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, tauroliothocholate-3-sulfate, and chenodeoxycholate-24-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- to 4-fold, although a few patients did not show any increment in the AUC ratios of lithocholate-3-sulfate, glycolithocholate-3-sulfate, and tauroliothocholate-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 increase of the plasma concentrations of endogenous OATP1B biomarkers after a 3-hour infusion (200 mg/m²) of paclitaxel, a time-dependent inhibitor of OATP1B, 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; Maeda et al., 2015). A few patients did not show any increment in the AUC ratios of lithocholate-3-sulfate, glycolithocholate-3-sulfate, and tauroliothocholate-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.

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Supplemental material to this article can be found at: http://dmd.aspetjournals.org/content/suppl/2020/02/29/dmd.119.089474.DC1

This article has supplemental material available at dmd.aspetjournals.org.
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 antimicrotubule 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 time-dependent inhibition of CYP2C8 (Floyd et al., 2012); patients who received both clopidogrel and paclitaxel exhibited higher incidence of severe paclitaxel neuropathy than those treated with paclitaxel and low-dose aspirin, although the impact on paclitaxel pharmacokinetic parameters remains to be determined (Agergaard et al., 2017). In contrast, paclitaxel was also suggested to inhibit OATP1B based on comparison of in vitro Ki values for OATP1B1 and OATP1B3 with its clinically relevant unbound concentrations (Marada et al., 2015; Murata et al., 2019). However, 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 chenodeoxycholate-3-sulfate (GCDCA-S) (Takehara et al., 2017, 2018), other sulfated bile acids (Thakare et al., 2017; Mori et al., 2019a; Takehara et al., 2019), coproporphyrin I (CPI) and CPII (Lai et al., 2016; Barnett et al., 2019), and tetradecanedioate and hexadecanedioate (Yee et al., 2018; Rodrigues et al., 2018) 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 OATP1B1 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-n-glucuronide ([E217βG] and [3H]cholic-acid-labeled compounds were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Authentic compounds including stable isotope-labeled compounds

**Table 1**

<table>
<thead>
<tr>
<th>Non-small cell lung cancer patient information</th>
<th>Patient information is summarized in the table. In subject PTX-6, paclitaxel administration was discontinued due to paclitaxel allergy. Alb, albumin; AGP, haptoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; T-Bil, total bilirubins; D-Bil, direct bilirubins; SCr, serum creatinine; eGFR, estimated glomerular filtration rate.</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTX-1</td>
<td>M 74 159.5 54.3 *1b/*1b 4.3 52.0 37 21 200 354 1.3 0.1 0.81 71.0 Alcoholic hepatitis, hyperuricemia, —</td>
<td>—</td>
</tr>
<tr>
<td>PTX-2</td>
<td>F 75 161.5 56.9 *1a/*1b 4.0 65.0 21 14 180 216 0.9 &lt;0.01 0.68 63.3 Diabetic retinopathy, hypertension, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-3</td>
<td>M 58 169 73.2 *1b/*15 3.3 144.0 32 24 269 216 0.5 &lt;0.01 0.89 68.7 Hypertension, atrial fibrillation, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-4</td>
<td>M 57 164.5 53.5 *1b/*15 4.3 73.0 12 6 165 135 1.6 0.1 0.72 87.1 Diastolic blood pressure —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-5</td>
<td>M 48 169 58.6 *1b/*15 3.9 76.0 28 25 190 265 0.9 &lt;0.01 0.10 76.4 Hypertension, angina, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-6</td>
<td>M 78 169 73.2 *1b/*1b 3.4 144.0 32 24 269 216 0.5 &lt;0.01 0.89 68.7 Hypertension, atrial fibrillation, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-7</td>
<td>M 66 165 63.9 *1b/*15 3.9 84.0 35 49 284 305 0.5 0.1 0.10 76.4 Hypertension, angina, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-8</td>
<td>M 67 163.5 46.6 *1b/*15 3.9 180.0 18 6 209 216 0.7 0.1 0.09 87.1 Hypertension, angina, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-9</td>
<td>M 67 163.5 46.6 *1b/*15 3.9 180.0 18 6 209 216 0.7 0.1 0.09 87.1 Hypertension, angina, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-10</td>
<td>M 68 170 63.9 *1b/*1b 3.7 180.0 18 6 209 216 0.7 0.1 0.09 87.1 Hypertension, angina, —</td>
<td>Hypertension</td>
</tr>
<tr>
<td>PTX-11</td>
<td>M 52 160 53 *1b/*15 4.3 73.0 12 6 165 135 1.6 0.1 0.72 87.1 Diastolic blood pressure —</td>
<td>Hypertension</td>
</tr>
</tbody>
</table>
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 transfected with OATP1B1, OATP1B3, 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-s-ornithine (50 mg/ml). After 48 hours, the medium was substituted by Dulbecco’s modified Eagle’s medium containing 5 mM sodium butyrate. After 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.

For evaluation of the inhibition potency of paclitaxel or 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.

**Western Blotting to Detect OATP1B1 and OATP1B3 in Whole Cells and Cell Surface.** Cells were harvested by cell scraper and collected by centrifugation (12,000 g, 4°C). They were lysed by cell lysis buffer (Cell Signaling, Danvers, MA). The samples were centrifuged at 12,000 g for 10 minutes at 4°C, and then the supernatants were used in the analysis. The 4× SDS sample buffer (12% SDS, 25% glycerol, 150 mM Tris-HCl pH 7.0, 0.05% bromophenol blue, 200 mM sodium butyrate) was added to 50 µg of protein, and heated at 60°C for 5 minutes. After heating, the samples were separated by SDS-PAGE and transferred to a PVDF membrane. Blotting Detection Kit (Amersham Biosciences, Piscataway, NJ) was used in this study. The membranes were then incubated in the blocking solution for 1 hour at room temperature, and then the membranes were incubated with antibodies of interest. After washing, the membranes were incubated with the secondary antibodies, and developed by chemiluminescence reagents (Amersham Biosciences, Piscataway, NJ). After developing, the membranes were scanned and analyzed using an imaging system (Molecular Dynamics, Sunnyvale, CA). The protein expression levels were normalized to levels of GAPDH.

**Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).** For quantitation of the radioactivity, the cells were solubilized with 0.1% formic acid/50% acetonitrile/50% water using a scraper and disrupted using a bioruptor (Sonic-bio, 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 (20,000g for 5 minutes at 4°C), and the supernatants were used for the following liquid chromatography-tandem mass spectrometry analyses. For quantification of the radioactivity, the cells were solubilized with 200 µl 0.2 N NaOH overnight at 4°C and then neutralized with 100 µl 0.4 N HCl. Aliquots of the lysates (200 µl) were transferred to scintillation vials containing a scintillation cocktail (Clear-sol I; Nacalai Tesque, Kyoto, Japan), and the radioactivity was measured in a liquid scintillation counter (LS6000SE; Beckman Instruments, Inc., Fullerton, CA). The protein concentration was determined using the protein assay method of Lowry et al. (1951), using bovine serum albumin as the protein standard.

**Clinical Study Design.** This was a prospective study performed with Japanese patients with non–small cell lung cancer who received the first cycle of paclitaxel and carboplatin regimen. To evaluate the difference in plasma concentration profiles of the endogenous substrates with or without the paclitaxel infusion, the blood samples were collected on two consecutive days at the same time points. On day 1, plasma samples were collected before and after the paclitaxel infusion as shown below, whereas on day 0, plasma samples were collected at the same time points as day 1. The study was conducted in accordance with the principles of the Declaration of Helsinki. This study protocol was approved by the Institutional Review Board of Showa University and Graduate School of Pharmaceutical Sciences, The University of Tokyo. All patients were asked for written informed consent for their peripheral blood samples and medical information to be used for research purposes. The study was registered at the University Hospital Medical Information Network-Clinical Trials Registry Japan (UMIN000032370).

**Eligibility.** All patients 20 years or older with metastatic or recurrent and histologically confirmed non–small cell lung cancer who received paclitaxel had an Eastern Cooperative Oncology Group performance status of 0 to 1 and no history of chemotherapy within 4 weeks were eligible. Each patient was confirmed to have adequate bone marrow function (absolute neutrophil count, at least 1.5 × 10^9/l; platelet count at least 150 × 10^9/l), liver function (serum bilirubin level less than 1.5 mg/dl; transaminases less than 2.5 times the upper limit of normal), and renal function (serum creatinine level less than 1.5 mg/dl) (Table 1).

**Treatment.** The therapeutic dose of paclitaxel (200 mg/m²) and carboplatin (area under the blood concentration-time curve of 6 mg/ml × min) was administered to patients with non–small cell lung cancer. Combination chemotherapy with bevacizumab (15 mg/kg) was permitted. Paclitaxel was administered as intravenous infusion over a period of 3 hours at 240 mg/m², and carboplatin was administered as an intravenous infusion over a period of 1 hour. In the patients receiving trametrexate with oral 5 mg of diphenhydramine followed by intravenously given 3 mg of granisetron, 50 mg of ranitidine, and 20 mg of dexamethasone at the course of 10 minutes (Supplemental Fig. 1). Thereafter, carboplatin was given as an intravenous drip infusion over the course of 1 hour. The patients were given a dietary supplement containing an equivalent amount of total daily lipids to minimize the influence of these dietary ingredients on the synthesis or disposition of the endogenous substrates of OATP1B1 and OATP1B3, such as bile acids. Note that regular meals were served to patients at 8:00 AM and 12:00 PM with the same menu on day 0 and day 1, and paclitaxel infusion was performed from 11:00 AM to 2:00 PM.

**Blood Sampling.** Blood samples were taken from the arm opposite the infusion site at the beginning of paclitaxel infusion and 0, 2, 4, and 7 hours after the end of the 3-hour infusion of paclitaxel on day 1. Blood samples were also collected at the same time points on day 0 to assess the difference in plasma concentration profiles of the endogenous substrates in the presence or absence of paclitaxel infusion. The blood samples were immediately centrifuged, and the plasma was stored at −80°C until analysis.

**Genotyping of SLCO1B1 in Patients.** This study focused on two SLCO1B1 single nucleotide polymorphisms, 388G>A and 521T>C, that form haplotypes *1a, *1b, and *15. The genotypes were determined as described previously (Akiyama et al., 2012) using genomes prepared from peripheral blood cells of the patients.

**Determination of the Unbound Fraction of Paclitaxel in the Plasma.** The unbound fraction of paclitaxel in the plasma (fu) was determined at 0 and 7 hours using a High Throughput Dialysis Model HTD96b and Dialysis Membrane Strips MWC0 (12–14 kDa) obtained from HTDialysis, LLC (Gales Ferry, CT).

**Quantification of Paclitaxel and 6α-Hydroxy Paclitaxel in the Plasma.** A plasma aliquot (20 µl) was added to acetonitrile (180 µl), vortexed, and centrifuged at 20,000g for 5 minutes for protein precipitation. The supernatant (10 µl) was mixed with 0.1% formic acid/acetonitrile = 1/1 solvents (150 µl) and analyzed using liquid chromatography-tandem mass spectrometry. Chromatography was performed on a Prominen Ultra Fast Liquid Chromatography system (Shimadzu, Kyoto, Japan), and separation was achieved as described in Supplemental Methods. Data were collected on an AB Sciex API5000 (QTRAP) mass spectrometer (Foster City, CA) using positive Turbo IonSpray electrospray ionization and Multiple Reaction Monitoring mode (Supplemental Methods). Data acquisition and processing were carried out with Analyst software version 1.6.2. (Applied Biosystems/MDS Sciex, Foster City, Canada).

**Quantification of Endogenous OATP1B Biomarkers and Bile Acids in the Plasma Specimens.** A QTRAP5500 mass spectrometer (AB Sciex) equipped with Prominen Ultra Fast Liquid Chromatography, system (Shimadzu) or Nexera Ultra High Performance Liquid Chromatography (Shimadzu) was used
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, GCDC-S and GDCA-S, and GCDC-G and GDCA-G, the chromatographic separation was modified as described in the Supplemental Methods. 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 (Ki) of Paclitaxel and 6α-Hydroxy Paclitaxel for OATP1B1 and OATP1B3. Iterative nonlinear least-squares method using GraphPad Prism8 (GraphPad Software, San Diego, CA) was conducted to determine the Ki values of paclitaxel or 6α-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:

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

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 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 [3H]E217βG in a concentration-dependent manner (Fig. 1), with Ki 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 Ki to 0.154 ± 0.031 μM (Fig. 1). Paclitaxel showed lower inhibition potency to OATP1B3 than OATP1B1 (Fig. 1), with the estimated Ki 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, 6α-hydroxy paclitaxel, were determined in the patients (Fig. 2). Plasma concentrations of 6α-hydroxy paclitaxel accounted for 1/10 of those of paclitaxel (Fig. 2). The f0 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 fp and Cmax at 0 hours. It was somewhat below the Ki for paclitaxel 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, GLCA-S, LCA-S, TLCA-S, GDCA-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, GDCA-24G; 4 hours postinfusion, GCDCA-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).
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
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, 6α-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). Shitara and Sugiyama

**TABLE 2**

Effect of paclitaxel on AUC of endogenous OATP1B substrates AUC of the endogenous OATP1B substrates were calculated. AUC are presented as mean ± S.E.M. Fold changes are presented as GMR and 90% CI (n = 10). GMR, geometric mean ratio.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Control</th>
<th>+ Paclitaxel</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPI (nM × h)</td>
<td>22.1 ± 1.7</td>
<td>61.4 ± 4.3</td>
<td>2.8 (2.5, 3.1)</td>
</tr>
<tr>
<td>CPIII (nM × h)</td>
<td>4.48 ± 0.34</td>
<td>14.1 ± 1.3</td>
<td>3.1 (2.6, 3.7)</td>
</tr>
<tr>
<td>GDCA-S (μM × h)</td>
<td>2.06 ± 0.45</td>
<td>5.71 ± 1.0</td>
<td>2.9 (2.2, 3.9)</td>
</tr>
<tr>
<td>LC-S (μM × h)</td>
<td>0.501 ± 0.09</td>
<td>2.2 ± 0.45</td>
<td>4.0 (2.8, 5.8)</td>
</tr>
<tr>
<td>GLCA-S (μM × h)</td>
<td>4.15 ± 1.4</td>
<td>10.4 ± 3.3</td>
<td>2.2 (1.4, 3.6)</td>
</tr>
<tr>
<td>TLCA-S (μM × h)</td>
<td>0.857 ± 0.22</td>
<td>1.94 ± 0.57</td>
<td>2.0 (1.3, 3.0)</td>
</tr>
<tr>
<td>GCDCA-G (nM × h)</td>
<td>20.6 ± 4.1</td>
<td>52.2 ± 10</td>
<td>2.5 (1.8, 3.4)</td>
</tr>
<tr>
<td>GDCA-G (nM × h)</td>
<td>94.4 ± 29</td>
<td>211 ± 46</td>
<td>2.5 (1.8, 3.4)</td>
</tr>
<tr>
<td>CDCA-24G (nM × h)</td>
<td>141 ± 33</td>
<td>369 ± 79</td>
<td>3.0 (2.2, 4.0)</td>
</tr>
</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.
(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]cholecytokinin 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 \( f_u \) and \( C_{\text{max}} \), with its \( K_i \) 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. The highest AUC ratio of GCDCA-S (13) and GCDCA-G (6.4) compared with the most rapid decline after the end of the infusion (within 2 hours) of the endogenous substrates tested, PBPK model-based simulation will enable implementation of an appropriate dosing schedule to avoid significant DDIs.

Paclitaxel Effects on the Endogenous OATP1B Biomarkers

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