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ARTICLE

Effects of 5-HT_3 receptor antagonists on cisplatin-induced kidney injury

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Abstract

Nausea, vomiting, and renal injury are the common adverse effects associated with cisplatin. Cisplatin is excreted via the multidrug and toxin release (MATE) transporter, and the involvement of the MATE transporter in cisplatin-induced kidney injury has been reported. The MATE transporter is also involved in the excretion of ondansetron, but the effects of 5-HT_3 receptor antagonists used clinically for cisplatin-induced renal injury have not been elucidated. Therefore, the aim of this study was to investigate the effects of 5-HT_3 receptor antagonists in a mouse model of cisplatin-induced kidney injury and to validate the results using medical big data analysis of more than 1.4 million reports and a survey of 3000 hospital medical records. The concomitant use of a first-generation 5-HT_3 receptor antagonist (ondansetron, granisetron, or ramosetron) significantly increased cisplatin accumulation in the kidneys and worsened renal damage. Conversely, the concomitant use of palonosetron had no effect on renal function compared with the use of cisplatin alone. Furthermore, an analysis of data from the US Food and Drug Administration Adverse Event Reporting System and retrospective medical records revealed that the combination treatment of cisplatin and a first-generation 5-HT_3 receptor antagonist significantly increased the number of reported renal adverse events compared with the combination treatment of cisplatin and a second-generation 5-HT_3 receptor antagonist. These results suggest that compared with the first-generation antagonists, second-generation 5-HT_3 receptor antagonists do not worsen cisplatin-induced acute kidney injury. The findings should be validated in a prospective controlled trial before implementation in clinical practice.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The involvement of the multidrug and toxin release (MATE) transporter in cisplatin-induced kidney injury has been reported. The MATE transporter is involved in the excretion of not only cisplatin but also ondansetron, a 5-HT_3 receptor antagonist used

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as an antiemetic; however, the effects of 5-HT$_3$ receptor antagonists used clinically for cisplatin-induced renal injury have not been elucidated.

WHAT QUESTION DID THIS STUDY ADDRESS?

The aim of this study was to investigate the effects of 5-HT$_3$ receptor antagonists in a mouse model of cisplatin-induced kidney injury and to validate the results using medical big data analysis of more than 1.4 million reports and a survey of 3000 hospital medical records.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The results suggest that compared with the first-generation antagonists, second-generation 5-HT$_3$ receptor antagonists do not worsen cisplatin-induced acute kidney injury.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Promoting the use of second-generation 5-HT$_3$ receptor antagonists is expected to reduce the number of patients who develop cisplatin-induced renal damage.

INTRODUCTION

Cisplatin, a platinum-based anticancer agent, is a key drug in various regimens. However, the adverse effects associated with cisplatin are often problematic in clinical practice, and they include renal impairment, hearing impairment, nausea, vomiting, and bone marrow suppression.[1] Renal impairment, a dose-limiting toxicity of cisplatin, occurs in approximately one-third of patients treated with cisplatin. It can cause irreversible damage, thereby reducing the quality of life of patients and necessitating cisplatin dosage reduction, if treatment for renal impairment is not administered.[2] Therefore, it is important to ensure that the renal function of patients does not worsen during cisplatin administration to maintain treatment intensity. Hydration methods are strongly recommended in the guidelines for renal damage treatment during anticancer therapy.[3] Studies have suggested that this approach reduces the frequency of renal damage.[4] However, despite prophylactic treatment use, several patients receiving cisplatin experience a decline in renal function.[5] There are no clinically effective therapies for the prophylaxis or treatment of cisplatin-induced renal injury, despite the completion of various studies in this regard.[1,5,6] Cisplatin is a renally excreted drug, and more than 90% of the drug is excreted in the urine over 24 h after intravenous injection.[7] The concentration of cisplatin in the kidneys is approximately five-fold higher than that in the serum and the accumulation of cisplatin in the kidneys significantly contributes to nephrotoxicity.[8–10] Cisplatin-induced kidney injuries include severe damage to tubular cells, and nephrotoxicity originates in the proximal tubules.[11] Preclinical studies have elucidated the mechanisms underlying cisplatin-induced kidney injury.[5–7] Two transporters implicated in the development of cisplatin-induced kidney injury are organic cation transporter 2 (OCT2), located on the basement membrane side of the proximal renal tubules, and multidrug and toxin extrusion (MATE) protein, located on the brush border membrane side of the proximal renal tubules. Cisplatin is taken up from the blood into the proximal renal tubular cells via OCT2, and then excreted into the tubules from the proximal renal tubular cells via MATE.[12–14]

The cisplatin transport activity of MATE is considerably lower than that of OCT2, and this difference leads to cisplatin accumulation in the proximal renal tubules.[15,16] Therefore, the inhibition of OCT2 expression may reduce cisplatin accumulation in the tubules, whereas the inhibition of MATE expression may increase cisplatin accumulation. Notably, cimetidine, a substrate of OCT2, has been reported to limit cisplatin-induced renal damage,[17] whereas the MATE inhibitor pyrimethamine has been reported to exacerbate renal damage.[15]

Cisplatin is designated as highly emetogenic in the Guidelines for the Proper Use of Antiemetic Drugs, with nausea and vomiting occurring in more than 90% of patients.[18] Nausea and vomiting can be controlled with the appropriate use of antiemetics. Because nausea and vomiting associated with chemotherapy are caused by the combined stimulation of 5-HT$_3$ receptors in the gastrointestinal tract and NK-1 receptors in the chemoreceptor trigger zone, these receptor antagonists and steroids are indicated as antiemetic agents. Notably, a three-drug combination, comprising an NK-1 receptor antagonist, a 5-HT$_3$ receptor antagonist, and a steroid, has been recommended for use with cisplatin[18] and incorporated as a supportive therapy in various regimens. The 5-HT$_3$ receptor antagonists include the first-generation agents, ondansetron and granisetron, and second-generation agent, palonosetron, and each regimen is used differently. The 5-HT$_3$ receptor antagonists have been reported to be substrates for OCT2 and MATE.[19,20] Furthermore, a preclinical

study using a mouse model of cisplatin-induced kidney injury demonstrated that ondansetron, a first-generation drug, may exacerbate cisplatin-induced kidney injury by inhibiting MATE.[16] However, the effects of 5-HT_3 receptor antagonists used clinically to prevent cisplatin-induced nausea and vomiting on cisplatin-induced kidney injury remain unknown. Therefore, here, we investigated the effects of various 5-HT_3 receptor antagonists in a mouse model of cisplatin-induced kidney injury and validated the results obtained using data from retrospective clinical trials and from the analysis of medical data.

MATERIALS AND METHODS

Animal model of cisplatin-induced nephrotoxicity

All experimental procedures were performed in accordance with the guidelines of the Animal Research Committee of Tokushima University Graduate School, and the protocol was approved by the Institutional Review Board of Tokushima University Graduate School for Animal Protection (Permit Number: T30-85). Nine-to-ten-week-old C57BL/6 J mice (weighing 24–27 g) were purchased from Nippon CLEA (Tokyo, Japan) and maintained with ad libitum access to water and food (type NMF; Oriental yeast, Tokyo, Japan). The relative humidity in the breeding room was set at 50% ± 10% and the room temperature was 26°C ± 1°C, with a 12-h light/dark cycle (lights on at 8:00 a.m., lights off at 8:00 p.m.). The mice were randomly divided into six groups (n = 7–9 per group): vehicle-injected, cisplatin-injected, and first-generation 5-HT_3 receptor antagonists (ondansetron [1 mg/kg], granisetron [30 mg/kg], or ramosetron [1 mg/kg]) or second-generation 5-HT_3 receptor antagonists (palonosetron [1.5 mg/kg]) administered to the cisplatin-injected group. The mice were intraperitoneally injected with cisplatin (15 mg/kg) or vehicle. Thirty minutes before cisplatin injection, the mice were administered various 5-HT_3 receptor antagonists intraperitoneally, including first- or second-generation 5-HT_3 receptor antagonists, or vehicle. At 4, 24, and 72 h after cisplatin injection, the mice were euthanized by an overdose of anesthesia, and samples were collected for the subsequent analysis. The doses were based on those used as antiemetic agents in animal models.[21–25]

Determination of blood and urine creatinine and blood urea nitrogen levels

Blood urea nitrogen (BUN), serum creatinine (serum Cr), and urine creatinine (urine Cr) levels in the serum and urine samples were determined by Oriental Yeast Industries (Shiga, Japan). Creatinine clearance (CrCL) was calculated as follows:

CrCL (ml/min/kg) = urine volume (ml/min/kg) × urine Cr concentration (mg/dl) / serum Cr concentration (mg/dl).

Real-time polymerase chain reaction using the kidney tissues from mice with cisplatin-induced kidney injury

The kidneys were collected from mice with cisplatin-induced kidney injury. RNA was extracted from the kidney samples using an RNA extraction solution (NIPPON GENE) according to the manufacturer's instructions. The cDNA was reverse transcribed using the PrimeScript RT Reagent kit (Takara Bio) and a polymerase chain reaction (PCR) Thermal Cycler Dice (Takara Bio). The cDNA from each sample was mixed with forward and reverse primers and THUNDERBIRD SYBR qPCR Mix (Toyobo); PCR was performed using an Applied Biosystems StepOnePlus machine (Applied Biosystems). Fold changes in gene expression relative to those of the control group were evaluated using mouse glyceraldehyde 3-phosphate dehydrogenase as the internal standard. The primer sets used for PCR are shown in Table S1.

Histological analysis

Renal tubular injury was assessed as previously described.[21] Briefly, the kidney samples were fixed with 4% paraformaldehyde and embedded in paraffin. The samples were cut into 4-μm sections, which were then stained with hematoxylin and eosin. Tubular damage was scored blindly by 3 or more individuals other than the experimenter, according to the percentage of damage (tubular necrosis, brush border loss, cast formation, tubular dilation, and tubular degeneration), as follows: 0, normal; 1, less than 25%; 2, 25%–50%; 3, 50%–75%; and 4, greater than 75%. Ten random microscopic fields per kidney section were used for quantification using the BX53 microscope (Olympus).

Determination of platinum concentration

To determine the concentration of platinum, 20 mg of the kidney sample or 20 μl of whole blood was incubated with 200 μl of 60% nitric acid (Wako) for 1 h at 95°C. The lysates were centrifuged at 5000 *g* for 10 min, and 50 μl of the supernatant was diluted with nitric acid. Platinum concentration was assessed using a polarized Zeeman atomic absorption spectrophotometer (Model Z-5710; Hitachi High-Technologies Co.). The absolute concentration of

platinum in the kidneys and whole blood was determined using a calibration curve.

Uptake study in HEK293 cells stably expressing hMATEs

Human embryo kidney cells were obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). The cells stably expressing hMATE1 (2.0×10^5 cells/well) were grown in 96-well plates for 24 h and used in the DAPI-update assay. The cells were preincubated with 0.1 ml of DAPI-free uptake buffer (130 mM KCl, 2 mM K_2HPO_4, 1.2 mM $MgSO_4$, 1.0 mM $CaCl_2$, 5 mM glucose, and 20 mM HEPES; pH 8.0) for 10 min. The uptake assay was initiated by replacing the DAPI-free uptake buffer with a buffer containing DAPI (0.5 μM) (0.1 ml) for 10 min. The optimal experimental conditions were determined after preliminary experiments based on a previous study.[26] All procedures were performed at 37°C. The reactions were terminated by adding ice-cold DAPI-free uptake buffer (0.1 ml), and the cells were washed twice with 0.1 ml of the same buffer. Thereafter, each well was filled with 0.1 ml of ice-cold DAPI-free uptake buffer, and the intensity of DAPI fluorescence was measured using a fluorescence plate reader (SpectraMaxi3; Molecular Devices; 345 nm excitation wavelength and 455 nm emission wavelength) to evaluate uptake. Cellular protein content was determined as described previously[27] using bovine serum albumin as the standard. The uptake assay was also performed in mock cells, which were transfected with an empty pcDNA5.1 vector, to determine nonspecific uptake. The half-maximal inhibitory concentration (IC_{50}) was calculated using the probit analysis with SPSS (SPSS Inc.).

Assessing the effects of concomitant drugs

From January 2004 to June 2020, 14,524,065 spontaneous adverse event reports were submitted to the US Food and Drug Administration's (FDA) Adverse Event Reporting System (FAERS), and they were downloaded from the FDA website.[28] Duplicate data were excluded in accordance with the FDA recommendations, and the remaining 12,192,072 reports were used in the analysis. SQLite was used to process the data, and R version 3.2.1 (R Foundation for Statistical Computing) was used for statistical analyses. Acute renal failure was defined using 47 terms of "acute renal failure (SMQ 20000003)" excluding neonatal and pediatric diseases (Table S2) according to Medical Dictionary for Regulatory Activities (MedDRA) version 23.1.

The risk of adverse events was assessed using the reporting odds ratio (ROR) and 95% confidence interval (CI). Patients who received cisplatin were classified into four groups: (1) patients who used drug A and reported acute renal failure; (2) patients who used drug A and did not report acute renal failure; (3) patients who did not use drug A and reported acute renal failure; and (4) patients who did not use drug A and did not report acute renal failure. Based on the following equation, the ROR and 95% CI were calculated.

$$\mathrm{ROR} = \frac{AD}{BC}$$

$$95\,\%\ \mathrm{CI} = \exp\left(\ln \mathrm{ROR} \pm 1.96\sqrt{\frac{1}{A}+\frac{1}{B}+\frac{1}{C}+\frac{1}{D}}\right)$$

All tests were two-tailed, and results with p values less than 0.05 were considered statistically significant.

Study design and population

We performed a retrospective case-control study in patients who received cisplatin at Tokushima University Hospital, a tertiary academic teaching hospital in Tokushima, Japan, between January 1, 2007, and December 31, 2019, under the approval of the Ethics Committee of Tokushima University Hospital (reference number 3820). This study was conducted according to the principles set forth in the Declaration of Helsinki. Because the present study was retrospective in nature and used existing information, an opt-out option was displayed on the institution website as a surrogate for informed consent for eligible patients according to the Japanese Governmental guidelines.[29] Patients were included if they had received a single dose of cisplatin greater than or equal to 60 mg/m^2 during one course of treatment, according to previous studies.[30,31] Patients were excluded if they were under 18 years of age, registered in an industry-initiated clinical trial, or had insufficient renal function (baseline serum creatinine [SCr] level of ≥1.5 mg/dl and/or estimated glomerular filtration rate [eGFR] of <60 ml/min/1.73 m^2).[32,33]

Data collection and definitions

We collected baseline data related to physiological conditions, laboratory results, concomitant medication use, and disease history upon the initiation of cisplatin using electronic medical records from Tokushima University Hospital. Cisplatin-induced nephrotoxicity (CIN) was defined as an increase in the SCr level by greater than or equal to 0.3 mg/dl (26.5 μmol/L) or 50% of the baseline level at 7 days after cisplatin administration.[34,35] Data on the proportion of patients using other antiemetic agents, such as NK1R antagonists, olanzapine, were not obtained because these drugs do not affect renal function in patients with an eGFR of greater than or equal to 60 ml/min/1.73 m^2.[36–39]

TABLE 1 Body weight, kidney weight, and renal function in vehicle-treated mice and cisplatin-treated mice with or without 5-HT$_3$ receptor antagonists

	Vehicle	CDDP	CDDP + ond	CDDP + gra	CDDP + ramo	CDDP + palo
Initial body weight (g)	26.1 ± 0.6	26.4 ± 0.7	26.2 ± 0.3	28.4 ± 0.4	27.6 ± 1.0	26.5 ± 0.3
Post body weight (g)	25.2 ± 0.5	20.7 ± 0.5†	20.3 ± 0.3†	21.9 ± 0.6†	21.1 ± 0.9†	20.9 ± 0.7†
Kidney weight (mg)	189.0 ± 7.0	156.9 ± 4.4	171.9 ± 8.0	194.7 ± 5.1	181.0 ± 7.4	173.6 ± 5.0
Urine volume (ml)	1.60 ± 0.25	0.95 ± 0.10†	0.85 ± 0.17†	0.92 ± 0.17†	0.85 ± 0.14†	0.93 ± 0.10†
BUN (mg/dl)	21.22 ± 0.69	81.86 ± 5.47†	180.23 ± 21.56 **$^{\#\#}$	123.80 ± 13.42	123.59 ± 17.6	90.80 ± 19.04
CrCL (ml/min/kg)	10.89 ± 1.05	4.94 ± 0.70†	1.29 ± 0.43 **$^{\#\#}$	1.75 ± 0.20 *$^{\#}$	1.58 ± 0.20 *$^{\#}$	4.83 ± 0.92

Note: Data are presented as mean ± SEM.

Abbreviations: BUN, blood urea nitrogen; CrCL, creatinine clearance; CDDP, cisplatin; gra, granisetron; ond, ondansetron; palo, palonosetron; ramo, ramosetron.

$^{\dagger}p < 0.05$ versus vehicle mice, $*p < 0.05$ and $**p < 0.01$ versus CDDP mice. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus CDDP + palonosetron mice; $n = 7$–9 in each group.

The eGFR was calculated as follows[40]:

eGFR (ml/min/1.73 m^2) = 194 × serum creatinine - 1.094 × age - 0.287 × 0.739 (if female).

Propensity score matching analysis

As patients in this retrospective case-control study were not randomized as patients treated with the first or second-generation antiemetic agents, propensity score matching (PSM) was performed to decrease the variability between treatment cohorts. Fourteen factors (sex, age, body surface area [BSA], eGFR, cisplatin dosing/BSA, serum creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, serum albumin, and number of patients concomitantly using magnesium sulfate and with chronic heart disease, hypertension, and diabetes mellitus) were selected as factors associated CIN and/or kidney injury or were deemed a priori to be important demographic characteristics for use in a logistic regression model, which was used to calculate propensity scores.[41–43] A 1:1 PSM analysis was performed using the nearest neighbor matching method with a caliper of 0.20 to reduce the potential bias of patient characteristics.[44] Matching was performed without a replacement treatment, and cases not meeting the matching criteria were excluded.

Statistical analysis

Differences between groups were analyzed using the parametric unpaired *t*-test or nonparametric Mann–Whitney *U* test, as applicable. Categorical variables were compared using Fisher's exact test or χ^2 test. Results with a *p* value of less than 0.05 were considered statistically significant. Statistical analysis of data of the retrospective case-control study was performed using JMP version 15.0 software (SAS Institute Inc.). For comparisons among three or more groups, a one-way analysis of variance was performed. Tukey's test was also performed as a post hoc analysis. R version 3.2.1 for Windows was used for statistical analyses, and results with a two-tailed *p* value of less than 0.05 were considered statistically significant.

RESULTS

Effects of 5-HT$_3$ receptor antagonists on cisplatin-induced kidney injury in mice

Cisplatin-treated mice presented a decrease in body weight, but no change in kidney weight (Table 1). Cisplatin-treated mice exhibited *KIM-1* and *Lcn2* mRNA expression, markers of renal tubular damage, presented elevated plasma BUN, and showed a significant decrease in CrCL, which indicated kidney damage (Table 1, Figure 1h,i). Cisplatin in combination with ondansetron, granisetron, or ramosetron exacerbated cisplatin-induced kidney damage (Table 1, Figure 1h,i); whereas, combination treatment with palonosetron did not exacerbate cisplatin-induced renal damage.

Histological evaluation

Cisplatin treatment was associated with the degeneration and disruption of renal tissue, including nuclear loss, proximal tubular cell emptying, and tubular dilation. Mice treated with the first-generation 5-HT$_3$ receptor antagonists presented more extensive damage, such as tubular dilation and tubular cell necrosis, than mice treated with cisplatin alone. However, there was no difference between the groups that received combination treatment of cisplatin and palonosetron and cisplatin alone (Figure 1g).

The first-generation 5-HT$_3$ receptor antagonist group had a significantly higher score than the cisplatin only group. The

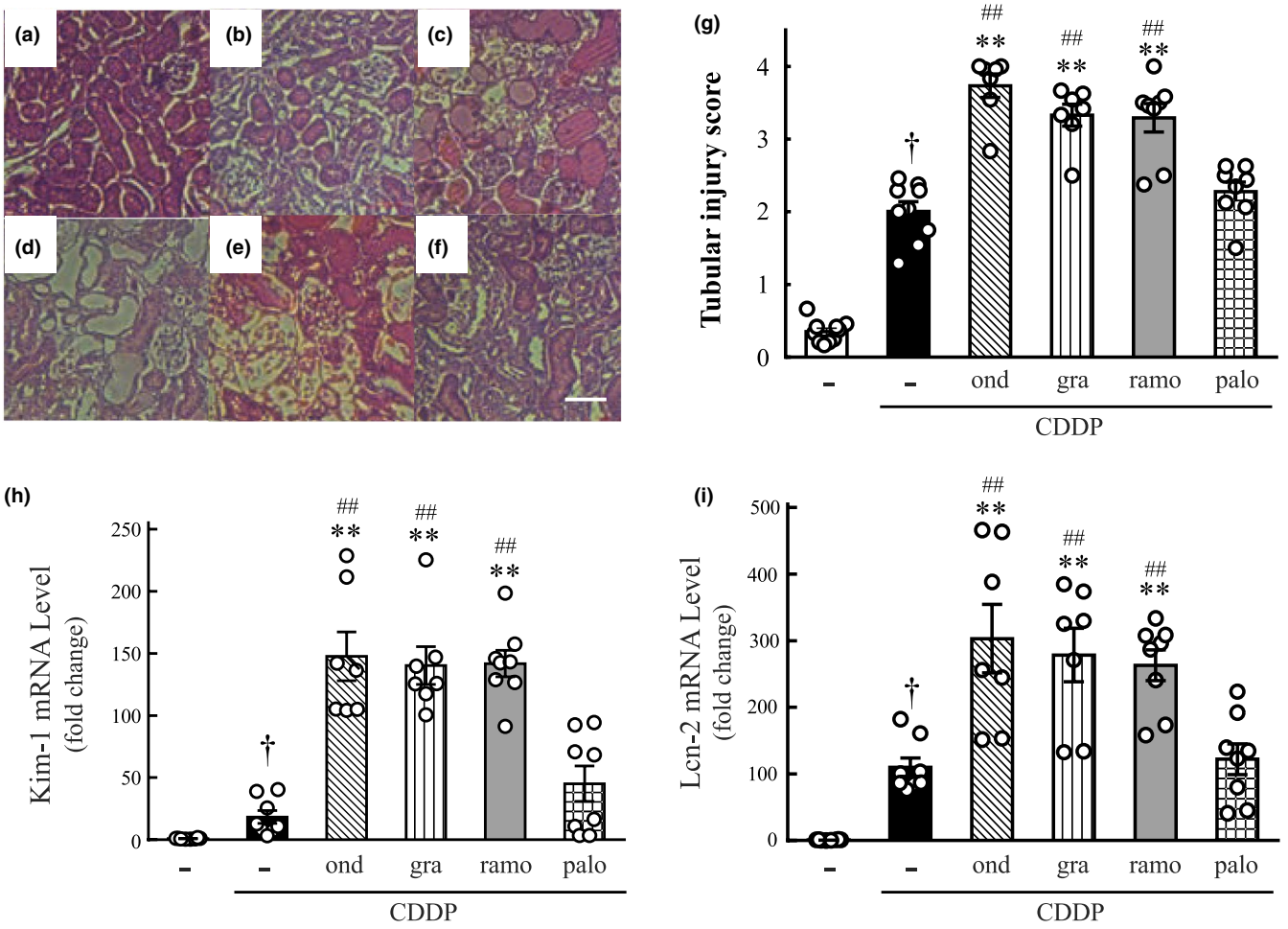

FIGURE 1 Effects of 5-HT$_3$ receptor antagonists on cisplatin-induced nephrotoxicity. (a–f) Representative hematoxylin and eosin staining (H&E) of the kidney section of the control mice, cisplatin (CDDP)-injected mice with vehicle or a 5-HT$_3$ receptor antagonist. (a) vehicle, (b) CDDP, (c) CDDP + ondansetron, (d) CDDP + granisetron, (e) CDDP + ramosetron, (f) CDDP + palonosetron. (g) Quantitative analysis of renal damage scores. Values are expressed as mean ± SEM. ond; ondansetron, gra; granisetron, ramo; ramosetron, palo; palonosetron, †$p < 0.05$ vs. vehicle mice, **$p < 0.01$ versus CDDP mice. $^{\#\#}p < 0.01$ versus CDDP + palonosetron mice; $n = 7$–9 in each group. (h), (i) The mRNA expression levels of kidney injury markers (Kim-1 [h] and Lcn-2 [i]) in the kidneys of mice in each group. Values are expressed as mean ± SEM. ond; ondansetron, gra; granisetron, ramo; ramosetron, palo; palonosetron, †$p < 0.05$ versus vehicle mice, **$p < 0.01$ versus CDDP mice. $^{\#\#}p < 0.01$ versus CDDP + palonosetron mice; $n = 7$–9 in each group

score for combination treatment with palonosetron was similar to that for cisplatin alone (Figure 1g).

Effects of 5-HT$_3$ receptor inhibitors on the platinum concentration in the whole blood and kidneys

The renal platinum concentration significantly increased in mice treated with ondansetron, granisetron, or ramosetron at 4 h after cisplatin administration (Figure 2a). In contrast, palonosetron administration had no effect on the renal platinum concentration (Figure 2a). There was no difference in the platinum concentration in whole blood at 4 h after cisplatin administration in all groups (Figure 2b). At 24 h after cisplatin administration, renal accumulation of cisplatin (CDDP only, 14.40 ± 2.74 μg/g, $n = 6$) was less than half of that at 4 h, and no effect of concomitant medication was observed (data not shown). These results suggest that the first-generation 5-HT$_3$ receptor inhibitors may increase platinum accumulation in the kidneys.

Uptake study in HEK293 cells stably expressing hMATEs

The *cis*-inhibition analysis with DAPI revealed that the first-generation inhibitors had an IC$_{50}$ equal to or lower than that of cimetidine, a typical MATE inhibitor. The first-generation inhibitors were found to have a lower IC$_{50}$ than the second-generation inhibitors (Table 2, Figure S1).

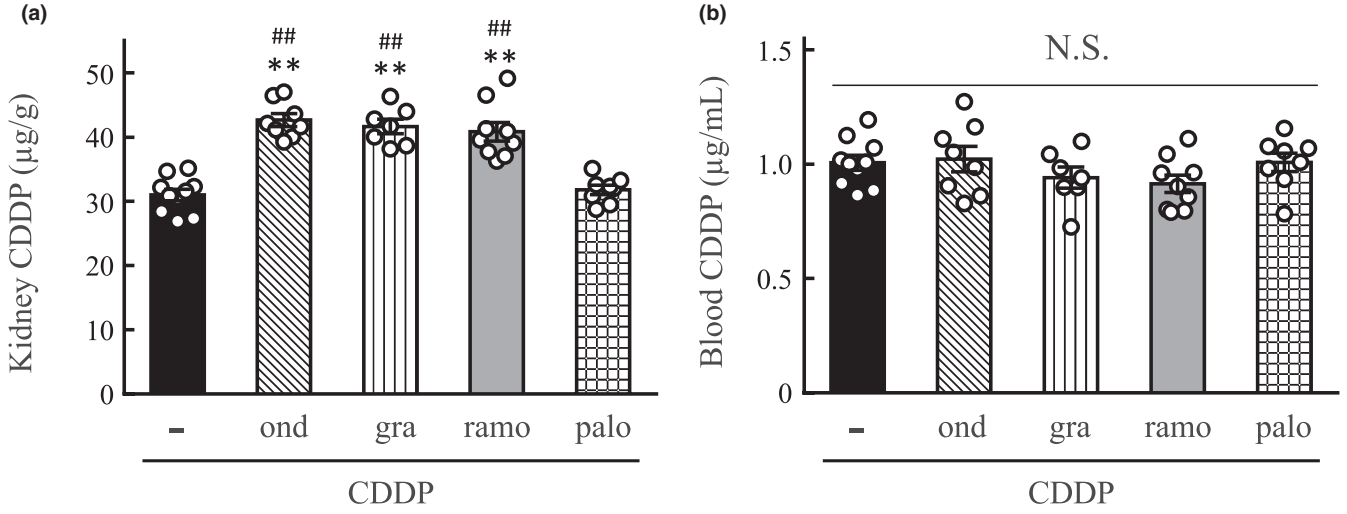

FIGURE 2 Platinum content in the kidney (a) and whole blood (b) at 4 h after cisplatin (CDDP) treatment. Data are expressed as mean ± SEM. ond; ondansetron, gra; granisetron, ramo; ramosetron, palo; palonosetron, $^{**}p < 0.01$ versus CDDP mice. $^{\#\#}p < 0.01$ versus CDDP +palonosetron mice, N.S.: not significant; $n = 7–9$ in each group

TABLE 2 IC_{50} value of 5-HT_3 receptor antagonists for hMATE1

Drug	IC_{50} (μM)
Ondansetron	0.75 ± 0.10
Granisetron	24.70 ± 4.81
Ramosetron	4.42 ± 1.03
Palonosetron	55.79 ± 6.13
Cimetidine	17.94 ± 4.71

Note: Values are expressed as mean ± SEM.

Abbreviation: IC_{50}, half-maximal inhibitory concentration.

FAERS analysis results

Among 14,524,065 spontaneous adverse event reports submitted from January 2004 to June 2020, 44,678 cases involved cisplatin administration. Based on these reports, the ROR for acute renal failure was compared among cases reporting combination treatment with the first- or second-generation 5-HT_3 receptor antagonists. In total, 8.0% of the patients who received cisplatin reported acute renal failure. The OR of the first-generation 5-HT_3 receptor antagonists in combination with cisplatin was 1.26 (95% CI: 1.140–1.398), and that of the second-generation 5-HT_3 receptor antagonists was 0.79 (95% CI: 0.607–1.02). The combination of cisplatin with the first-generation 5-HT_3 receptor antagonists significantly increased the reported OR for cisplatin-related nephropathy ($p < 0.01$; Table 3).

Patient characteristics

Among 2664 patients treated with CDDP, between January 1, 2007 and December 31, 2019, 53 patients met the inclusion criteria but had insufficient clinical data to allow the evaluation of patient characteristics listed in Table 1; thus, 431 fulfilled the inclusion and exclusion criteria and were enrolled. CIN occurred in 40 of the 431 enrolled patients (Figure 3). Among these, 126 patients received a concomitant first-generation antiemetic (first group) and 305 received a concomitant second-generation antiemetic (second group). There was no significant difference in the incidence of CIN between the groups (first group, 11.9%; second group, 8.2%; Table 4). Conversely, there was a significant difference in age, eGFR, SCr, serum albumin, and proportion of patients concomitantly using magnesium sulfate between the groups.

Propensity score-adjusted results

The area under the curve of the propensity score for second-generation antiemetic treatment, with 14 covariates, was 0.812. We obtained 111 pairs by matching the propensity score at a caliper value of 0.2. The significant differences in age, eGFR, and percentage of patients using Mg sulfate that affected CIN development observed in the 111 pairs before matching were eliminated in the pairs after matching. After the 1:1 PSM analysis, CIN in the first group (15/111, 13.5%) was significantly lower than that in the second group (6/111, 5.4%) in the 111 matched pairs. Patients using antiemetics with different pharmacokinetics, such as hepatic metabolism and renal excretion, were included. There were no significant differences in hepatic and renal functions between the groups after matching the 111 pairs. The proportion of patients who received steroids to improve renal function did not differ between the groups in the 111 pairs after PSM matching (first group, 109/111, 98.2%; second group, 111/111, 100.0%, not significant). There was no significant difference in other patient characteristics, except for CIN incidence among the 111

TABLE 3 Effect of 5-HT$_3$ receptor antagonists on the occurrence of cisplatin-induced ARF using the FAERS data analysis

Drug	ARF (%) without the drug	ARF (%) with the drug	ROR (95% CI)	*p* value
First-generation 5-HT$_3$ receptor antagonist[a]	9.66% (3952/40899)	12.20% (461/3779)	1.26 (1.140–1.398)	<0.001[c]
Second- generation 5-HT$_3$ receptor antagonist[b]	9.92% (4350/43870)	7.80% (63/808)	0.79 (0.607–1.018)	0.072[c]

Abbreviations: ARF, acute renal failure; CI, confidence interval; FAERS, US Food and Drug Administration's Adverse Event Reporting System; ROR, reporting odds ratio.

[a]Ondansetron, Granisetron, and Ramosetron.

[b]Palonosetron.

[c]Fisher's exact test.

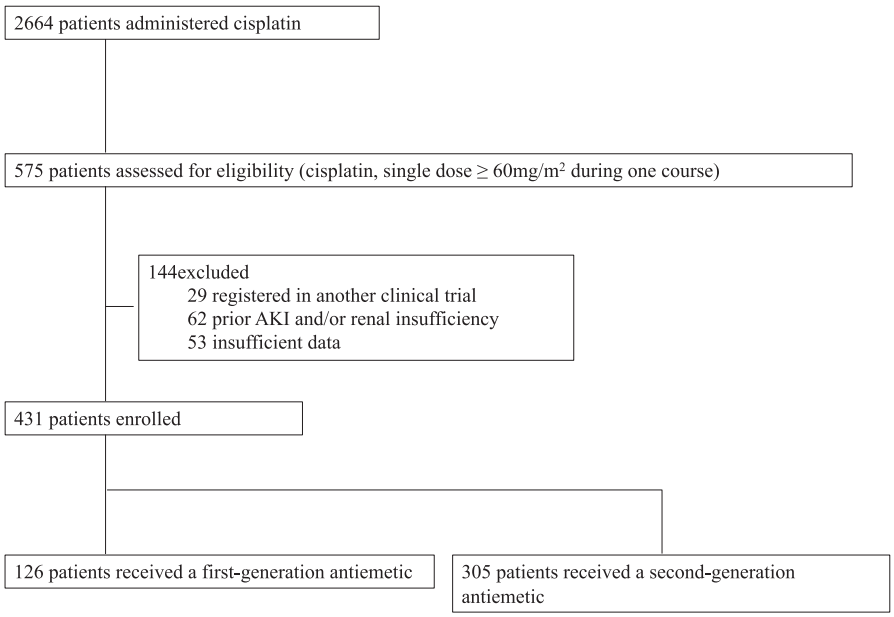

FIGURE 3 Flow chart of patient selection. AKI, acute kidney injury

matched pairs (Table 4). The PSM analysis indicated the efficacy of second-generation antiemetic drugs for CIN.

DISCUSSION

Our results show that the first-generation 5-HT$_3$ receptor antagonists exacerbate cisplatin-induced kidney injury by increasing cisplatin accumulation in the kidneys. Conversely, the second-generation 5-HT$_3$ receptor antagonists do not adversely affect the kidneys. The results indicate that the higher inhibitory effect of the first-generation 5-HT$_3$ receptor antagonists on MATE affects the increase in cisplatin accumulation in the kidneys in response to these agents. A retrospective analysis of reports in an adverse event-reporting database revealed a difference in the effects between the first- and second-generation 5-HT$_3$ receptor antagonists on cisplatin-induced kidney injury. A retrospective chart review of clinical patient data suggested that patients receiving the second-generation 5-HT$_3$ receptor antagonists may be at a lower risk of exacerbation of cisplatin-induced kidney injury than those receiving the first-generation drugs. Therefore, the second-generation 5-HT$_3$ receptor antagonists may be useful as antiemetic agents as they do not exacerbate cisplatin-induced kidney injury.

Considerable progress has been made in cancer treatment. New anticancer agents with novel action mechanisms, such as tyrosine kinase inhibitors and immune checkpoint inhibitors, have been developed and used in clinical practice. However, several anticancer therapies with proven effectiveness continue to be used, including cisplatin, a platinum-based agent used in chemotherapy for various malignant solid tumors. Renal impairment is a dose-limiting toxicity of cisplatin and is a challenge to manage in clinical practice. Despite several studies, there are no clinically effective drugs for the prevention and treatment of cisplatin-induced renal impairment; therefore, it is important to ensure that the renal function does not worsen during cisplatin treatment in order to maintain treatment intensity. Cisplatin is classified as a drug with a high emetogenic risk, and guidelines recommend the concomitant use of antiemetic agents. Previous studies in mouse models have reported that ondansetron exacerbates cisplatin-induced kidney injury. Therefore, 5-HT$_3$ receptor antagonists currently used in clinical practice may affect the incidence of

TABLE 4 Patient characteristics of the overall series and propensity score matched pairs at baseline

	Before propensity score matching			After propensity score matching		
Variable	**1st (n = 126)**	**2nd (n = 305)**	***p* value**	**1st (n = 111)**	**2nd (n = 111)**	***p* value**
CIN incidence (%)	15 (11.9)	25 (8.2)	0.273	15 (13.5)	6 (5.4)	0.039
Sex (male/female)	92/34	238/67	0.264	83/28	85/26	0.876
Age (year)	61.0 (9.2)	63.6 (9.5)	0.009	61.5 (9.1)	63.0 (10.1)	0.247
Body weight (kg)	58.0 (11.6)	59.4 (11.4)	0.238	58.7 (11.7)	58.2 (11.8)	0.763
Height (cm)	162.3 (8.8)	163.7 (8.2)	0.112	162.5 (8.8)	162.5 (8.7)	0.999
BSA (m^2)	1.61 (0.18)	1.64 (0.18)	0.149	1.62 (0.19)	1.61 (0.19)	0.815
Cisplatin dosing (mg/m^2/course)	78.2 [70.8–79.9]	76.9 [70.0–79.9]	0.650	78.2 [70.8–79.9]	74.9 [68.8–79.4]	0.826
eGFR (ml/min/1.73 m^2)	84.4 [74.4–97.4]	79.8 [70.6–90.4]	0.001	83.3 [73.8–96.5]	81.2 [73.6–91.0]	0.475
Serum creatinine (mg/dl)	0.68 [0.59–0.76]	0.73 [0.62–0.84]	0.003	0.69 [0.60–0.76]	0.72 [0.60–0.78]	0.527
AST (IU/L)	23.0 (15.4)	24.4 (13.2)	0.343	23.9 (16.1)	23.2 (12.9)	0.754
ALT (IU/L)	21.5 (19.1)	21.8 (16.8)	0.903	22.5 (20.0)	21.4 (19.4)	0.678
Total bilirubin (mg/dl)	0.64 (0.29)	0.64 (0.25)	0.965	0.63 (0.29)	0.64 (0.26)	0.941
Serum albumin (g/dl)	3.4 (0.6)	3.6 (0.5)	0.012	3.4 (0.6)	3.5 (0.6)	0.504
Concomitant use of magnesium sulfate (%)	10 (7.9)	153 (50.2)	<0.001	10 (9.0)	8 (7.2)	0.807
Comorbidities						
Chronic heart disease (%)	0 (0.0)	7 (2.3)	0.112	0	0	NA
Hypertension (%)	26 (20.6)	65 (21.3)	1.000	24 (21.6)	25 (22.5)	1.000
Diabetes mellitus (%)	40 (31.8)	111 (36.4)	0.377	39 (35.1)	34 (30.6)	0.568

Note: Data are presented as mean (SD), median [interquartile range], or number of patients (%).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, body surface area; CIN, cisplatin-induced nephrotoxicity; eGFR, estimated glomerular filtration rate; NA, not applicable.

cisplatin-induced renal injury. In a mouse model of cisplatin-induced renal injury, combination treatment with cisplatin and a first-generation 5-HT_3 receptor antagonist exacerbated renal damage. Histological evaluation of the renal sections revealed extensive proximal tubular cell necrosis.

To determine the cause of cisplatin-induced renal damage exacerbated by the concomitant use of first-generation 5-HT_3 receptor antagonists, we measured cisplatin accumulation in the kidneys at 4 or 8 h after cisplatin administration. The concomitant use of a first-generation 5-HT_3 receptor antagonist significantly increased the renal cisplatin accumulation; however, there was no difference in accumulation between animals that received combination therapy with palonosetron and those that received cisplatin monotherapy. Combination treatment with first- and second-generation agents did not affect the blood levels of cisplatin. Thus, the first-generation 5-HT_3 receptor antagonists altered cisplatin accumulation in the kidneys without affecting cisplatin pharmacokinetics.

The excretion of cisplatin from renal tubular cells is mediated by MATE1, which has been reported to be involved in the CIN development.[45] Ondansetron is a MATE transporter inhibitor.[46,47] Ondansetron inhibited MATE and exacerbated cisplatin-induced kidney injury in mice.[16] Li et al.[16] showed that ondansetron inhibits MATE activity in vitro and increases the blood concentration and renal accumulation of metformin, a substrate of MATE transporters, in vivo, suggesting a correlation between the inhibition of MATE transporters and inhibition of renal excretion of MATE substrates. It has also been reported that there is an association between transporter inhibitory activity, as determined in vitro, and clinical symptoms.[48] Thus, a comparison of the MATE inhibitory activity of various 5-HT_3 receptor antagonists revealed that the second-generation inhibitor palonosetron had weaker MATE inhibitory activity than the three first-generation agents. Furthermore, the most notable difference between palonosetron and the first-generation drugs is their affinity for 5-HT_3 receptors (~ 10-fold lower inhibition constant values than those of ondansetron). In addition, the half-life is significantly increased, to ~ 35 h compared with 5 h for the first-generation drugs. Therefore, the difference in pharmacokinetics can be attributed to a difference in elimination competition with cisplatin.

There were some limitations to this study. First, the results of the experiments conducted in cells and animals do not fully reflect the effect in humans. Second, the FAERS database accumulates spontaneous reports, which may be subject to bias such as under- or over-reporting. Therefore, we conducted a retrospective study using electronic medical records, which provide detailed patient information. The results suggest that the first-generation 5-HT$_3$ receptor antagonists may increase the incidence of cisplatin-induced kidney injury. Our retrospective study using medical electronic records involved a small number of patients, and we could not obtain data about some potential factors of CIN, such as other antiemetics, other nephrotoxins, and hydration status. Additionally, palonosetron is a more efficacious antiemetic than the first-generation 5-HT$_3$ receptor antagonists and, therefore, the hydration status may be improved. Consequently, our results may not be fully generalizable without further prospective study in a larger population. Thus, although each method has its limitations, they can be overcome using multiple methods and information obtained through databases. The results of basic experiments can be validated for drug efficacy using clinical data to predict clinical effects and safety. The results suggest that the concomitant use of first-generation 5-HT$_3$ receptor antagonists may be a risk factor for cisplatin-induced renal damage. However, second-generation 5-HT$_3$ receptor antagonists appeared to have less effect. Therefore, renal injury risk in high-risk patients may be reduced by selecting a second-generation 5-HT$_3$ receptor antagonist if the use of antiemetic agents is necessary.

CONFLICT OF INTEREST

All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

M.G. and Y.I. wrote the manuscript. M.G., Y.I., and K.I. designed the research. M.K., T.Y., A.Y., Y.M., T.N., and M.C. performed the research. M.G., Y.Z., F.A., H.H., N.O., K.Y., and M.C. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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