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Association of *ABCC2* polymorphisms with cisplatin disposition and efficacy

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Abstract

ABCC2 (MRP2; cMOAT) expression has been implicated in cisplatin resistance *in vitro*. In mice, cisplatin disposition and toxicity were unaffected by *Abcc2* knockout. Moreover, in cancer patients (n=237), cisplatin pharmacokinetics (P>0.12) and efficacy (P>0.41) were not associated with 7 SNPs in *ABCC2*. These SNPs were also not correlated with *ABCC2* expression in the NCI60 panel (P>0.26) or cisplatin-induced cytotoxicity (P=0.21). These findings highlight the importance of verifying drug-transporter interactions from *in vitro* tests in humans.

Keywords

Cisplatin; *ABCC2* (MRP2); Pharmacogenetics; Lung cancer

Cisplatin is among the most widely used anticancer drugs and has been approved for use in treatment of gastric, non-small cell lung (NSCLC), ovarian, and testicular cancers. The pharmacokinetic profile of cisplatin is characterized by extensive renal elimination and limited metabolism or biliary excretion¹, with up to 4-fold differences in drug clearance between patients.² This degree of variability has important toxicological and therapeutic ramifications. In particular, it was previously demonstrated that systemic exposure to cisplatin is the major determinant of response to treatment in patients with head-and-neck cancer³ or NSCLC.⁴

The unpredictable response to cisplatin remains a major challenge of modern chemotherapy. Recent population pharmacokinetic analyses have attempted to identify demographic and physiologic factors that may influence clearance of cisplatin.⁵ However, the magnitude of interindividual pharmacokinetic variability of this agent remains unexplained. A critical determinant of this variability is likely associated with differential expression of polymorphic transporters at sites of elimination. *ABCC2*, a ubiquitously expressed ATP-binding cassette transporter, has previously been implicated in cellular efflux of cisplatin.⁶

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CONFLICT OF INTEREST

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Localization of ABCC2 is observed at the brush border membrane of renal proximal tubes and the canalicular membrane of hepatocytes where it is believed to regulate secretion of organic anions.^{7,8} Although a role of ABCC2 in luminal secretion of transported substrates in the kidney remains unclear, its biological function and localization indicates that differential expression or activity may affect systemic exposure to cisplatin, and thus may indirectly alter drug response. Interestingly, clinical evidence exists indicating that common ABCC2 polymorphisms may affect disposition or efficacy of drugs which are known ABCC2 substrates.⁹

In the current study, we evaluated the extent to which transport of cisplatin by ABCC2 is an important source of interindividual variability in disposition, toxicity, and efficacy of cisplatin-based chemotherapy using *in vitro* and *in vivo* experimental approaches involving cell lines with variable expression levels of ABCC2, transporter-knockout mice,^{7,10} and 2 cohorts of white cancer patients carrying variants of ABCC2 with reduced function.^{11,12}

RESULTS

Mouse studies

To provide *in vivo* insights into a role of ABCC2 in pharmacokinetics and toxicity of cisplatin, we first assessed the urinary excretion profile of cisplatin in mice. Following a single dose of 10 mg/kg, the cumulative percentage of the administered dose was similar in Abcc2-knockout [Abcc2(–/–)] mice compared with age-matched wildtype mice (Figure 1A). Since expression of the related transporter Abcc4 is upregulated in the kidney of Abcc2(–/–) mice,¹³ we considered the possibility that these two transporters work in concert to eliminate cisplatin. However, urinary excretion of cisplatin was also unchanged in Abcc4(–/–) mice (Figure 1B). The lack of a significant impact of the individual Abcc2 and Abcc4 knockouts on elimination of cisplatin is consistent with the finding that cisplatin-induced nephrotoxicity, measured by histological grading of kidney slices, was also unchanged in Abcc2(–/–) and Abcc4(–/–) mice (Figures 1C–D).

Clinical studies

We next assessed the association of common germline variants in *ABCC2* with various pharmacokinetic and -dynamic endpoints in 2 separate cohorts of cancer patients receiving cisplatin. We focused specifically on 7 common SNPs in *ABCC2* because of their association with decreased transport of other ABCC2 substrates and relatively high allelic frequency in the target populations.¹⁴ In line with the murine data, the unbound clearance of cisplatin or changes in serum creatinine levels following treatment, used as a marker of nephrotoxicity, were not significantly linked with any of the common *ABCC2* genotypes analyzed in 112 cancer patients (Table 1). Furthermore, no significant changes in renal clearance of cisplatin were found among the various *ABCC2* genotypes (Supplementary Figure 1) or 18 identified haplotypes (Supplementary Table 1).

The possible association of response to cisplatin-based treatment was considered for 4 of the *ABCC2* variants in a second cohort of 125 patients with NSCLC. However, no statistically significant associations were identified with response rate, progression-free survival or overall survival (Table 2). These results are in line with a gene expression analysis done on the NCI60 cancer cell line panel (Supplementary Figure 2A), indicating that *ABCC2* mRNA levels were not dependent on the presence of the SNPs of interest in *ABCC2* (Supplementary Figure 2B). In addition, we found that *ABCC2* expression was not associated with the growth inhibitory potential of cisplatin in culture ($R=-0.05$; $P=0.21$).

DISCUSSION

In the current study we evaluated the association of common variants in the transporter *ABCC2*¹² with cisplatin-induced nephrotoxicity, differential clearance of cisplatin, or differential response and survival rates. The incentive for this investigation was based on the long-held premise that *ABCC2* might be a major contributor to cisplatin elimination, as result of its ability to export glutathione-conjugated cisplatin out of cells⁶ and its high expression levels in the proximal tubule of the kidney.^{7,8}

Using a mouse model with a genetically-engineered knockout of *Abcc2*, we found that urinary excretion of cisplatin or severity of cisplatin-induced nephrotoxicity were not critically dependent on this transporter or *Abcc4*, which is known to be upregulated in *Abcc2*-knockout mice. *Abcc4* may play a redundant role in cisplatin excretion since its overexpression has been linked with reduced accumulation of cisplatin and a cisplatin resistance phenotype in certain cancer cells.^{13,15} While these findings indicate that loss of *Abcc2* or *Abcc4* function does not result in significant phenotypic changes following cisplatin administration, future studies should focus on the pharmacological role of *Abcc2* and *Abcc4* collectively rather than individually.

In agreement with our mouse studies, cisplatin clearance, severity of nephrotoxicity, progression-free and overall survival did not differ in patients carrying individual SNPs in *ABCC2* with known or suspected alteration in biological function or expression. Furthermore, multiple *ABCC2* haplotype and diplotype combinations also did not significantly improve associations with cisplatin pharmacokinetics and -dynamics. In light of the relatively few individuals identified in our patients with variant genotypes, it should be pointed out that the currently observed lack of significant relationships between the studied *ABCC2* variants and cisplatin endpoints has relatively limited statistical power. It is also theoretically possible that additional genetic variants or haplotypes in *ABCC2* of importance to the disposition and/or efficacy of cisplatin in the studied populations are yet to be discovered, and/or that larger numbers of patients are needed to more precisely quantify genotype-phenotype associations. Nonetheless, in conjunction with our murine data, the current findings support the contention that *ABCC2* by itself is not an important contributor to interindividual variability in the pharmacokinetics and -dynamics of cisplatin.

In this context, it is noteworthy that results of our study are in apparent conflict with several previous studies indicating that the *ABCC2*-24C>T variant was correlated with higher response rates following irinotecan or platinum-based treatments and with decreased response to antiepileptic pharmacotherapy.^{16–18} The -24C>T variant has been previously linked with decreased mRNA levels; however, no changes in protein levels were observed and additional follow-up studies were unable to show altered mRNA expression.^{19–21} An additional study involving a population of Japanese patients with NSCLC receiving platinum-based therapy concluded that the -24C>T variant correlated with decreased response, although not with toxicity.²² Instead these investigators found that the 3927C>T variant, a silent mutation,^{19,23} correlated with increased overall toxicity in the female sub-set of the studied population, or only thrombocytopenia when both males and females were combined. Unfortunately, the above studies did not separate patients based on the applied chemotherapeutic regimen, which included either cisplatin or carboplatin. Since it is currently unknown whether *ABCC2* transports cisplatin and carboplatin with equal efficiency, their failure to properly stratify patients could explain inconsistencies with our findings. Furthermore, patients did not receive identical treatment regimens in the individual studies which could have compromised results. For example, patients in the previously published reports were eligible to receive a taxane-platinum based combination regimen, whereas in our study all patients received a cisplatin-gemcitabine duplet. Since taxanes are

efficiently transported by ABCC2,²⁴ it is conceivable that the reported associations of *ABCC2* genotypes with treatment outcome are due to associations with taxanes, and not with platinum compounds. Taken together, our results highlight the importance of verifying drug-transporter interactions from *in vitro* tests in follow-up clinical studies. The current study indicates that, although ABCC2 overexpression may affect cisplatin transport *in vitro*, this transporter by itself is unlikely to contribute substantially to the urinary excretion, toxicity and efficacy of cisplatin. Instead other transporters, such as the solute carriers OCT2 or MATE1, may be more appropriate for further study of interindividual variability as these transporters have both been independently implicated in altering platinum-pharmacokinetics in murine knockout models.^{25,26} In conclusion, further study is warranted to determine the individual and collective contribution of additional, potentially redundant, transporters to the pharmacokinetics and -dynamics of cisplatin.

METHODS

Animal experiments

Adult (8–12 week old) male wildtype and *Abcc2*($-/-$) mice, both on an FVB background, were obtained from Taconic (Germantown, NY). *Abcc4*($-/-$) mice, also on an FVB background, were kindly donated by Drs. John Schuetz and Clinton Stewart. Housing, handling, treatment, sample collection and analysis were performed as previously described.²⁷ The experiments were approved according to guidelines of the IACUC of St Jude Children's Research Hospital (Memphis, TN).

Pharmacogenetic association studies

Pharmacokinetic data for cisplatin and cisplatin-induced nephrotoxicity data were obtained from 112 white patients with solid tumors, and parameter estimates in these patients were determined as described.²⁸ Efficacy endpoints were evaluated in a second cohort of 125 white patients with NSCLC receiving cisplatin in combination with gemcitabine.²⁹ A baseline whole-body computed tomography scan was performed at baseline within 28 days before study entry and then every 2 months for objective tumor response (complete response [CR], partial response [PR], stable disease [SD], and progressive disease [PD]) according to Response Evaluation Criteria in Solid Tumors. Time to tumor progression and survival were defined as the period for the start of gefitinib treatment to date of disease progression or death, respectively, or last follow-up.

Genotyping procedures

DNA from all 237 patients was isolated and then amplified as described.²⁹ Variation in the *ABCC2* promoter at 7 different loci, including the $-24C>T$ substitution (rs717620) in the promoter region, was determined using direct nucleotide sequencing, as described.³⁰ These variants were selected on the basis of their relatively high allelic frequency and/or the known or suspected influence on functional properties of the encoded proteins. The recorded genotype was termed "variant" if it differed from the reference sequence for the SNP position as acquired by GenBank.

DNA and RNA from the NCI60 cancer cell lines 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) according to manufacturer's recommendations. Gene transcripts were quantified in triplicate using SYBR Green PCR mastermix (Qiagen) and primers, followed by normalization to the housekeeping gene, *GAPDH*.

Statistical considerations

Pharmacokinetic data obtained from the murine studies were analyzed using a t-test with a cutoff for statistical significance set at $P < 0.05$. The clinical studies were exploratory in nature and no power calculations for sample size were performed *a priori* before statistical analysis of the data. For the genotype association analysis, clinical endpoints of interest were clearance of unbound cisplatin, nephrotoxicity, determined from changes in serum creatinine, response rate (PR/CR *versus* other), time to tumor progression or progression-free survival, and overall survival. Statistical significance of associations between variant genotypes and categorical response rate was determined for individual parameters by a Fisher exact test, while other associations were evaluated using a Kruskal-Wallis test. Associations between variant genotypes with time to progression or overall survival were assessed by the Kaplan-Meier method, using an exact log-rank test and a Cox proportional hazard model. The cutoff for statistical significance was set at $P < 0.001$ for two-tailed tests adjusted for multiple comparisons, and were calculated using NCSS 2004 (J. Hintze, Number Cruncher Statistical Systems, Kaysville, UT).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Influence of Abcc2 or Abcc4 knockout on cisplatin endpoints in mice, showing urinary excretion of cisplatin in wildtype mice (solid line) and Abcc2^{-/-} mice (broken line) (**A**) or Abcc4^{-/-} mice (broken line) (**B**) and renal histopathology of kidney slices removed from wildtype or Abcc2^{-/-} mice (**C**) and wildtype mice or Abcc4^{-/-} mice (**D**). Cisplatin was administered at a single dose of 10 mg/kg and kidney slices for histological examination were obtained 72 hours later.

Table 1

Association of ABCC2 genotypes with cisplatin pharmacokinetics and nephrotoxicity

Genotype	Median unbound clearance (95% CI; L/h)				P	Geometric mean % change in serum creatinine (95% CI; L/h)			
	REF	HET	VAR	P		REF	HET	VAR	P
-1549G>A	27.3 (25.6–29.6)	26.1 (23.9–29.4)	32.7 (24.8–36.1)	0.28	14.2 (8.7–23.2)	29.9 (17.2–51.9)	17.2 (7.9–37.2)	0.07	
-1019A>G	27.3 (24.9–29.6)	25.9 (24.9–29.4)	32.7 (24.8–36.1)	0.15	14.8 (8.8–24.9)	23.5 (13.6–40.8)	18.4 (6.9–49.4)	0.6	
-34T>C	26.6 (25.3–28.7)	33.0 (24.4–39.7)	N/A	0.12	20.2 (14.7–27.8)	4.35 (3.8–5.0)	N/A	0.17	
-24C>T	26.8 (25.6–28.7)	28.9 (23.9–30.7)	27.7 (12.2–45.2)	0.99	17.0 (11.8–24.6)	20.9 (9.86–44.2)	43 (N/A)	0.37	
1249G>A	26.6 (24.9–29.4)	27.7 (25.8–30.0)	26.6 (24.9–29.4)	0.69	20.5 (13.9–30.3)	16.7 (9.2–30.3)	26.7 (N/A)	0.98	
3972C>T	26.8 (24.9–27.9)	26.6 (24.8–30.7)	29.3 (23.2–46.0)	0.28	13.1 (8.1–21.1)	22.9 (15.2–34.5)	32 (5.2–196)	0.04	
4544G>A	26.9 (25.6–28.9)	31.2 (N/A)	N/A	0.42	19.6 (14.2–26.9)	4.3 (N/A)	N/A	0.39	

Patients exposed to cisplatin-based treatments were assessed for ABCC2 variants and classified as homozygous (REF), heterozygous (HET) and homozygous for the variant (VAR). Median unbound clearance of cisplatin (L/h) and mean % change in serum creatinine levels were measured for each population and significance was determined by Kruskal-Wallis test. Confidence intervals (CI) are provided for each sample unless not available (N/A) due to limited sample size (N=1 or N=2).

Table 2

Association of ABCC2 variants with cisplatin efficacy

Genotype	Response							
	REF				HET			
	N	PD/SD	PR/CR	P	PD/SD	PR/CR	PD/SD	P
-1549G>A	125	11 (36%)	7 (18%)	0.74	44 (51%)	18 (46%)	31 (36%)	0.63
-34T>C	112	58 (74%)	26 (76%)	0.63	17 (22%)	5 (15%)	3 (4%)	0.42
-24C>T	108	50 (65%)	24 (77%)	0.68	21 (27%)	6 (19%)	6 (8%)	0.41
4544G>A	90	49 (82%)	22 (73%)	0.86	10 (17%)	6 (20%)	1 (1%)	0.41
Median Progression-Free Survival (95% CI; days)				Median Overall Survival (95% CI; days)				
Genotype	REF	HET	VAR	P	REF	HET	VAR	P
-1549G>A	138 (81–207)	99 (83–129)	91 (71–134)	0.74	242 (184–367)	262 (215–320)	242 (159–296)	0.88
-34T>C	100 (91–134)	70 (44–138)	125 (55–143)	0.63	242 (202–266)	270 (150–398)	271 (102–376)	0.87
-24C>T	101 (81–138)	79 (48–132)	91 (77–167)	0.68	231 (184–270)	262 (159–336)	360 (198–369)	0.81
4544G>A	129 (92–145)	49 (40–138)	143 (122–154)	0.86	266 (227–346)	270 (170–391)	271 (230–471)	0.85

Patients exposed to cisplatin-based treatments were assessed for Abcc2 variants and classified as homozygous (REF), heterozygous (HET) and homozygous for the variant (VAR) (N=sample size). Patients were also separated into progressive disease (PD)/stable disease (SD) and partial responders (PD)/complete responders (CR). Significance of correlations were determined by Chi-square probability level. Patients with variants of Abcc2 were also assessed for progressive-free and overall survival (media; days). Significance was determined by log-rank test from Kaplan-Meier analysis.