5.29 ± 3.87 μM, respectively. Preincubation potentiated its inhibitory effect on both OATP1B1 and OATP1B3, with Kᵢ of 0.154 ± 0.031 and 0.624 ± 0.183 μM, respectively. Ten patients with non–small cell lung cancer who received 200 mg/m² of paclitaxel by a 3-hour infusion were recruited. Plasma concentrations of 10 endogenous OATP1B biomarkers—namely, coproporphyrin I, coproporphyrin III, glycochenodeoxycholate-3-sulfate, glycochenodeoxycholate-3-glucuronide, glycodeoxycholate-3-sulfate, glycodeoxycholate-3-glucuronide, lithocholate-3-sulfate, glycolithocholate-3-sulfate, taurocholate-3-sulfate, and chenodeoxycholate-3-glucuronide—were determined in the patients with non–small cell lung cancer on the day before paclitaxel administration and after the end of paclitaxel infusion for 7 hours. Paclitaxel increased the area under the plasma concentration-time curve (AUC) of the endogenous biomarkers 2–4-fold, although a few patients did not show any increment in the AUC ratios of lithocholate-3-sulfate, glycolithocholate-3-sulfate, and tauroolithocholate-3-sulfate. Therapeutic doses of paclitaxel for the treatment of non–small cell lung cancer (200 mg/m²) will cause significant OATP1B1 inhibition during and at the end of the infusion. This is the first demonstration that endogenous OATP1B biomarkers could serve as surrogate biomarkers in patients.

SIGNIFICANCE STATEMENT

Endogenous biomarkers can address practical and ethical issues in elucidating transporter-mediated drug–drug interaction (DDI) risks of anticancer drugs clinically. We could elucidate a significant increment of the plasma concentrations of endogenous OATP1B biomarkers after a 3-hour infusion (200 mg/m²) of paclitaxel, a time-dependent inhibitor of OATP1B1, in patients with non–small cell lung cancer. The endogenous OATP1B biomarkers are useful to assess the possibility of OATP1B–mediated DDIs in patients and help in appropriately designing a dosing schedule to avoid the DDIs.

Introduction

Organic anion–transporting polypeptides 1B1/SLC01B1 (OATP1B1) and 1B3/SLC01B3 (OATP1B3) are specifically expressed on the sinusoidal membrane of hepatocytes to mediate the uptake of their substrates from the blood circulation into hepatocytes for further elimination to the bile and metabolism (Maeda, 2015; Patel et al., 2016; Lee and Ho, 2017; McFeely et al., 2019). OATP1B1 and OATP1B3 are multispecific transporters that recognize various anionic drugs and play key roles in the clearance of such drugs. Therefore, OATP1B–mediated drug–drug interactions (DDIs via induction or inhibition) could modulate the pharmacokinetics of drugs and, thereby, their pharmacological effect or toxicity (Maeda, 2015; Patel et al., 2016;
Lee and Ho, 2017; Asaumi et al., 2019; McFeely et al., 2019). Regulatory agencies in Japan, the United States, and the European Union strongly recommend pharmaceutical industries in their guidelines to assess the OATP1B-mediated DDI risks of their new chemical entities routinely in vitro, and when the predicted risk is above the regulatory thresholds, to evaluate the DDI impact clinically.

The principle of DDI prediction is to compare the unbound concentration at the site of DDI and inhibition constants (Kᵢ) (Giacomini et al., 2010). However, there is a limitation in the current DDI risk prediction for improvement in preclinical stages to avoid false-positive and false-negative predictions (Vaidyanathan et al., 2016). Endogenous biomarkers for drug transporters have emerged as surrogate DDI probes to strengthen the in vitro DDI prediction (Chu et al., 2018; Müller et al., 2018; Rodrigues et al., 2018). Using the typical drug transporter inhibitors, the magnitude of changes of endogenous biomarkers in the area under the plasma concentration-time curves (AUC) and/or renal clearance have been reported for multidrug and toxic compound extrusions, OAT1 and OAT3, and OATP1B1 and OATP1B3. The most important advantage of endogenous biomarkers is that researchers are freed from administration of probe drugs, which could increase the opportunity to assess the DDI risk in humans.

An antimitotubule agent, paclitaxel, has been used in chemotherapy of various tumors, such as non–small cell lung cancer, breast cancer, and ovarian cancer. The major elimination pathway of paclitaxel from the blood circulation is CYP2C8-mediated metabolism to form 6α-hydroxy paclitaxel (Sonichsen et al., 1995). DDI with paclitaxel as victim drug has been studied in patients who received clopidogrel, which caused time-dependent inhibition of CYP2C8 (Floyd et al., 2012); patients who received both clopidogrel and paclitaxel exhibited higher incidence of inhibition by paclitaxel has not been confirmed because of ethical and practical issues in conducting clinical DDI studies in healthy subjects or patients. We and other groups identified glycochenodeoxycholate-3-sulfate (GCDCA-S) (Takehara et al., 2017, 2018), coproporphyrin I (CPI) and CPIII (Lai et al., 2016; Barnett et al., 2016, 2019), and tetradecanedioate and hexadecanedioate (Yee et al., 2019) as endogenous biomarkers for drug transporters have emerged as surrogate DDI probes. The clinical relevance of OATP1B inhibition by paclitaxel has not been confirmed because of ethical and practical issues in conducting clinical DDI studies in healthy subjects or patients. We and other groups identified glycochenodeoxycholate-3-sulfate (GCDCA-S) (Takehara et al., 2017, 2018), coproporphyrin I (CPI) and CPIII (Lai et al., 2016; Barnett et al., 2016, 2019), and tetradecanedioate and hexadecanedioate (Yee et al., 2019) as endogenous biomarkers for OATP1B1/1B3. Accumulation of clinical data in healthy subjects to support the suitability of these OATP1B-endogenous biomarkers could allow us to evaluate the OATP1B-mediated DDI risks of anticancer drugs in patients.

This study was a clinical study designed to assess the changes in plasma concentrations of such endogenous OATP1B biomarkers in patients with non–small cell lung cancer who received paclitaxel chemotherapy (paclitaxel 200 mg/m² given intravenously by a 3-hour infusion plus dexamethasone, diphenhydramine, and famotidine given 30 minutes prior to paclitaxel administration). In addition, the patients also received drug therapy for treatment of their complications (Table 1), but there was no drug that was administered to all patients.

Materials and Methods

Chemicals. [3H]Estradiol 17β-glucuronide (E217βG) and [3H]cholecystokinin octapeptide were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Authentic compounds including stable isotope-labeled compounds

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<tr>
<th>ID</th>
<th>Sex</th>
<th>Age</th>
<th>Height</th>
<th>b.wt.</th>
<th>OATP1B1 haplotype</th>
<th>d.wt.</th>
<th>Ab</th>
<th>ACP</th>
<th>ALT</th>
<th>LDH</th>
<th>ALP</th>
<th>T-Bil</th>
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<td>0.79</td>
<td>87.1</td>
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</table>

This study was a clinical study designed to assess the changes in plasma concentrations of such endogenous OATP1B biomarkers in patients with non–small cell lung cancer who received paclitaxel chemotherapy (paclitaxel 200 mg/m² given intravenously by a 3-hour infusion plus dexamethasone, diphenhydramine, and famotidine given 30 minutes prior to paclitaxel administration). In addition, the patients also received drug therapy for treatment of their complications (Table 1), but there was no drug that was administered to all patients.
used in this study were all commercially available (Supplemental Materials). Other reagents and organic solvents were of a commercially available analytical grade.

In Vitro Transport Study Using Transporter-Expressing Cells. HEK293 cells, which were stably transduced with OATPB1, OATPB3, OAT1, OAT3, or empty vector, were established previously (Deguchi et al., 2004; Hirano et al., 2004). The transport study was performed according to the previous reports (Deguchi et al., 2004; Hirano et al., 2004). Briefly, HEK293 cells were seeded at 2 × 10^5 cells per well in a 24-well plate coated with poly-l-lysine (50 mg/ml) and poly-l-ornithine (50 mg/ml). After 48 hours, the medium was substituted by Dulbecco’s modified Eagle’s medium containing 5 mM sodium butyrate. For 24 hours, the transport study was conducted. Cells were washed twice and preincubated with Krebs-Henseleit (KH) buffer at 37°C for 5 minutes. Then, substrates were added to initiate the uptake. At the designated times, incubation buffer was removed, and ice-cold KH buffer was added to terminate the uptake.

To evaluate the inhibition potency of paclitaxel and 6α-hydroxy paclitaxel, either paclitaxel or 6α-hydroxy paclitaxel was added simultaneously with the test substrate. When the time dependence of the inhibitory effect was assessed (shown in Fig. 1 and Supplemental Fig. 8), cells were preincubated in the presence of paclitaxel or 6α-hydroxy paclitaxel for 15 minutes. In a separate experiment (shown in Supplemental Fig. 6A), cells were preincubated in KH buffer containing paclitaxel for the designated periods (5, 10, 30, 60 minutes) and then washed. After washing, uptake was initiated in the absence of inhibitor. To assess the duration of the inhibitory effect (shown in Supplemental Fig. 6B), cells were preincubated with KH buffer containing paclitaxel for 30 minutes and incubated with paclitaxel-free KH buffer for the designated periods (0, 10, 30, 60 minutes). Then, uptake was initiated in the absence of paclitaxel.

To evaluate the time dependence of the inhibitory effect of paclitaxel and 6α-hydroxy paclitaxel, the cells were collected in Milli-Q water using a scraper and disrupted using a bioruptor (Sonicsbio, Kanagawa, Japan). A 20-μl aliquot was mixed with 60 μl of acetonitrile containing the corresponding internal standard solution. After vortex mixing, the mixtures were centrifuged (20,000 g) for 10 minutes and the supernatant was subjected to Western blot analysis. The samples were subjected to Western blot analysis. (12% SDS, 25% glycerol, 150 mM Tris-HCl pH 7.0, 0.05% bromophenol blue, containing the corresponding internal standard solution. After vortex mixing, the mixtures were centrifuged (20,000 g) for 10 minutes and the supernatant was subjected to Western blot analysis. The samples were subjected to Western blot analysis.
for quantification of endogenous compounds. Quantification of C4, bile acids, and bile acid sulfates (LCA-S, TLCA-S, and GLCA-S) was performed as described previously (Mori et al., 2019a,b). The measurement method is summarized in Supplemental Methods. For separation of stereoisomers, such as CPI and CPIII, GCDCA-S and GDCA-S, and GCDCA-G and GDCA-G, the chromatographic separation was modified as described in the Supplemental Methods. 

Calculation of AUC of the Endogenous Compounds. The AUC was calculated from –3 to 10 hours using the linear trapezoidal method using Excel. 

Determination of the Inhibition Constants (K_i) of Paclitaxel and 6a-Hydroxy Paclitaxel for OATP1B1 and OATP1B3. Iterative nonlinear least-squares method using GraphPad Prism8 (GraphPad Software, San Diego, CA) was conducted to determine the K_i values of paclitaxel or 6a-hydroxy paclitaxel. The uptake of test compound determined in the presence and absence of inhibitor [CL_uptake (+inhibitor) and CL_uptake (control)] respectively was fitted to the following equation:

\[
\frac{CL_{\text{uptake (+inhibitor)}}}{CL_{\text{uptake (control)}}} = \frac{1}{1 + \frac{I}{K_i}}
\]

where I represents the inhibitor concentration.

Statistics. Paired t test was used to examine statistical significance in the effect of paclitaxel administration on the plasma concentrations of endogenous OATP1B biomarkers using GraphPad Prism8 (GraphPad Software). Geometric means and 95% confidence of intervals of the AUC ratio were calculated using GraphPad Prism8.

Results

Effect of Paclitaxel on OATP1B1- and OATP1B3-Mediated Uptake in HEK293 Cells. Paclitaxel inhibited the OATP1B1-mediated uptake of \[^{3}H\]E217βG in a concentration-dependent manner (Fig. 1), with K_i of 0.579 ± 0.107 μM with a competitive manner (Supplemental Fig. 2). A 15-minute preincubation with paclitaxel potentiated its inhibitory effect, reducing the K_i to 0.154 ± 0.031 μM (Fig. 1). Paclitaxel showed lower inhibition potency to OATP1B3 than OATP1B1 (Fig. 1), with the estimated K_i of 0.624 ± 0.183 and 5.29 ± 3.87 μM with or without preincubation, respectively (Fig. 1). OATP1B1 and OATP1B3 were not inhibited by drugs administered prior to paclitaxel administration (Supplemental Fig. 3). Paclitaxel did not inhibit either OAT1 or OAT3 at 10 μM (Supplemental Fig. 4).

Plasma Concentration-Time Profile of Paclitaxel in Patients with Non–Small Cell Lung Cancer. Plasma concentrations of paclitaxel and its hydroxy metabolite, 6a-hydroxy paclitaxel, were determined in the patients (Fig. 2). Plasma concentrations of 6a-hydroxy paclitaxel accounted for 1/10 of those of paclitaxel (Fig. 2). The f_p of paclitaxel at 0 and 7 hours was determined in all of the subjects. The mean values were 0.0158 ± 0.0042 and 0.0354 ± 0.0247, respectively, which were similar to the previously reported values (0.036–0.079) (Brouwer et al., 2000). The unbound concentration of paclitaxel was calculated using individual f_p and C_max at 0 hours. It was somewhat below the K_i for OATP1B1 with preincubation (Supplemental Fig. 5).

Effect of Paclitaxel Administration on the Plasma Concentrations of the Endogenous OATP1B Biomarkers. Plasma concentrations of 10 endogenous biomarkers, three porphyrin metabolites (CPI and CPIII), and bile acid glucuronides or sulfate (GCDCA-S, GCDCA-G, GDCA-S, GDCA-G, LCA-S, GLCA-S, TLCA-S, CDCA-24G) were determined on day 0 (baseline) and day 1 (before and after paclitaxel administration) (Fig. 3). During the observed time (–3 to 7 hours), there was no obvious change in the plasma concentrations on day 0. After a 3-hour infusion of paclitaxel, the plasma concentrations of the endogenous biomarkers were higher than the predose level or baseline (day 0). The time to reach maximum concentration differed among the biomarkers: end of infusion, CPI, CPIII, and CDCA-24G; 0–2 hours postinfusion, GCDCA-S, GDCA-S, LCA-S, GLCA-S, TLCA-S, CDCA-24G; 4 hours postinfusion, GDCA-G and GDCA-G. The increased plasma concentrations did not return to the corresponding baseline levels by 7 hours postinfusion. Note that LCA-S, GLCA-S, and TLCA-S could not be quantified in one patient (PTX-5).

Fig. 1. Inhibitory effect of paclitaxel on the OATP1B1- and OATP1B3-mediated uptake in HEK293 cells. The uptake of \[^{3}H\]E217βG by HEK293 cells stably expressing OATP1B1 and that of pitavastatin (0.3 μM) by HEK293 cells stably expressing OATP1B3 for 3 minutes with (○) or without (●) paclitaxel preincubation (15 minutes) was determined in the absence and presence of paclitaxel at the designated concentrations or rifampicin (20 μM). The solid line represents the fitted line obtained by nonlinear regression analysis as described in the Materials and Methods section. The dotted line represents the mean value of the uptake of \[^{3}H\]E217βG in the presence of rifampicin. Each symbol represents the mean value with S.E.M. (n = 3). Co, coincubation; Pre+Co, preincubation plus coincubation; Rif, rifampicin.

Fig. 2. Plasma concentrations of paclitaxel and 6a-hydroxy paclitaxel. Plasma concentrations of paclitaxel (closed symbols) and 6a-hydroxy paclitaxel (open symbols) were determined at designated time points in patients with non–small cell lung cancer after a 3-hour intravenous infusion of paclitaxel (200 mg/m^2) from −3 to 0 hours. Each symbol represents the mean value, and error bars representing S.E.M. (n = 10) were within the symbol.
The interindividual difference in the response to paclitaxel administration is shown as AUC in Fig. 4. The mean and range of the AUC ratio are shown in Table 2. There were nonresponders (AUC ratio < 1.25) to paclitaxel for LCA-S, GLCA-S, and TLCA-S; LCA-S, PTX-7, and PTX-10; GLCA-S, PTX-7, and PTX-9; and TLCA-S, PTX-7, PTX-8, and PTX-9. Because of these nonresponders, the mean ratio of AUC was somewhat smaller for these metabolites. The mean ratio of AUC was similar among the other metabolites (Table 2).

**Effect of Paclitaxel Administration on the Plasma Concentrations of the Intermediate Metabolites of Bile Acid Synthesis and Bile Acids.** The plasma concentrations of C4 and bile acids were also measured in the patients with or without paclitaxel administration. The plasma concentrations of C4 on day 0 were similar throughout the study, whereas those on day 1 decreased significantly over time (Fig. 5A). Plasma concentrations of bile acids, including the precursor of the glucuronide and sulfate conjugates, such as GCDCA and GDCA, were also determined. The mean values increased at the end of infusion for TCA, GCA, GCDCA, GDCA, and TDCA, followed by a rapid decline to the baseline levels, whereas there was no difference in the plasma concentrations of CDCA and DCA with and without paclitaxel administration (Fig. 5A). There was a clear interindividual difference in the

![Fig. 3: Effect of paclitaxel on the plasma concentrations of endogenous OATP1B substrates.](image)

**Fig. 3.** Effect of paclitaxel on the plasma concentrations of endogenous OATP1B substrates. Plasma concentrations of CPI, CPIII, sulfate-conjugated bile acids (GCDCA-S, GDCA-S, LCA-S, GLCA-S, TLCA-S), and glucuronide-conjugated bile acids (GCDCA-G, GDCA-G, CDCA-24G) were determined at designated time points in patients with non-small cell lung cancer treated with or without an intravenous dose of paclitaxel (200 mg/m²). Each symbol represents the mean value, and error bars represent S.E.M. (n = 10). *P < 0.05; **P < 0.01; ***P < 0.001.

![Fig. 4: Area under the plasma concentration-time curves for endogenous OATP1B substrates with and without paclitaxel administration.](image)

**Fig. 4.** Area under the plasma concentration-time curves for endogenous OATP1B substrates with and without paclitaxel administration. The symbols represent AUC values in 10 patients calculated using the linear trapezoidal rule from time -3 to 7 hours. *P < 0.05; **P < 0.01; ***P < 0.001.
baseline levels of bile acids and effect of paclitaxel administration on AUC of bile acids (Fig. 5B).

**Discussion**

DDI risk assessment of anticancer drugs remains mainly limited to in vitro or animal data because elucidating the clinical relevance of the inhibition at their therapeutic dose ranges is ethically and practically difficult. Paclitaxel is one of the anticancer drugs that were suggested to inhibit OATP1B by conventional prediction methods (Marada et al., 2015; Murata et al., 2019). Taking advantage of endogenous biomarkers, circumventing the need for investigators to administer probe drugs, we assessed OATP1B1 and OATP1B3 inhibition potency of paclitaxel at its therapeutic dose for the treatment of non–small cell lung cancer.

Consistent with previous reports, the in vitro inhibition study showed the concentration-dependent inhibition of OATP1B1 and OATP1B3 by paclitaxel (Fig. 1). Competitive inhibition was confirmed for OATP1B1 inhibition (Supplemental Fig. 2), whereas absence of paclitaxel effect on renal organic anion transporters, OAT1 and OAT3 (Supplemental Fig. 4), excludes nonspecific effects, at least during the experiments. Furthermore, the inhibition of OATP1B1 and OATP1B3 by paclitaxel was time-dependent (Fig. 1; Supplemental Fig. 6), which had been reported for cyclosporin A (Shitara and Sugiyama, 2017; Tátrai et al., 2019). A metabolite of paclitaxel, 6a-hydroxy paclitaxel, retained a time-dependent effect on OATP1B1 with similar inhibition potency, whereas it no longer showed time-dependent inhibition of OATP1B3 (Supplemental Fig. 8). The time dependence of OATP1B1 inhibition appears to be reversible because the activity partially recovered after incubation in the absence of paclitaxel (Supplemental Fig. 6). The possibility that paclitaxel treatment decreased the expression of OATP1B1 and OATP1B3 proteins in whole cells and on the cell surface was excluded (Supplemental Fig. 7).

**TABLE 2**

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<tr>
<th>Compounds</th>
<th>Control (nM × h)</th>
<th>+ Paclitaxel (nM × h)</th>
<th>Fold Change</th>
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<tr>
<td>CPI</td>
<td>22.1 ± 1.7</td>
<td>61.4 ± 4.3</td>
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<td>CPIII</td>
<td>4.48 ± 0.34</td>
<td>14.1 ± 1.3</td>
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<td>GCDCA-S</td>
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<td>LCA-S</td>
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<td>GLCA-S</td>
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<td>10.4 ± 3.3</td>
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<td>TLCA-S</td>
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<td>CDCA-24G</td>
<td>141 ± 33</td>
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</tbody>
</table>

Fig. 5. Plasma concentrations of C4 and bile acids with and without paclitaxel administration. (A) Plasma concentrations of C4 and bile acids were determined at designated time points in patients with non–small cell lung cancer treated with or without an intravenous dose of paclitaxel (200 mg/m²). Each symbol represents the mean value, and error bars represent S.E.M. (n = 10). *P < 0.05; **P < 0.01. (B) Area under the plasma concentration-time curves for C4 and bile acids. The symbols represent individual values of AUC calculated using the trapezoidal rule from time –3 to 7 hours. *P < 0.05; **P < 0.01.
Paclitaxel Effects on the Endogenous OATP1B Biomarkers

(2017) proposed a *trans*-inhibition model to account for the time-dependent effect of cyclosporin A (Shitara and Sugiyama, 2017). Paclitaxel may also have an inhibitory effect on OATP1B1 in the plasma membrane or from the cytosolic compartment, where paclitaxel accumulated during incubation. It is also possible that paclitaxel inhibits the maintenance of the driving force for OATP1B1 and OATP1B3, which has not been identified, or post-translational modifications such as phosphorylation, as has been proposed for other drug transporters (Sprowl et al., 2016). It should be noted that, as pointed out for OATP1B1 (Izumi et al., 2015), the inhibitory effect of paclitaxel for OATP1B3 was substrate-dependent; the inhibitory effect of paclitaxel on OATP1B3-mediated [3H]cholecystokinin octapeptide uptake was weaker than that on pitavastatin uptake (Supplemental Fig. 9). Careful selection of test OATP1B3 probe is required for conservative DDI risk assessment.

Comparison of the unbound concentration of paclitaxel in the plasma, estimated as the product of fp and Cmax, with its Ki for OATP1B1 and OATP1B3 (Supplemental Fig. 5), suggests that at this dose regimen, paclitaxel inhibits OATP1B1 more potently than OATP1B3. The plasma concentrations of the endogenous OATP1B biomarkers were significantly higher at the end of paclitaxel infusion than the two control values; baselines were determined on the day before paclitaxel administration, and values were determined before starting the administration of paclitaxel (confirming the interday difference) (Fig. 3). The magnitude of changes in CPI concentrations induced by paclitaxel administration was similar to that previously observed in the subjects with *SLCO1B1* *15* homozygotes (Mori et al., 2019a) and those who received 300 mg of rifampicin (Takehara et al., 2018). Previously, the AUC ratio of GCDCAs (4.3) when 300 mg of rifampicin was administered was greater than that of CPI (2.3) (Takehara et al., 2018), whereas the AUC ratios of CPI and GCDCAs were similar in this study (Table 2). Such a difference may arise from the in vivo inhibition potency of OATP1B1 and OATP1B3 between rifampicin and paclitaxel or disease-related factors. The AUC ratio of LCA-S, GLCA-S, and TLCA-S did not change in two to three patients in this study. Such nonresponse of sulfate conjugates of secondary bile acids to OATP1B1 inhibition was also observed in the rifampicin study (Mori et al., 2019b). The reason for this remains unknown, but using such metabolites as surrogate OATP1B1 probes should be avoided.

The plasma concentrations of bile acids were also determined on both days. Those on day 1 at some time points were higher than the predose levels (Fig. 5; Supplemental Table 1). One patient who showed a higher AUC ratio of GCDCAs (6.8) than others (0.8–1.7) also showed the highest AUC ratio of GCDCAs (13) and GCDCAs-G (6.4) compared with others (2.4–6 and 1.2–3.1, respectively), but the AUC ratio of CPI was similar. Therefore, the AUC ratio of GCDCAs and GCDCAs-G might be influenced not only by OATP1B1 inhibition but also by the interday variation or effect of paclitaxel and/or the concomitantly administered drug on GCDCAs. The possibility that paclitaxel inhibits sodium-taurocholate co-transporting polypeptide can be excluded because of absence of the effect on sodium-taurocholate co-transporting polypeptide-mediated uptake of [3H]taurocholate at 10 μM (data not shown). In addition, C4, an intermediate metabolite of bile acid synthesis from cholesterol, continued to decline on day 1 after paclitaxel administration (Fig. 5). Paclitaxel therapy might affect bile acid synthesis.

Although limited in patient number, effect of *SLCO1B1* genotypes (wild-type homozygotes and *15* heterozygotes) and biochemical test results were shown in Supplemental Figs. 10 and 11. A nonsynonymous single nucleotide polymorphism of *SLCO1B1* (c. 521T>C, p.Val174Ala) is well-known to be associated with higher systemic exposure of OATP1B1 substrate drugs (Lee and Ho, 2017; Yee et al., 2018). In Japanese people, this mutation is associated in a haplotype (*15*) with another nonsynonymous mutation (c. 388A>G, p.Asn130Asp) (Nishizato et al., 2003). Japanese individuals homozygous for the *15* allele showed higher plasma concentrations of CPI and GCDCAs-S than homozygotes of reference types and heterozygotes (Mori et al., 2019a). Consistent with our previous report, individuals heterozygous for this mutation did not show any difference in the plasma concentrations of these substrates (Supplemental Fig. 10), whereas the AUC ratio of CPIII, but not CPI, appeared to be *SLCO1B1* genotype–dependent (Supplemental Fig. 10), which requires further confirmation in future studies. There was no significant effect of biochemical test results on the AUC ratio of CPI (Supplemental Fig. 11) and other test compounds (data not shown).

Together with previously reported practices (Lai et al., 2016; Yee et al., 2016; Shen et al., 2017; Takehara et al., 2017, 2018; King-Ahmad et al., 2018; Kunze et al., 2018; Liu et al., 2018; Müller et al., 2018; Cheung et al., 2019), this study provides more convincing evidence to expand application of the endogenous OATP1B biomarkers to assess in vivo OATP1B inhibition under conditions in which administration of probe drugs is limited or under those closer to actual clinical practice. In addition to paclitaxel, mitoxantrone and etoposide were suggested to cause significant inhibition of OATP1B3 (Marada et al., 2015), and tyrosine kinase inhibitors might cause OATP1B1 inhibition via inhibition of phosphorylation (Sprowl et al., 2016). Endogenous OATP1B biomarkers will be able to demonstrate their clinical relevance in future studies in patients. Whereas the patients generally received drugs other than the potential perpetrator drug to treat their complications, these could have included drugs for which the clinical risk of OATP1B-mediated DDI has not been adequately assessed. In this study, drugs commonly used to treat all patients comprised dexamethasone, diphenhydramine, and famotidine, which at least in vitro, did not inhibit OATP1B1 (Supplemental Fig. 3), but the possibility of OATP1B1-mediated DDIs with these drugs needs to be confirmed in further studies.

The changes in endogenous OATP1B biomarkers induced by perpetrator drugs must be extrapolated to the changes in the AUC of OATP1B1 substrate drugs to suggest the risk of OATP1B1-mediated DDI. An important tool for this purpose is the use of a physiologically based pharmacokinetic (PBPK) model. Yoshikado et al. (2008) constructed a PBPK model of CPI that could account for the plasma concentration-time profiles of CPI after rifampicin administration (Yoshikado et al., 2018). Once an appropriate PBPK model has been constructed for paclitaxel, by analogy with that for CPI, it could be used to help estimate the in vivo Ki of paclitaxel for OATP1B1 that would be required to simulate DDIs between paclitaxel and OATP1B1 substrate drugs, including in cases using other dose regimens for paclitaxel medication. In addition, because the plasma concentrations of CPI and CPIII showed the most rapid decline after the end of the infusion (within 2 hours) of the endogenous substrates tested, PBPK model-based simulation will indicate whether OATP1B1 inhibition by paclitaxel is significant during infusion and for at least 2 hours after the end of infusion, which would enable implementation of an appropriate dosing schedule to avoid significant DDIs.

In conclusion, the present study showed that paclitaxel could inhibit OATP1B1 and OATP1B3 at the dose for the non–small cell lung cancer treatment (200 mg/m² by a 3-hour infusion). This study increases the feasibility of transporter-mediated DDI risk assessment in clinical settings.

**Authorship Contributions**

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Wrote or contributed to the writing of the manuscript: Mizuno, Fujita, Kusuhara.

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