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Contribution of Organic Cation Transporter 2 (OCT2) to Cisplatin-Induced Nephrotoxicity

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Cisplatin is one of the most widely used anticancer agents for the treatment of solid tumors. The clinical use of cisplatin is associated with dose-limiting nephrotoxicity, which occurs in one-third of patients despite intensive prophylactic measures. Organic cation transporter 2 (OCT2) has been implicated in the cellular uptake of cisplatin, but its role in cisplatin-induced nephrotoxicity remains unknown. In mice, deletion of Oct1 and Oct2 resulted in significantly impaired urinary excretion of cisplatin without an apparent influence on plasma levels. Furthermore, the Oct1/Oct2-deficient mice were protected from severe cisplatin-induced renal tubular damage. Subsequently, we found that a nonsynonymous single-nucleotide polymorphism (SNP) in the OCT2 gene *SLC22A2* (rs316019) was associated with reduced cisplatin-induced nephrotoxicity in patients. Collectively, these results indicate the critical importance of OCT2 in the renal handling and related renal toxicity of cisplatin and provide a rationale for the development of new targeted approaches to mitigate this debilitating side effect.

Cis-diamminedichloroplatinum (cisplatin) is among the most widely used cytotoxic anticancer agents, and it has a broad spectrum of activity against various cancers, including those of the lung, head and neck, bladder, germ cell, ovary, endometrium, and uterine cervix.[1] In the conventional 3- or 4-weekly treatment regimens, dose-limiting side effects associated with cisplatin-based chemotherapy regimens are observed, including renal tubular dysfunction (nephrotoxicity), peripheral neuropathy, and hearing loss (ototoxicity), whereas hematologic toxicity becomes dose-limiting in the more dose-dense regimens in which the drug is administered on a weekly basis.[2]

Severe and irreversible damage to the kidney is the most important complication of cisplatin treatment—it may limit further treatment or even threaten life. This side effect primarily affects the S3 segment of the renal proximal tubules and occurs in one-third of patients despite intensive prophylactic measures.[3] Furthermore, ~20% of all acute renal failure cases among hospitalized patients are attributable to cisplatin-containing chemotherapy.[4] Despite having been the focus of intense investigation for many decades, the exact pathogenesis of cisplatin-related nephrotoxicity, in which quiescent proximal tubular cells are selectively damaged, remains unclear.[5] Recent studies have suggested that inflammation, oxidative stress, and apoptosis probably explain part of the tissue injury, although the initiating event leading to tubular damage and the details of the mechanism of renal handling of the drug are still poorly understood.[6]

We as well as others have recently reported that the organic cation transporter 2 (OCT2), encoded by the *SLC22A2* gene, can actively transport cisplatin *in vitro*.[7–10] This transporter is predominantly expressed in human kidney at the basolateral membrane of renal proximal tubules and is involved in the secretion of various cationic substances from the circulation into tubular cells. All experiments to date have assessed a putative role of OCT2 in cisplatin-related nephrotoxicity in cultured cells. The identification of substrates for renal transporters using heterologous *in vitro* expression systems provides valuable information for the prediction of drug–drug and drug–protein interactions.[11] However, the well-recognized limitation of these preliminary determinations is that they do not indicate the true relevance of a transporter in handling a substrate in the context of whole-body disposition, renal and extrarenal transport, and glomerular filtration. Indeed, before attributing an abnormality in normal physiology to transporter perturbation, the relevance of the transporter to the disposition of a drug must first be determined *in vivo*. The aim of the current investigation was to compare the effects of cisplatin treatment on the pharmacokinetics, urinary excretion, and extent of nephrotoxicity in groups of mice

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lacking the ortholog transporters Oct1 (Oct1(−/−) mice), Oct2 (Oct2(−/−) mice), or both Oct1 and Oct2 (Oct1/2(−/−) mice). In addition, we evaluated the association of genetic variations in the *SLC22A2* gene with nephrotoxicity seen in patients treated with cisplatin.

RESULTS

SLC22A2 expression in normal human tissue and human tumor tissue

To provide further insight into the tissue-specific expression profile of the OCT2 gene *SLC22A2*, real-time PCR analysis was performed on a panel of normal human tissues. As expected, the tissue with the highest expression level was the kidney, whereas low expression levels were noted in a variety of other tissues, including the intracranial artery (**Figure 1a**). *SLC22A2* expression was further evaluated in a select series of human tumors, and we found a low level of expression in a number of kidney tumor types. Interestingly, the expression of *SLC22A2* was absent in breast, colon, liver, lung, ovarian, prostate, and thyroid tumors, particularly when compared with the baseline expression in the normal human kidney (**Figure 1b**). In line with a previous observation,[12] very low to absent expression levels of *SLC22A2* were found in most cell lines of the NCI60 human tumor panel, with the exception of a single ovarian cancer cell line, SKOV-3 (data not shown).

Urinary excretion of cisplatin in Oct1- and Oct2-deficient mice

We next assessed the urinary excretion profile of cisplatin in mice following a single dose of 10 mg/kg. We found that the cumulative percentage of the administered dose excreted was very similar in both Oct1(−/−) mice (**Supplementary Figure S1a** online) and Oct2(−/−) mice (**Supplementary Figure S1b**), as compared with those in age-matched wild-type mice. Consistent with previous findings,[13] the majority of the cisplatin dose was excreted in urine within the first 24 h after drug administration. The lack of a significant impact of the individual Oct1 and Oct2 deficiencies on the elimination of cisplatin is in line with earlier observations made for the cationic substance tetraethylammonium in the same animal models[14,15] and suggests that in the mouse cisplatin is likely recognized as a substrate by both Oct1 and Oct2. There is strong agreement about the finding that, whereas mice express substantial levels of both Oct1 and Oct2 in the kidney, in humans OCT2 dominates renal organic cation transport and OCT1 dominates hepatic organic cation transport.[16] Therefore, we used Oct1/2(−/−) mice in all subsequent experiments as the most appropriate animal model.

Cisplatin pharmacokinetics in Oct1/2(−/−) double-knockout mice

At 24 h after cisplatin administration, a cumulative urinary excretion amounting to 45% of the administered dose was observed in Oct1/2(−/−) mice, as compared with 91% in wild-type animals ($P = 0.0008$); in the entire sample collection period (72 h), only 56% of the dose was recovered in Oct1/2(−/−) mice ($P = 0.0016$) (**Figure 2a**). The marked reduction in the rate of the cumulative urinary excretion of cisplatin the Oct1/2(−/−) mice confirms that organic cation transporters play a direct role in the renal handling of cisplatin. Microarray analysis of kidney samples revealed that the *Slc22a8* gene, encoding the organic anion transporter 3 (OAT3), was specifically upregulated (up to 11-fold for the two probe sets) in the Oct1/2(−/−) mice (**Figure 2b**). This transporter is also localized on the basolateral membrane of renal proximal tubule cells and has been identified as an important contributor to xenobiotic and endogenous organic anion secretion.[17] In order to rule out the possibility that OAT3 plays a role in the renal tubular transport of cisplatin, *in vitro* transport of cisplatin was assessed in mammalian cells engineered to overexpress human OAT3 or murine Oat3. These studies indicated, however, that cisplatin is not a substrate of either transporter ($P = 0.093$ and $P = 0.14$, respectively) (**Figure 2c**), and it was therefore concluded that this factor was not confounding the results of the murine pharmacokinetic studies.

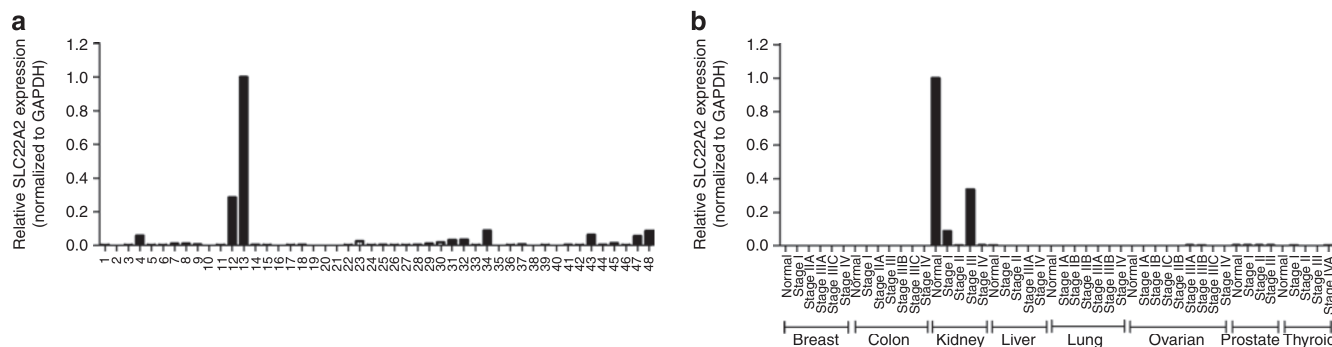

Figure 1 Expression of *SLC22A2*, normalized to the housekeeping gene *GAPDH*, in (**a**) human normal tissues and (**b**) human tumor samples. *SLC22A2* was predominantly expressed in kidney, and expression was low to absent in tumor samples. Tissue and tumor plates containing cDNA tissues were used for real-time PCR analysis. Data are shown as mean values (bars) of duplicate plates and expressed relative to values observed in normal human kidney, which were set to a value of 1. Numbers represent tissue from (1) adrenal gland, (2) bone marrow, (3) brain, (4) cervix, (5) colon, (6) descending duodenum, (7) epididymis, (8) esophagus, (9) fat, (10) heart, (11) small intestine, (12) intracranial artery, (13) kidney, (14) liver, (15) lung, (16) lymph node, (17) peripheral blood leukocyte, (18) mammary gland, (19) muscle, (20) nasal mucosa, (21) optic nerve, (22) ovary, (23) oviduct, (24) pancreas, (25) penis, (26) pericardium, (27) pituitary, (28) placenta, (29) prostate, (30) rectum, (31) retina, (32) seminal vesicles, (33) skin, (34) spinal cord, (35) spleen, (36) stomach, (37) testis, (38) thymus, (39) thyroid, (40) tongue, (41) tonsil, (42) trachea, (43) urethra, (44) urinary bladder, (45) uterus, (46) uvula, (47) vagina, and (48) vena cava.

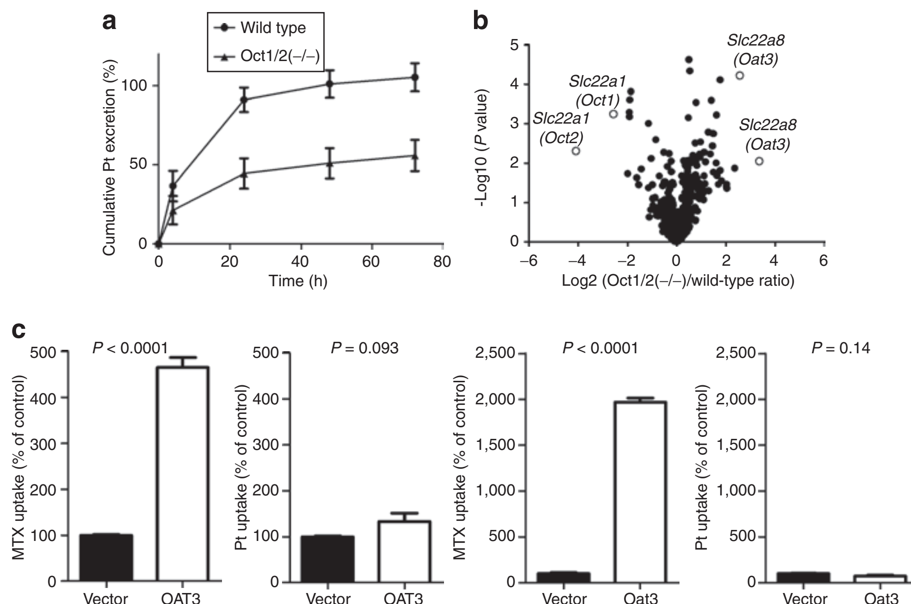

Figure 2 Urinary platinum (Pt) excretion and changes in gene expression in Oct1(–/–) and Oct2(–/–) mice. (**a**) Effect of simultaneous Oct1 and Oct2 deficiency on renal handling of cisplatin in mice. The cumulative excretion of cisplatin was reduced in Oct1/2(–/–) mice as compared with wild-type mice (n = 11–12/group) after drug administration (10 mg/kg; intraperitoneal). Data are shown as mean values; error bars represent SE. (**b**) Differential gene expression in the kidneys of male Oct1/2(–/–) mice relative to wild-type FVB mice (n = 3/group), assessed using the Affymetrix Mouse 430v2 GeneChip array. Select genes on the volcano plot include enzymes, nuclear receptors, adenosine triphosphate–binding cassette transporters, and solute carriers. (**c**) The intracellular uptake of methotrexate (MTX), a positive control, and Pt was assessed in human embryonic kidney 293 cells transfected with human OAT3 and mouse Oat3 following incubation with 10 or 500 µmol/l MTX or 500 µmol/l cisplatin for 30 min. Data are expressed relative to drug accumulation in cells transfected with an empty vector, which was set to 100%. Data are shown as mean values with SE.

The reduced renal excretion of cisplatin in the Oct1/2(−/−) mice was not accompanied by measurable changes of cisplatin concentrations in plasma (**Figure 3a**). The observed peak concentration of cisplatin was slightly higher in Oct1/2(−/−) mice than in wild-type mice (36.4 ± 8.47 and 26.7 ± 9.73 µg/ml, respectively; P = 0.20), although the total area under the concentration–time curve was very similar in the two groups (54.9 and 50.4 µg·h/ml, respectively). These values are comparable with the average area under the curve of cisplatin in 270 cancer patients receiving the drug at a dose of 100 mg/m^2 (54.9 ± 12.4 µg·h/ml).[18] The unbound fraction of cisplatin was also not significantly different between plasma samples obtained from Oct1/2(−/−) mice and wild-type mice (10.3% vs. 11.1%; P = 0.46) (**Figure 3b**)—suggesting that possible differences in the extent of drug binding to serum proteins did not contribute to the impaired urinary excretion.

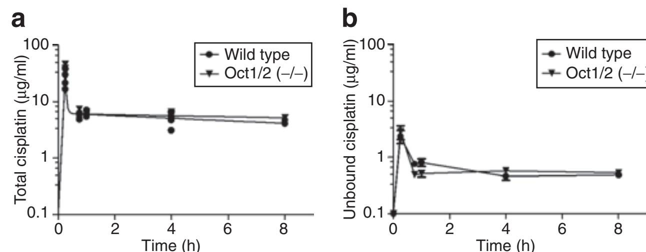

Figure 3 Plasma pharmacokinetics in mice after cisplatin treatment. (**a**) Comparative concentration–time profiles of cisplatin in plasma of Oct1/2(–/–) mice (inverted triangles) and wild-type mice (circles) (n = 4/group) after the administration of cisplatin (10 mg/kg; intraperitoneal (i.p.)). Data are shown as mean values with SE along with a curve fitted from a two-compartment model. (**b**) Comparative concentration–time profiles of unbound platinum in plasma of Oct1/2(–/–) mice (inverted triangles) and wild-type mice (circles) (n = 4/group) after the administration of cisplatin (10 mg/kg, i.p.). Data are shown as mean values with SE.

Histopathology and serum chemistry analysis

To evaluate the pharmacodynamic implications of the Oct1/2 genotype–dependent urinary excretion pattern of cisplatin, we performed several additional analyses. As predicted on the basis of the excretion data, we found that severe, acute renal tubular necrosis was observed in the kidneys of all wild-type mice (n = 8) but not in those of the Oct1/2(−/−) mice (n = 11) (**Figure 4**). The lesions observed in cisplatin-treated wild-type mice were characterized by dilated tubules filled with necrotic tubular epithelial cells, cellular debris, and proteinaceous casts, whereas the glomeruli, which do not express Oct1 or Oct2, were histologically normal. There was no evidence of toxicity in 18 other tissues in the Oct1/2(−/−) mice, including liver, bone marrow, and intestine (**Supplementary Figure S2** online), indicating that the impaired renal excretion in this strain does not cause exacerbated cisplatin-related side effects elsewhere.

Consistent with the histopathological changes observed in the cisplatin-treated wild-type mice, several physiologic hallmarks of cisplatin-related nephrotoxicity in humans—such as changes in levels of serum alkaline phosphatase, blood urea nitrogen, glucose, and serum creatinine—were specifically found to be altered in wild-type mice undergoing cisplatin treatment (**Table 1**).

Table 1 Altered serum chemistry in wild-type mice 72 h after cisplatin administration (10 mg/kg, intraperitoneal)

	Untreated (median (range))		Treated (median (range))		
Variable	Wild type	Oct1/2(−/−)	Wild type	Oct1/2(−/−)	P^a
Albumin (g/dl)	3.4 (3.2–3.4)	3.6 (3.3–4.1)	3.2 (1.8–4.5)	3.7 (3.4–4.3)	0.41
Alkaline phosphatase (U/l)	139 (120–154)	138 (108–167)	55 (2–68)[b]	75 (51–91)	0.00048
Alanine aminotransferase (U/l)	68 (50–98)	63 (9–273)	250 (108–493)[b]	139 (90–304)	0.018
Bilirubin (mg/dl)	0.30 (0.3–0.3)	0.35 (0.3–0.4)	0.20 (0.1–0.3)	0.30 (0.2–0.4)	0.070
Blood urea nitrogen (mg/dl)	24 (22–27)	22 (18–28)	157 (62–199)[b]	80 (31–180)	0.00096
Calcium (mg/dl)	9.4 (9.3–9.9)	9.8 (9.5–10.1)	11.4 (7.1–12.7)	9.8 (9.6–11.7)	0.13
Creatinine (mg/dl)	0.25 (0.20–0.30)	0.35 (0.20–0.50)	0.90 (0.40–1.60)[b]	0.45 (0.20–0.70)	0.0080
Globulin (g/dl)	1.9 (1.8–1.9)	2.1 (1.9–2.4)	2.3 (1.2–2.8)	2.2 (1.3–2.6)	0.13
Glucose (mg/dl)	215 (198–238)	183 (172–655)	149 (35–205)[b]	177 (135–223)	0.034
Phosphorus (mg/dl)	8.5 (8.4–10.1)	9.4 (8.9–18.1)	15.0 (5.4–24.2)	6.8 (5.9–9.0)	0.088
Potassium (mmol/l)	6.9 (6.8–8.0)	6.9 (3.0–7.8)	7.9 (6.8–9.7)	8.5 (6.8–9.7)	0.10
Sodium (mmol/l)	151 (147–151)	152 (151–152)	148 (110–152)	152 (148–154)	0.054
Total protein (g/dl)	5.3 (4.9–5.3)	5.6 (5.5–5.6)	5.6 (3.0–7.1)	5.6 (3.1–6.9)	0.19

[a]Kruskal–Wallis test followed by a Kruskal–Wallis *Z*-test with Bonferroni correction. [b]Different from untreated.

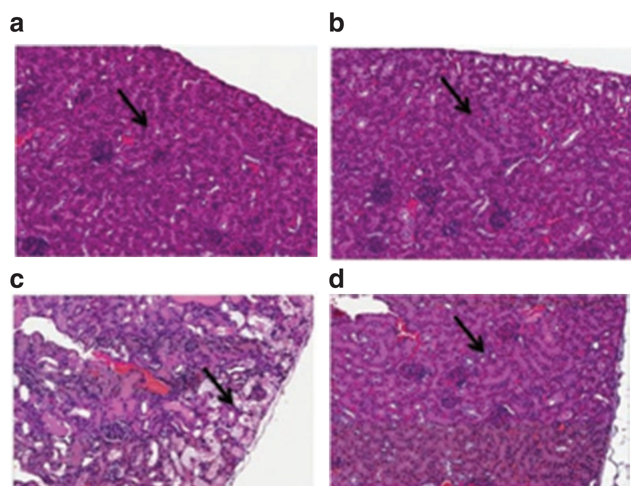

Figure 4 Cisplatin-related nephrotoxicity in (**a**) untreated wild-type and (**b**) untreated Oct1/2(−/−) mice as compared with that in (**c**) treated wild-type and (**d**) treated Oct1/2(−/−) mice 72 h after the administration of cisplatin (10 mg/kg, intraperitoneal), from representative animals. Severe renal tubular necrosis, characterized by dilated tubules filled with necrotic tubular epithelial cells, was observed in kidneys of all wild-type mice but in none of the Oct1/2(−/−) mice (*n* = 8/group). Arrows indicate tubules.

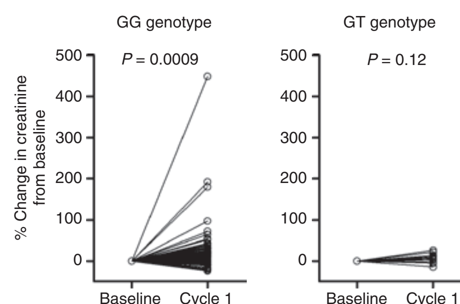

Figure 5 Changes in serum creatinine level, a marker of acute nephrotoxicity, measured at baseline and after the first cycle of cisplatin treatment in cancer patients as a function of *SLC22A2* 808G>T genotype (GG; *n* = 68, GT; *n* = 10).

SLC22A2 genotype–toxicity associations

Consistent with the view that cisplatin-related nephrotoxicity may be linked to how the kidney transports cisplatin is the finding of high cisplatin content in human proximal tubules[19] and the correlation of nephrotoxicity with concentrations of cisplatin in autopsied kidney cortex samples.[20] In order to provide evidence for the involvement of OCT2 in this process, we next assessed the association of a common germline variant in *SLC22A2* with changes in serum creatinine level in a cohort of cancer patients receiving cisplatin. We focused on the single-nucleotide polymorphism (SNP) at the 808G>T locus (rs316019) because it is known to be associated with decreased transport of other OCT2 substrates[21] and because it has a relatively high allelic frequency.[10]

Changes in serum creatinine level, a marker of acute nephrotoxicity, were measured at baseline (the day before the first cisplatin administration) and after the first cycle of cisplatin treatment (1–8 days after the first cisplatin administration), and they were assessed as a function of *SLC22A2* 808G>T genotype. We found that patients carrying a copy of this SNP (*n* = 10; 13%) experienced no change in serum creatinine after cisplatin treatment (*P* = 0.12), whereas patients carrying the reference sequence showed a significant increase in serum creatinine level (*n* = 68; *P* = 0.0009) (**Figure 5**). Differences in serum creatinine levels between baseline and cycle 1 in patients with the *SLC22A2* 808GG genotype remained statistically significant after eliminating from the analysis the data relating to the three individuals with the largest increases (*P* = 0.00041). As predicted on the basis of the results obtained in Oct1/2(−/−) mice, we found that the rs316019 SNP was not associated with systemic clearance (**Figure 6a**) or with plasma concentrations of cisplatin (**Figure 6b**). In support of the observed genotype–toxicity association in our patient population, we also found that, in a panel of human cell lines, rs316019

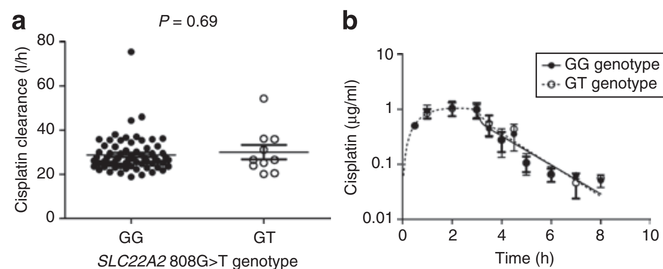

Figure 6 Plasma pharmacokinetics in patients after cisplatin treatment. (**a**) Unchanged systemic clearance of unbound cisplatin in cancer patients as a function of *SLC22A2* 808G>T genotype status (GG; $n = 68$, GT; $n = 10$). Each point represents a patient and mean values are indicated by lines. (**b**) Concentration–time profile of unbound cisplatin as a function of *SLC22A2* 808G>T genotype status (GG; $n = 68$, GT; $n = 10$). Data are shown as mean values ± SE along with a curve fitted from a two-compartment model (lines).

is associated with decreased expression of *SLC22A2* in a gene dosage–dependent fashion, although group differences were not statistically significant (data not shown).

DISCUSSION

In this study, we found that the urinary excretion of cisplatin and drug-induced damage to kidneys are dependent on organic cation transporter–mediated renal tubular transport. This process was found to be mediated by the two closely related murine transporters Oct1 and Oct2, which are functionally redundant and together fulfill a role equivalent to that of OCT2 in humans. Furthermore, we noted that individuals carrying an inherited, reduced-function variant in *SLC22A2*, the gene encoding OCT2, are at decreased risk of experiencing cisplatin-induced nephrotoxicity. Our data complement previous knowledge on the interaction of cisplatin with organic cation transporters obtained using *in vitro* model systems, and they may have important practical implications for optimal use of cisplatin.

As mentioned previously, cisplatin has been a mainstay for therapy of multiple solid tumors, but its clinical use is hampered by the occurrence of severe and unpredictable side effects to normal tissues, most notably the kidney. Although it has been speculated for several decades that net tubular secretion of cisplatin may be the initiating event in renal tubular damage,[6] our demonstration that Oct1/2(−/−) mice exhibit impaired urinary elimination of cisplatin substantiates—for the first time—the existence of an important role for organic cation transporters in the *in vivo* renal handling of this compound. The most plausible explanation for the observed differences between Oct1/2(−/−) mice and wild-type mice with regard to urinary excretion of cisplatin is that, in the knockout strain of mice, the uptake of the drug in renal tubular cells is impaired, and consequently the renal tubular secretion into urine is reduced. It is important to note that compensatory upregulation of various transporter genes, most notably of the Oat3 gene *Slc22a8*, occurred in the kidney of Oct1/2(−/−) mice. However, cisplatin was not found to be transported by either human OAT3 or mouse Oat3, thereby suggesting that, in this particular mammalian model, the degree of compensation is not sufficient to counteract the effect of simultaneous deficiency of Oct1 and Oct2.

Despite the observed changes in renal handling of cisplatin in the Oct1/2(−/−) mice, we found that the apparent plasma clearance of cisplatin and measures of systemic exposure to both total cisplatin and unbound cisplatin were unaltered in this mouse model. This paradox might be explained by the possibility that the high expression of Oct1 and Oct2 in renal tubular cells can directly control local drug levels and thereby alter the urinary excretion of cisplatin without affecting measures of systemic exposure. The finding that the impaired urinary excretion of cisplatin in the Oct1/2(−/−) mice was not accompanied by any evidence of other organ damage could possibly indicate a shunting of the primary pathway of cisplatin elimination as opposed to a dramatically altered drug distribution. A similar phenomenon has been reported for the anticancer and antirheumatic drug methotrexate—the deficiency of Abcc2 (Mrp2), a transporter regulating the biliary secretion of this agent, was found to be associated with increases in the extent of urinary excretion in mice deficient in this transporter as compared with wild-type mice.[22] Additional investigation is required to confirm the possibility that cisplatin is predominantly eliminated by the hepatobiliary route in the Oct1/2(−/−) mice.

One cannot entirely exclude the possibility that other renal tubular uptake transporters are involved in the urinary excretion of cisplatin. For example, a recent investigation indicated that the copper transporter 1 (Ctr1; *Slc31a1*) is localized on the basolateral side of both proximal and distal tubular cells in the kidney of C57BL/6 mice and that downregulation of Ctr1 expression by small interfering RNA was associated with decreased cisplatin uptake *in vitro*.[23] In this context, it is interesting to note that the expression of *Slc31a1* was not different in kidney samples obtained from wild-type mice and Oct1/2(−/−) mice, and it was also not affected by treatment with cisplatin (**Supplementary Figure S3** online). This result indicates that there is limited involvement of the Ctr1 transporter in the pharmacokinetic and pharmacodynamic changes observed in our particular mouse model.

Recent re-sequencing of the human OCT2 transporter gene *SLC22A2* has revealed a number of allelic variants that affect the activity of the gene product. Some of these genetic variants may potentially modulate the phenotypic activity of the transporter in patients and thereby affect their predisposition to toxicity and response to substrate drug treatment. In particular, an SNP in exon 4 of the *SLC22A2* gene has been described (rs316019), in which a 808G>T transversion results in an amino acid change of serine to alanine at codon 270.[24] Although a detailed analysis of the potential functional consequences of this *SLC22A2* variant has not yet been performed, *in vitro* studies have indicated altered substrate specificity and function of the mutant protein relative to the wild-type protein—for example, in relation to the OCT2 substrate drug metformin.[21] An expression analysis of *SLC22A2* in the NCI60 cell line panel suggests that the rs316019 polymorphism is associated with a decreased gene expression. This change in expression could partially explain the decrease in function observed with this variant, although a more comprehensive evaluation is necessary to draw definite conclusions.

In this study, we found that white patients with cancer who had the rs316019 polymorphism ($n = 10$; 13%) did not experience a significant change in serum creatinine after the first cycle of cisplatin treatment, whereas patients with the reference allele showed indications of renal damage. To the best of our knowledge, this is the first evidence suggesting that inherited genetic variation contributes to interindividual variability in cisplatin-induced nephrotoxicity. Although this observation requires confirmation in a larger cohort of patients as well as further exploration in other ethnic populations, these findings are in line with those obtained in the Oct1/2(−/−) mice. They also suggest that *a priori* genetic testing could be employed to identify patients who would be at increased risk for developing acute nephrotoxicity.

This work on the mechanisms underlying cisplatin-induced nephrotoxicity also opens up new avenues for further evaluation of pharmacologic approaches to renoprotection involving the targeting of OCT2. In particular, the use of OCT2 inhibitors in combination with cisplatin might prevent high concentrations of cisplatin from reaching the renal tubular cells, thereby possibly reducing the development of nephrotoxicity. Over the past few decades, numerous approaches have been reported for providing renoprotection during cisplatin treatment.[25] However, most of these approaches have not separately considered the possible implications with regard to the anticancer actions of cisplatin in tumors. This is surprising, considering the overlap and degree of similarity in the critical target genes for cisplatin in the kidney and tumor cell. Indeed, using a therapeutic approach to avoid the occurrence of renal tubular damage following cisplatin treatment is a Pyrrhic victory if this comes accompanied by diminished antitumor activity. Our findings indicate that the expression of OCT2 is either very low or absent in solid tumors, particularly in those for which treatment with cisplatin is indicated—e.g., lung and ovarian tumors—and that OCT2 is unlikely to play a role in the transport of cisplatin into tumor cells *in vivo*. We are currently investigating whether the administration of an OCT2 inhibitor can ameliorate cisplatin-induced nephrotoxicity in patients with cancer without influencing circulating concentrations of cisplatin.

Our demonstration that OCT2 plays an important role in cisplatin-related nephrotoxicity reveals a new host factor that contributes to enhanced interindividual variation in tolerability to this drug. These findings might be beneficial in planning future therapeutic interventions comprising cisplatin-containing regimens involving the use of specific inhibitors of OCT2.

METHODS

Cellular transport. Human embryonic kidney 293 cells overexpressing human OCT2 were produced using the Flp-In transfection system (Invitrogen, Carlsbad, CA) as previously described.[10] Mouse Oat3 cDNA was transfected into human embryonic kidney 293 cells. Human embryonic kidney 293 cells overexpressing human OAT3 were provided by Yuichi Sugiyama (Tokyo, Japan). Measurements of cellular accumulation of cisplatin were carried out in six-well plates with monolayer cultures at 37 °C, as previously reported.[10]

Animal experiments. Adult (8–12-week-old) male Oct1(−/−), Oct2(−/−), or Oct1/2(−/−) (Taconic, Germantown, NY) mice were used along with age-matched male FVB wild-type mice. Mouse genotypes were determined from tail biopsies using real-time PCR with specific probes designed for each gene (Transnetyx, Cordova, TN). A previous investigation demonstrated that there is no significant difference in glomerular filtration rate, assessed using inulin clearance, between Oct1/2(−/−) mice and FVB wild-type mice.[15] All the animals were housed and handled in accordance with the guidelines of the Institutional Animal Care and Use Committee of St Jude Children's Research Hospital. Animals were housed in a temperature-controlled environment with a 12-h light cycle, and they were given a standard diet and water *ad libitum*.

The animals were allowed to acclimatize to their metabolic cages 5 days prior to the administration of cisplatin followed by determination of platinum excreted in the urine. On the day of drug administration, baseline urine samples of the mice were collected 16 h after the onset of light, and then the animals were given a single intraperitoneal injection of cisplatin at a dose of 10 mg/kg. Urine was again collected 4, 24, 48, and 72 h after drug administration, diluted with nitric acid (0.2%), and immediately analyzed for total platinum, using flameless atomic absorption spectrometry.[10] At the end of the study period, the mice were killed and the tissues were removed for determination of gene expression and histological examination. In a separate group of animals, the plasma pharmacokinetics of cisplatin (10 mg/kg, intraperitoneal) was determined using a terminal sampling procedure.

Gene expression analysis of mouse kidney. RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA). RNA samples were amplified from three animals per group and analyzed using the Mouse 430v2 GeneChip array (Affymetrix, Santa Clara, CA). Volcano plots were constructed using select genes, including those that encode enzymes ($n = 27$ probe sets), nuclear receptors ($n = 27$), adenosine triphosphate–binding cassette transporters ($n = 56$), and solute carriers ($n = 293$).

Peripheral blood analysis. Serum chemistry analysis was performed on serum using a VetScan autoanalyzer (Abaxis, Union City, CA) to determine levels of albumin, alkaline phosphatase, alanine aminotransferase, amylase, total bilirubin, blood urea nitrogen, calcium, phosphorus, creatinine, glucose, sodium, potassium, and total protein.

Histological evaluation of mouse tissues. After collection, tissues were fixed overnight in 10% neutral-buffered formalin. Next, the tissues were processed routinely, embedded in paraffin, sectioned (4 μm), and stained with hematoxylin and eosin. Microscopic evaluation was performed by an experienced veterinary pathologist blinded as to the composition of the groups. Toxicities, including acute renal tubular necrosis, were scored as absent, rare, mild, moderate, or severe.

Real-time PCR and identification of *SLC22A2* variants in human cell lines. RNA and DNA from the NCI anticancer screening panel were provided by the National Cancer Institute tumor repository (Bethesda, MD). RNA was reverse transcribed using SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen) in accordance with the manufacturer's recommendations. Tissue plates containing cDNA from 48 normal human tissues and 96 human cancer tissues were obtained from OriGene (Rockville, MD). Gene transcripts were quantified using SYBR Green PCR Master Mix (Qiagen) and the primers previously described.[26] As previously reported, reactions were carried out in triplicate unless otherwise stated.[10]

The genomic sequence of *SLC22A2* was obtained from GenBank (accession no. NM003058), and primers were designed as previously described.[10] An SNP associated with an amino acid change at position Ala270Ser (exon 4) was evaluated. Optimal reaction and cycle conditions were determined for each amplicon in a 25 μl reaction volume. As previously described,[10] samples were cleaned with ExoSAP-IT reagent (USB, Cleveland, OH) following PCR and sequenced in both

forward and reverse directions using BigDye Terminator (version 3.1) Chemistry on Applied Biosystems 3730XL DNA analyzers (Foster City, CA). Sequencher software (version 4.7; Gene Codes, Ann Arbor, MI) was used for sequencing analysis.

Clinical studies. Eligibility criteria, study protocols, and pharmacokinetic analysis for determination of plasma clearance and urinary excretion have been documented in detail previously.[27] All the patients were treated at the Erasmus MC–Daniel den Hoed Cancer Center (Rotterdam, The Netherlands), the study protocols were reviewed and approved by the Erasmus MC review board, and patients provided written informed consent.

Acute cisplatin-related nephrotoxicity was assessed on the basis of serum creatinine analysis. These measurements were performed on serum samples obtained immediately before the first administration of cisplatin (baseline) and on the first serum samples obtained for diagnostic purposes after the first administration of cisplatin. The median time at which the latter samples were obtained was 1 day after start of the cisplatin infusion (range, 1–8 days). Changes in serum creatinine level following the administration of cisplatin were expressed as a percentage relative to the baseline values.

Statistical calculations. All graphical data are presented as mean values (symbols or bars) with SE (error bars). Group differences in median pharmacokinetic parameters of cisplatin as a function of mouse genotype or human OCT2-variant status and differences in intracellular drug concentrations in the various cell types were evaluated using a Mann–Whitney *U*-test. The variability in serum chemistry values in the studied mouse strains, as a function of treatment, was assessed using a Kruskal–Wallis test followed by a Kruskal–Wallis *Z*-test. Changes in serum creatinine level before and after cisplatin treatment in patients were assessed as a function of OCT2-variant status, using a Wilcoxon's signed-ranks test. Two-tailed P values of <0.05 were considered statistically significant. All statistical calculations were performed using the NCSS software package, version 2004 (Number Cruncher Statistical System, Kaysville, UT).

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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CONFLICT OF INTEREST

Oct1/2 knockout mice are made commercially available through the company Taconic, and the research group of A.H.S. benefits from a fraction of the revenue generated. The other authors declared no conflict of interest.

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