first multiplex genotyping analysis of patients with advanced NSCLC enrolled in a phase III clinical trial. Such an approach will be important for future evaluation of the clinical impact of specific genetic alterations and predictive biomarkers. Our data indicate that MassARRAY-based multiplex genetic testing both for somatic mutations and for *ALK*, *ROS1*, and *RET* fusion genes performed well with nucleic acid (DNA and RNA) extracted from FFPE tumor specimens obtained from patients with advanced NSCLC.

METHODS

Patients and sample collection

The design and results of the LETS study have been described previously [19,20]. In brief, the study subjects comprised patients aged 20 to 74 years with a histopathologic diagnosis of stage IIIB or IV NSCLC, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and preserved function of major organ systems. They had not previously received chemotherapy, and they were randomly assigned in a 1:1 ratio to treatment with either carboplatin plus S-1 or carboplatin plus paclitaxel. The present study was designed retrospectively after completion of the first interim analysis of the LETS trial and was approved by the institutional ethics committee of each of the participating institutions. Archival FFPE tumor specimens were collected for diagnosis from the participants of the LETS study at 22 centers and were shipped to Kinki University Faculty of Medicine.

Sample processing

The collected FFPE specimens underwent histological review, and only those containing sufficient tumor cells as revealed by hematoxylin-eosin staining were subjected to nucleic acid extraction. DNA and RNA were purified with the use of an Allprep DNA/RNA FFPE Kit (Qiagen, Valencia, CA). The isolated RNA was subjected to reverse transcription with the use of a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The DNA and RNA samples were analyzed in the following order of priority: (1) multiplex analysis of somatic gene mutations (LungCarta Panel; Sequenom, San Diego, CA), (2) quantitative analysis of gene expression (results to be described elsewhere), and (3) characterization of *ALK*, *ROS1*, and *RET* fusion genes (LungFusion Panel).

Mutation detection by mass spectrometry

The genes in the LungCarta Panel are listed in Supplementary Table S1. Multiplex PCR was performed in a volume of 5 μL containing 1 U of Hotstart Taq polymerase (Sequenom), 1.1 to 10 ng of genomic DNA, the LungCarta PCR primer pool(Sequenom), and 500 μmol of each deoxynucleoside triphosphate (dNTP). The PCR protocol included incubation at 95°C for 15 min; 45 cycles of incubation at 94°C for 20 s, 56°C for 30 s, and 72°C for 60 s; and a final incubation at 72°C for 3 min. Unincorporated dNTPs were deactivated by incubation with 0.5 U of shrimp alkaline phosphatase (Sequenom) at 37°C for 40 min, after which the enzyme was inactivated by incubation for 5 min at 85°C. Single-base primer extension was performed with the LungCarta extension primer pool (Sequenom), 0.2 μL of mass-modified dNTPs (Sequenom), and 1.15 U of Thermosequenase enzyme (Sequenom). The extension protocol included incubation at 94°C for 30 s; 60 cycles of incubation at 94°C for 5 s, 52°C for 5 s, and 80°C for 5 s; and a final incubation at 72°C for 3 min. After the addition of a cation-exchange resin to remove residual salt followed by 41 μL of water, the extension products were spotted onto a matrix pad (3-hydroxypicolinic acid) of a SpectroCHIP II (Sequenom) for analysis with a Bruker MALDI-TOF mass spectrometer. Spectra were processed with SpectroREADER software (Sequenom) and transferred to the MassARRAY Typer 4 Analyzer (Sequenom) for further analysis.

Fusion gene detection by mass spectrometry

PCR and extension primers were designed to specifically amplify the breakpoint junction regions for 20 types of fusion gene (Supplementary Tables S3–S5) with the use of MassARRAY Assay Designer 3.1 (Sequenom). The detection technique has been described previously.[25] Reverse-transcribed cDNA was subjected to PCR in a volume of 5 μL containing 1 U of Taq polymerase (Sequenom), 500 μmol of each dNTP, and 200 nmol of each PCR primer. The PCR protocol included incubation at 95°C for 15 min; 45 cycles of incubation at 94°C for 20 s, 56°C for 30 s, and 72°C for 60 s; and a final incubation at 72°C for 3 min. Unincorporated dNTPs were deactivated by incubation with 0.5 U of shrimp alkaline phosphatase (Sequenom) at 37°C for 40 min, after which the enzyme was inactivated by incubation for 5 min at 85°C. Single-base primer extension was performed with the LungFusion extension primer pool (depending on the mass), 0.2 μL of mass-modified dNTPs (Sequenom), and 1 U of iPLEX enzyme (Sequenom). The extension protocol included incubation at 94°C for 30 s; 40 cycles of incubation at 94°C for 5 s, 52°C for 5 s, and 80°C for 5 s; and a final incubation at 72°C for 3 min. After the

addition of a cation-exchange resin to remove residual salt followed by 41 μL of water, the extension products were spotted onto a matrix pad (3-hydroxypicolinic acid) of a SpectroCHIP II (Sequenom) for analysis with a Bruker MALDI-TOF mass spectrometer. Spectra were processed with SpectroREADER software (Sequenom) and then transferred to the MassARRAY Typer 4 Analyzer (Sequenom) for further analysis.

Control vectors containing fusion sequences were constructed by In-Fusion PCR cloning (Clontech, Palo Alto, CA), with the exception of those for *EML4-ALK*, which were constructed as described previously [24]. Data analysis was performed with MassARRAY Typer software, version 4.0 (Sequenom). Positive samples were confirmed by subcloning and sequencing with the pTA2 vector (Toyobo, Osaka, Japan) and M13 universal primers.

FISH

FISH was performed to determine *MET* copy number in FFPE tumor specimens with the use of a c-Met/CEN7p Dual Color FISH Probe (GSP Laboratory, Kawasaki, Japan), where CEN7p is the centromeric region of chromosome 7p. After screening of all sections, images of tumor cells were captured and recorded, and the signals for at least 50 random nuclei were counted for an area in which individual cells were recognized in each of at least 10 representative images. Nuclei with a disrupted boundary were excluded from the analysis. Gene amplification was strictly defined on the basis of a mean *MET*/CEN7p copy number ratio of >2.2, as previously described (30). Polysomy or an equivocal *MET*/CEN7p ratio (1.8 to 2.2) was thus scored as negative for amplification.

Statistical analysis

OS in patients for each biomarker analysis was estimated with the Kaplan-Meier method and analyzed with a Cox proportional-hazard model. Differences in OS between genotypes were evaluated with the log-rank test. All statistical analysis was performed with SAS for Windows, release 9.2 (SAS Institute, Cary, NC), and JMP software (version 10, SAS Institute). A P value of <0.05 was considered statistically significant.

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Chemotherapeutic drugs that penetrate the blood–brain barrier affect the development of hyperactive delirium in cancer patients

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ABSTRACT

Objective: Delirium is a frequently encountered psychiatric disease in terminal cancer patients. However, the mechanism of delirium is unclear. The aim of our study was to investigate the relationship between administration of chemotherapy drugs that penetrate the blood–brain barrier (BBB) and the development of delirium in cancer patients.

Method: We retrospectively analyzed 166 cancer patients (97 males, 69 females) continuously who died between September of 2007 and January of 2010 using a review of medical charts. Multiple logistic regression analysis was employed to investigate the effects of antineoplastic drugs penetrating the BBB on development of delirium in cancer patients with control for other risk factors.

Results: In multivariate analysis, antineoplastic drugs that penetrated the BBB were significantly associated with development of delirium ($OR = 18.92$, $CI_{95} = 1.08–333.04$, $p < 0.001$).

Significance of results: The use of chemotherapy drugs that penetrate the BBB may be a risk factor for delirium. This information may allow palliative care doctors and medical oncologists to predict which patients are at increased risk for delirium.

KEYWORDS: Cancer patients, Chemotherapeutic drugs, Delirium, Blood–brain barrier, P glycoprotein

INTRODUCTION

Delirium is a frequent neurological complication in hospitalized cancer patients (Clouston et al., 1992) and occurs in up to 85% of cancer patients during their last weeks of life (Massie et al., 1983). The probability of developing delirium is determined by the combined effects of predisposing or vulnerability factors such as age and previous cognitive dysfunction or dementia; incident factors such as drug toxicity and metabolic abnormalities; and other conditions that are often associated with the severity of the underlying illness (Inouye et al., 1993; American Psychiatric Association., 2000). However, the mechanism of delirium remains unclear.

Metaanalyses have shown that women who undergo adjuvant chemotherapy for breast cancer may experience a subtle yet consequential cognitive

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decline (Falleti et al., 2005; Stewart et al., 2006). Thus, cancer patients who receive chemotherapy are at high risk of a treatment-induced decrease in cognitive function. However, few studies have investigated development of delirium following chemotherapy. Furthermore, most such studies are case reports or have no control group and are limited to specific antineoplastic drugs.

Chemotherapeutic agents including methotrexate, fluorouracil, vincristine, vinblastine, bleomycin, bischloronitrosourea, cisplatin, ifosfamide, interferon, asparaginase, and procarbazine have been reported to cause delirium in single case reports or studies with small populations (Brunner & Young, 1965; Holland et al., 1974; Stolinsky et al., 1974; Greenwald, 1976; Yamada et al., 1979; Berman & Mann, 1980; Priestman, 1980; Heim et al., 1981; Silberfarb, 1983). Some larger studies showed the possibility of developing delirium due to the use of vincristine and vinblastine, as well as combinations of vincristine and high-dose methotrexate plus citrovorum factor rescue (Frei et al., 1961; Holland et al., 1973; Allen & Rosen, 1978).

In an evaluation of the records of 100 consecutive hospitalized cancer patients referred for psychiatric consultation, another study found that delirium was frequently misdiagnosed as depression, was not recognized, or was recognized but undertreated (Levine et al., 1978). To avoid missing an organic brain syndrome, the importance of examining the mental status of all patients as a routine procedure was emphasized. However, this study did not take into account a possible relationship between chemotherapy and delirium.

Aging, systemic diseases, and ischemic brain injury can disrupt the blood–brain barrier (BBB) and result in a decline in overall BBB function and integrity, as shown by Zeevi and coworkers (2010). Their evidence linked deficits in the cerebral microvasculature and BBB integrity with dementia, medication-related cognitive decline, white matter disease, and related geriatric syndromes (including delirium and gait disorders). Temozolomide, lapatinib, topotecan, nitroso derivatives, tamoxifen, idarubicin and methotrexate can penetrate the BBB (Lin et al., 2004; Wong & Berkenblit, 2004), and capecitabine has been shown to cause changes in the brain by penetrating the BBB (Ekenel et al., 2007). However, the relationship between delirium and the use of chemotherapy drugs that penetrate the BBB has not been examined sufficiently. Therefore, the objective of our study was to investigate the effects of chemotherapy, in particular with agents that penetrate the blood–brain barrier, and other risk factors on the development of delirium in cancer patients.

DESIGN

We retrospectively analyzed continuous data for 166 cancer patients (97 males, 69 females) who were hospitalized and died at the palliative care unit at Kinki University Sakai Hospital between September of 2007 and January of 2010. Patients were ineligible if they had cognitive dysfunction (e.g., dementia). Two psycho-oncologists reviewed medical charts and diagnosed delirium according to DSM–IV–TR criteria. The effects of anticipated risk factors on development of delirium in cancer patients were investigated. Utilization of hormone therapy, a molecular-targeted drug, and an antineoplastic agent were considered to be chemotherapy. Patients treated with an antineoplastic drug as neoadjuvant or adjuvant therapy were also included in the chemotherapy group, and those treated at least once with a drug that penetrates the BBB were placed in the BBB group (Lin et al., 2004; Wong & Berkenblit, 2004; Ekenel et al., 2007).

Patients exposed to corticosteroids at daily doses greater than 15 mg had a 2.7-fold increase in the risk of developing delirium, compared with patients exposed to smaller doses (Gaudreau et al., 2005). Therefore, daily use of betamethasone in doses larger than 2 mg (almost equal effect to 15 mg corticosteroids) to treat an illness was considered "steroid use," but steroid treatment employed in combination with chemotherapy was defined as "non-steroid use," because daily steroid doses were not larger than 15 mg.

Many potentially important delirium risk factors within one week of onset of delirium were included in our analysis, including infections, anemia, and metabolic abnormalities (hepatic function, renal function, electrolyte imbalance, and dehydration), hypooxgenation, and intracranial disease. Patients with a specific cause of delirium and those with delirium induced by medication, such as that occurring immediately after opioid treatment (within one week before), were excluded to investigate the effects of chemotherapy alone.

Clinically, it is difficult for medical staff such as nurses and oncologists without expertise in delirium to discern hypoactive delirium. Therefore, we limited our study to hyperactive and mixed-type delirium. We also excluded cases of delirium occurring within two weeks before death, because it is difficult to identify a single cause of delirium in an end-term cancer patient (Lawlor et al., 2000).

Our study was approved by the institutional review board of the Kinki University Faculty of Medicine. Because this was a retrospective study using variables obtained during routine clinical practice, written informed consent was not required according

to the ethics guidelines for epidemiological studies developed by the Japanese Ministry of Labor, Health, and Welfare. Instead, the study was disclosed on the website of Kinki University Hospital built to receive requests for withdrawal from the study by a patient's family.

Measurements

Logistic regression analysis was performed using univariate and multivariate models with development of delirium as the dependent variable. Age, sex, use of steroids, use of opioids, use of antineoplastic drugs penetrating the BBB, use of antineoplastic drugs not penetrating the BBB, and ECOG performance status were included as independent variables. A two-sided significance level of 0.05 was utilized. All statistical analyses were conducted using SPSS software (v. 19.0; SPSS Japan Inc., Tokyo).

Table 1. *Demographic and clinical characteristics of patients* (n = *166)*

Variable	Data	
Age (years)[a]	68.4 ± 11.6	
Gender		
Male	97	58%
Female	69	42%
Use of steroid drugs		
Yes	116	70%
No	50	30%
Use of opioid drugs		
Yes	107	64%
No	59	36%
Use of psychotropic drugs		
Yes	56	34%
No	110	66%
Use of antiepileptic drugs		
Yes	49	30%
No	117	70%
Undergoing chemotherapy		
Yes	114	69%
No	52	31%
Development of delirium		
Yes	58	35%
No	108	65%
ECOG performance status		
1 to 2	132	80%
3 to 4	34	20%
Days from initiation of chemotherapy to development of delirium[b]	570	244–1262
Primary tumor site		
Lung	41	24.7%
Stomach	29	17.5%
Colon	29	17.5%
Breast	21	12.7%
Pancreas	9	5.4%
Urological	8	4.8%
Gynecological	7	4.2%
Liver	6	3.6%
Neck	4	2.4%
Gall bladder duct	3	1.8%
Esophagus	2	1.2%
Unknown	3	1.8%
Other	4	2.4%

Data are shown as a number and percentage, unless indicated as [a] mean ± *SD*, [b] median and interquartile range.

RESULTS

Patient Characteristics

The demographic, disease, and treatment information for the 166 cancer patients are shown in Table 1. Characteristics were assessed at time of death. Performance status was documented at the first medical examination. Some 114 patients received chemotherapy. The drugs employed for chemotherapy are shown in Table 2.

Risk Factors for Delirium (see Table 3)

In the multiple logistic regression model, antineoplastic drugs that penetrate the BBB were significantly associated with development of delirium (odds ratio, 18.92; CI_{95} = 1.08–333.04; $p < 0.001$). Patients suffering from metabolic abnormalities and dehydration were also significantly more likely to develop delirium in the multivariate model.

DISCUSSION

The present study demonstrated that chemotherapy with agents that penetrate the BBB may be a risk factor for development of delirium in cancer patients. There is growing evidence in the medical literature for increased incidence of cognitive decline—so-called "chemobrain" or "chemofog"—in cancer

Table 2. *Agents used in the 114 treatment groups*[a]

Variable	BBB	Number
Carboplatin/cisplatin	Nonpenetrating	63
Taxane	Nonpenetrating	60
Irinotecan	Nonpenetrating	34
Oxaliplatin	Nonpenetrating	27
Fluorouracil/S-1	Nonpenetrating	26
Capecitabine	Penetrating	26
Gemcitabine	Nonpenetrating	23
Anthracycline	Nonpenetrating	20
Vinorelbine	Nonpenetrating	12
Topotecan	Penetrating	5
Others	Penetrating	3
	Nonpenetrating	7

[a] Some patients received multiple drugs. BBB = blood–brain barrier.

Table 3. *Results of multiple logistic regression analysis*

Variable	Univariate Model			Multivariate Model		
	Odds Ratio	95% Confidence Interval	*p* Value	Odds Ratio	95% Confidence Interval	*p Value*
Age (years)						
<70	1.0			1.0		
≥70	1.07	0.56–2.02	0.884	1.46	0.49–4.39	0.496
Gender						
Female	1.0			1.0		
Male	1.29	0.67–2.47	1.288	1.21	0.39–3.75	0.740
Use of steroid drugs						
No	1.0			1.0		
Yes	1.21	0.60–2.44	0.602	1.08	0.31–3.82	0.90
Use of opioid drugs						
No	1.0			1.0		
Yes	4.69	2.10–10.50	<0.001	2.85	0.82–9.90	0.100
Use of psychotropic drugs						
No	1.0			1.0		
Yes	5.54	2.75–11.17	<0.001	1.71	0.58–5.03	0.328
Use of antiepileptic drugs						
No	1.0			1.0		
Yes	10.22	4.73–22.06	<0.001	3.42	0.58–20.01	0.173
Use of antihistamine drugs						
No	1.0			1.0		
Yes	7.47	2.96–18.86	<0.001	1.28	0.14–11.37	0.827
Hypooxgenation						
No	1.0			1.0		
Yes	3.46	1.68–7.14	<0.001	0.93	0.28–3.13	0.933
Metabolic abnormalities (electrolyte imbalance, hepatic dysfunction, renal dysfunction etc)						
No	1.0			1.0		
Yes	3.12	1.61–6.06	0.001	4.30	1.43–12.96	0.009
Infections						
No	1.0			1.0		
Yes	9.58	4.07–22.54	<0.001	2.83	0.79–10.12	0.112
Dehydration (BUN/Cr ratio > 20)						
No	1.0			1.0		
Yes	8.76	4.17–18.38	<0.001	5.16	1.83–14.59	0.002
Anemia						
No	1.0			1.0		
Yes	6.71	3.14–14.38	<0.001	2.83	0.11–3.69	0.612
Intracranial disease (brain metastases etc)						
No	1.0			1.0		
Yes	13.68	4.39–42.62	<0.001	3.16	0.56–17.82	0.193
ECOG performance status						
PS1-2 ($n = 132$)	1.0			1.0		
PS3-4 ($n = 34$)	1.16	0.52–2.58	0.72	1.05	0.24–4.56	0.947
No chemotherapy						
($n = 52$)	1.0			1.0		
Chemotherapy with drugs that do not penetrate the blood–brain barrier						
($n = 83$)	3.70	1.41–9.72	0.008	2.58	0.24–27.19	0.432
Chemotherapy with drugs that penetrate the blood–brain barrier						
($n = 31$)	31.94	9.32–109.50	<0.001	18.92	1.08–333.04	<0.001

survivors that results from chemotherapy (Argyriou et al., 2010). A study by Wefel and colleagues (2004) showed that at 3 weeks postchemotherapy, 61% of participants experienced a decline in certain cognitive skills, including verbal and visual memory, executive function, visuospatial ability, and information-processing speed. A prospective, multicenter, longitudinal study using 12 neuropsychological tests showed that chemotherapy-induced cognitive impairment affected 27% of 101 patients with breast cancer after neoadjuvant chemotherapy (Hermelink et al., 2007). Another review showed that impairment induced by chemotherapy significantly affected visual memory only (Jansen et al., 2005). These studies investigated the association between chemotherapy and slight cognitive dysfunction detected

by specialized tests. However, prior to our current study, descriptions of delirium after chemotherapy have been limited to case reports. Few macromolecules are transferred into the brain because vesicular transcytosis in the endothelial cells is considerably limited, and the tight junction is located between the endothelial cells. In addition, there are several types of influx or efflux transporters at the BBB, such as P-glycoprotein (P-gp), multidrug resistance-associated protein, and breast cancer-resistant protein (Cordon-Cardo et al., 1989; Ueno, 2009). The reason for the developing delirium might be a disruption of the BBB that leads chemotherapy drugs into brain tissue and results in accumulation of high levels of these drugs within the brain.

There are several limitations of our study. First, this study was not prospective but retrospective in design and employed chart review. The diagnosis of delirium could thus be unreliable, a factor that could not be overcome. Second, we did not investigate differences among duration of illness, presence of comorbidities, duration of the use of drugs, and the patient's psychosocial background (e.g., educational level and employment status). Third, several potentially important delirium risk factors were not taken into account in the analysis, including opioid dose and pain. Fourth, exposure to chemotherapy was not sufficiently examined, with no information included on dose, duration, and route, all of which may have had an impact on delirium onset. Fifth, the temporal link between chemotherapy and delirium that occurs 570 days later may be uncertain. Finally, we limited the study to cases of hyperactive and mixed-type delirium because of the difficulty involved in diagnosing hypoactive delirium by general medical staff. Further studies are needed to clarify the effects of these factors.

In conclusion, our findings suggest that chemotherapy agents that penetrate the BBB can be a risk factor for development of delirium. This information may allow palliative care doctors and medical oncologists to predict which patients are at increased risk of developing delirium.

DISCLOSURE STATEMENT

The authors have no competing financial interests to declare.

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Risk Factors for Cisplatin-Induced Nephrotoxicity and Potential of Magnesium Supplementation for Renal Protection

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Abstract

Background: Nephrotoxicity remains a problem for patients who receive cisplatin chemotherapy. We retrospectively evaluated potential risk factors for cisplatin-induced nephrotoxicity as well as the potential impact of intravenous magnesium supplementation on such toxicity.

Patients and Methods: We reviewed clinical data for 401 patients who underwent chemotherapy including a high dose (≥ 60 mg/m^2) of cisplatin in the first-line setting. Nephrotoxicity was defined as an increase in the serum creatinine concentration of at least grade 2 during the first course of cisplatin chemotherapy, as assessed on the basis of National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. The severity of nephrotoxicity was evaluated on the basis of the mean change in the serum creatinine level. Magnesium was administered intravenously to 67 patients (17%).

Results: Cisplatin-induced nephrotoxicity was observed in 127 patients (32%). Multivariable analysis revealed that an Eastern Cooperative Oncology Group performance status of 2 (risk ratio, 1.876; P = 0.004) and the regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) (risk ratio, 1.357; P = 0.047) were significantly associated with an increased risk for cisplatin nephrotoxicity, whereas intravenous magnesium supplementation was associated with a significantly reduced risk for such toxicity (risk ratio, 0.175; P = 0.0004). The development of hypomagnesemia during cisplatin treatment was significantly associated with a greater increase in serum creatinine level (P = 0.0025). Magnesium supplementation therapy was also associated with a significantly reduced severity of renal toxicity (P = 0.012).

Conclusions: A relatively poor performance status and the regular use of NSAIDs were significantly associated with cisplatin-induced nephrotoxicity, although the latter association was marginal. Our findings also suggest that the ability of magnesium supplementation to protect against the renal toxicity of cisplatin warrants further investigation in a prospective trial.

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Introduction

Cisplatin (*cis*-diammine-dichloroplatinum), an inorganic platinum chemotherapeutic drug, has been widely administered either alone or in combination with other agents for the clinical treatment of various solid tumors [1]. The efficacy of cisplatin is limited, however, by severe side effects such as nephrotoxicity, neurotoxicity, ototoxicity, and emetogenicity [2,3]. In particular, the nephrotoxicity of cisplatin is dose dependent and therefore limits the amount of drug that can be administered [4]. Procedures to reduce such toxicity include aggressive hydration with saline and simultaneous administration of mannitol, which is now accepted as the standard of care for individuals treated with regimens containing a high dose (≥60 mg/m^2) of cisplatin [5]. Unfortunately, renal toxicity still occurs even with such hydration, highlighting the need for more effective preventive strategies.

Another approach to limiting the nephrotoxicity of cisplatin is intravenous magnesium supplementation. Cisplatin-induced nephrotoxicity is accompanied by disturbance of the renal handling of

electrolytes. In particular, depletion of magnesium has emerged as a common event associated with the acute renal toxicity induced by the drug [6]. Whereas several studies have demonstrated the efficacy of magnesium supplementation for prevention of hypomagnesemia during cisplatin treatment [7–10], only two prospective studies, each featuring a relatively small number of patients, have evaluated its efficacy in terms of protection against cisplatin-induced nephrotoxicity [11,12]. Despite the dearth of evidence in support of a beneficial effect of magnesium supplementation therapy on the renal toxicity of cisplatin, intravenous administration of magnesium is currently recommended for outpatients receiving high-dose cisplatin with a short hydration regimen [13]. We have therefore recently applied this procedure to all patients who receive such chemotherapy. However, given that magnesium supplementation has not been accepted as the standard of care, at least in Japan, most patients who receive high-dose cisplatin are treated with aggressive hydration in the inpatient setting.

We have now assessed a large group of unselected consecutive patients in an attempt to identify potential biological or pharmacological parameters that might predispose individuals to cisplatin-induced nephrotoxicity. We also retrospectively evaluated the potential impact of intravenous magnesium supplementation on this side effect of cisplatin treatment.

Patients and Methods

Eligibility criteria

We reviewed the cases in our database and retrospectively examined the clinical data of patients who received therapy including a high dose ($\geq$60 mg/m^2) of cisplatin in the first-line setting at the Department of Medical Oncology, Kinki University Hospital, between January 2008 and August 2012. Patients were eligible if they had pathologically confirmed malignancies and an Eastern Cooperative Oncology Group performance status (PS) of 0 to 2. Patients were excluded from the study if they had a history of cisplatin treatment or had more than one cancer. The study protocol was approved by the ethics committee of Kinki University Hospital with the condition that all data be processed and analyzed anonymously, and written informed consent was provided by all patients. The study also conforms with the provisions of the Declaration of Helsinki.

Cisplatin administration

All regimens containing high-dose cisplatin were administered in the inpatient setting. Cisplatin was administered in 500 mL of 0.9% normal saline over 1 h. Most patients were prehydrated with 500 mL of one-quarter isotonic saline containing 5% glucose and 20 mEq of KCl, and they were posthydrated with 500 mL of 0.9% normal saline mixed with 500 mL of one-quarter isotonic saline containing 5% glucose, 20 mEq of KCl, and 10 mEq of sodium L-lactate, which was administered over 1 to 2 h and followed by 60 g of mannitol over 1 h and 20 mg of furosemide in 50 mL of 0.9% normal saline over 15 min. Antiemetic prophylaxis with 5-HT$_3$ serotonin receptor antagonists plus dexamethasone was administered 15 min before the onset of chemotherapy in all cases. A neurokinin 1 (NK1) receptor antagonist was added to the antiemetic cocktail from October 2010 in response to the approval of this drug in Japan. Magnesium sulfate (20 mEq) was administered with 500 mL of one-quarter isotonic saline over 1 h after cisplatin administration as magnesium supplementation therapy to all patients from July 2011.

Nephrotoxicity evaluation

According to a previous study [14], we adopted an increase in the serum concentration of creatinine as a measure of nephrotoxicity. The serum creatinine concentration was determined before the first course of cisplatin chemotherapy (baseline value) and weekly during chemotherapy. For evaluation of nephrotoxicity, the increase in the serum creatinine concentration was calculated as the maximum value during the first course of chemotherapy minus the baseline value. Given that the serum creatinine level is a denominator of the Cockcroft-Gault equation, changes in creatinine clearance over a short period are solely dependent on those in serum creatinine concentration. Nephrotoxicity was defined as an increase in the serum creatinine concentration of grade 2 or higher, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.0), during the first course of cisplatin chemotherapy.

Statistical analysis

To identify risk factors potentially associated with the occurrence of a nephrotoxicity event, each factor was compared by the unpaired Student's t test or Fisher's exact test. Factors in the analysis included age ($\geq$70 vs. <70 years) and PS (2 vs. 0 or 1), given that chemotherapy might be expected to result in excessive toxicity in patients with an age of $\geq$70 years or a PS of 2 [15]. The other factors were sex (male vs. female), tumor type, concurrent radiation treatment, hypoalbuminemia (serum albumin concentration of <3.0 g/dL), enteral or total parenteral nutrition, type 2 diabetes, hydration ($\leq$2000 mL), intravenous magnesium supplementation, oral intake of magnesium oxide as a laxative agent, use of antihypertensive medication, treatment with an NK1 receptor antagonist, and regular use of nonsteroidal anti-inflammatory drugs (NSAIDs). The risk factors were also evaluated in multivariable analysis with the Poisson regression model. The risk ratio with 95% confidence interval (CI) was calculated for the independent prognostic factors. The mean change in serum creatinine concentration was compared between groups with the use of box-and-whisker plots showing the range (maximum and minimum), median, and quartile range (75 and 25 percentiles) and was evaluated with the unpaired Student's t test. Statistical analysis was performed with the use of SAS software version 9.4 (SAS Institute, Cary, NC). A P value of <0.05 was considered statistically significant.

Results

Patient characteristics

A total of 401 patients who received chemotherapy including high-dose cisplatin were eligible for the analysis. Baseline characteristics of the eligible patients are summarized in Table 1. The median age was 65 years (range, 28–80), and most patients were male (77%) and had a good PS of 0 or 1 (94%). The most common malignancies were lung cancer (36%), head and neck cancer (23%), gastric cancer (19%), and esophageal cancer (16%). Median age, sex, PS, median serum creatinine concentration at baseline, median body surface area, median body mass index, and the median dose of cisplatin in the first course of chemotherapy did not differ significantly among the types of malignancy. The various chemotherapy regimens administered to the patients are shown in Table S1.

Table 1. Baseline characteristics of the 401 study patients.

Characteristic			All patients	Lung cancer	Head and neck cancer	Gastric cancer	Esophageal cancer	Other malignancies
			(*n*=401)	(*n*=144)	(*n*=92)	(*n*=78)	(*n*=65)	(*n*=22)
Sex								
	Male	*n* (%)	308 (77)	107	74	57	54	16
	Female	*n* (%)	93 (23)	37	18	21	11	6
PS								
	0–1	*n* (%)	375 (94)	139	89	67	61	19
	2	*n* (%)	26 (6)	5	3	11	4	3
Baseline Cr (mg/dL)								
	Median		0.69	0.67	0.68	0.73	0.72	0.71
	(range)		(0.23–1.31)	(0.39–1.11)	(0.24–1.15)	(0.23–1.31)	(0.40–1.10)	(0.49–1.08)
BSA (m^2)								
	Median		1.61	1.62	1.56	1.60	1.60	1.60
	(range)		(1.15–2.21)	(1.29–2.21)	(1.29–1.96)	(1.22–1.90)	(1.15–1.87)	(1.28–1.92)
BMI (kg/m^2)								
	Median (range)		21.1 (11.6–35.3)	22.2 (14.9–35.3)	20.9 (11.6–34.0)	20.9 (15.2–33.5)	20.5 (13.4–28.1)	20.6 (16.4–28.8)
Cisplatin dose (mg/m^2)								
	Median		78.0	78.7	80.0	60.0	70.0	79.8
	(range)		(60.0–105)	(60.0–80.3)	(60.0–105)	(60.0–84.0)	(60.0–80.0)	(60.0–100)
Age (years)								
	Median		65	64	62	67	67	64
	(range)		(28–80)	(33–80)	(30–79)	(28–80)	(51–78)	(37–75)
	≥70	*n* (%)	97 (24)	30	21	25	18	3
	<70	*n* (%)	304 (76)	114	71	53	47	19
Concurrent radiation								
	Yes	*n* (%)	167 (42)	45	60	1	50	11
	No	*n* (%)	234 (58)	99	32	77	15	11
Hypoalbuminemia (serum albumin, <3.0 g/dL)								
	Yes	*n* (%)	43 (11)	14	3	18	8	0
	No	*n* (%)	358 (89)	130	89	60	57	22
Enteral nutrition or TPN								
	Yes	*n* (%)	42 (10)	2	20	2	16	2
	No	*n* (%)	359 (90)	142	72	76	49	20
Type 2 diabetes								
	Yes	*n* (%)	99 (25)	39	30	15	11	4

Table 1. Cont.

Characteristic			All patients	Lung cancer	Head and neck cancer	Gastric cancer	Esophageal cancer	Other malignancies
			(*n* = 401)	(*n* = 144)	(*n* = 92)	(*n* = 78)	(*n* = 65)	(*n* = 22)
	No	*n* (%)	302 (75)	105	62	63	54	18
Hydration of ≤2000 mL								
	Yes	*n* (%)	34 (8)	0	23	6	1	4
	No	*n* (%)	367 (92)	144	69	72	64	18
Use of NK1 receptor antagonist								
	Yes	*n* (%)	230 (57)	66	68	46	38	12
	No	*n* (%)	171 (43)	78	24	32	27	10
Intravenous magnesium supplementation								
	Yes	*n* (%)	67 (17)	13	23	16	11	4
	No	*n* (%)	334 (83)	131	69	62	54	18
Oral intake of magnesium oxide as a laxative agent								
	Yes	*n* (%)	164 (41)	56	39	33	28	8
	No	*n* (%)	237 (59)	88	53	45	37	14
Regular use of antihypertensive								
	Yes	*n* (%)	157 (39)	55	44	24	28	6
	No	*n* (%)	244 (61)	89	48	54	37	16
Regular use of NSAIDs								
	Yes	*n* (%)	117 (29)	51	30	18	11	7
	No	*n* (%)	284 (71)	93	62	60	54	15

Drug administration variables refer to the first course of cisplatin chemotherapy. Abbreviations: PS, performance status; Cr, serum creatinine concentration; BSA, body surface area; BMI, body mass index; TPN, total parenteral nutrition; NK1, neurokinin 1; NSAIDs, nonselective nonsteroidal anti-inflammatory drugs.

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Table 2. Comparison of clinicopathologic characteristics as risk factors for cisplatin-induced nephrotoxicity.

Characteristic		Cisplatin nephrotoxicity		*P* value
		Yes (*n* = 127)	No (*n* = 274)	
		n (%)	*n* (%)	
Age (years)				
	Median	65	65	0.524
	(range)	(37–80)	(28–80)	
	≥70	31 (32)	66 (68)	0.944
	<70	96 (32)	208 (68)	
Sex				
	Male	97 (31)	211 (69)	0.899
	Female	30 (32)	63 (68)	
PS				
	0 or 1	111 (30)	264 (70)	**0.002**
	2	16 (62)	10 (38)	
Tumor type				
	Lung	40 (28)	104 (72)	**0.045***
	Head and neck	28 (30)	64 (70)	
	Gastric	23 (29)	55 (71)	
	Esophageal	31 (48)	34 (52)	
	Other	5 (23)	17 (77)	
Concurrent radiation				
	Yes	56 (34)	111 (66)	0.515
	No	71 (30)	163 (70)	
Hypoalbuminemia (serum albumin, <3.0 g/dL)				
	Yes	15 (35)	28 (65)	0.608
	No	112 (31)	246 (69)	
Enteral nutrition or TPN				
	Yes	17 (40)	25 (60)	0.220
	No	110 (31)	249 (69)	
Type 2 diabetes				
	Yes	26 (26)	73 (74)	0.214
	No	101 (33)	201 (67)	
Hydration of ≤2000 mL				
	Yes	13 (38)	21 (62)	0.441
	No	114 (31)	253 (69)	
Use of NK1 receptor antagonist				
	Yes	61 (27)	169 (73)	**0.013**
	No	66 (39)	105 (61)	
Intravenous magnesium supplementation				
	Yes	4 (6)	63 (94)	**<0.0001**
	No	123 (37)	211 (63)	
Oral intake of magnesium oxide as a laxative agent				
	Yes	48 (29)	116 (71)	0.445
	No	79 (33)	158 (67)	
Regular use of antihypertensive				
	Yes	51 (32)	106 (68)	0.826
	No	76 (31)	168 (69)	

Table 2. Cont.

Characteristic		Cisplatin nephrotoxicity		*P* value
		Yes (*n*=127)	No (*n*=274)	
		n (%)	*n* (%)	
Regular use of NSAIDs				
	Yes	44 (38)	73 (62)	0.125
	No	83 (29)	201 (71)	

Drug administration variables refer to the first course of cisplatin chemotherapy. Abbreviations: PS, performance status. TPN, total parenteral nutrition; NK1, neurokinin 1; NSAIDs, nonselective nonsteroidal anti-inflammatory drugs.
*P value for heterogeneity for the occurrence of nephrotoxicity among tumor types. P values of <0.05 are shown in bold.
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Clinicopathologic analysis of risk factors for cisplatin nephrotoxicity

Cisplatin-induced nephrotoxicity was observed in 127 (32%) of the 401 enrolled patients, including 108, 16, and 3 patients with nephrotoxicity of grade 2, 3, and 4, respectively. Among these patients, 55 individuals developed irreversible renal failure. Fisher's exact test revealed that a PS of 2 ($P=0.002$), the absence of intravenous magnesium supplementation ($P<0.0001$), and the lack of treatment with an NK1 receptor antagonist ($P=0.013$) were significantly associated with cisplatin nephrotoxicity (Table 2). We also detected significant heterogeneity in the occurrence of nephrotoxicity among tumor types ($P=0.045$). Examination of the possible impact of concurrent chemotherapy agents on the prevalence of nephrotoxicity (Table S2) revealed no significant association between the use of these agents and such toxicity ($P=0.373$).

Multivariable analysis of risk factors for cisplatin nephrotoxicity

To assess the contribution of each individual risk factor to cisplatin-induced nephrotoxicity, we performed multivariable analysis (Table 3). A PS of 2 (risk ratio, 1.876; 95% CI, 1.229–2.864; $P=0.004$) and regular use of NSAIDs (risk ratio, 1.357; 95% CI, 1.004–1.835; $P=0.047$) were significantly associated with an increased risk for cisplatin nephrotoxicity, whereas intravenous magnesium supplementation (risk ratio, 0.175; 95% CI, 0.066–0.462; $P=0.0004$) was associated with a significantly reduced risk. We also found that esophageal cancer was an independent risk factor for nephrotoxicity compared with lung cancer (risk ratio,

Table 3. Risk ratio in multivariable analysis of potential predisposing factors for cisplatin-induced nephrotoxicity (*n*=401).

Factor		Risk ratio	95% CI	*P* value
Age (≥70 vs. <70 years)		1.006	0.990–1.023	0.475
Sex (male vs. female)		0.947	0.683–1.314	0.745
PS (2 vs. 0 or 1)		1.876	1.229–2.864	**0.004**
Concurrent radiation		1.071	0.769–1.491	0.684
Serum albumin (≥3.0 vs. <3.0 g/dL)		0.897	0.693–1.165	0.419
Enteral nutrition or TPN		0.989	0.643–1.520	0.959
Type 2 diabetes		0.872	0.599–1.270	0.476
Hydration (≤2000 or >2000 mL)		0.801	0.536–1.200	0.283
Use of NK1 receptor antagonist		0.878	0.663–1.163	0.363
Intravenous magnesium supplementation		0.175	0.066–0.462	**0.0004**
Oral intake of magnesium oxide as a laxative agent		0.933	0.703–1.240	0.634
Regular use of antihypertensive		1.010	0.810–1.485	0.553
Regular use of NSAIDs		1.357	1.004–1.835	**0.047**
Tumor type				
	Lung	1.000		
	Head and neck[a]	1.301	0.845–2.010	0.232
	Gastric[a]	1.071	0.678–1.692	0.770
	Esophageal[a]	1.937	1.277–2.940	**0.002**
	Other[a]	0.810	0.360–1.823	0.610

Drug administration variables refer to the first course of cisplatin chemotherapy. Abbreviations: CI, confidence interval; PS, performance status; TPN, total parenteral nutrition; NK1, neurokinin 1; NSAIDs, nonselective nonsteroidal anti-inflammatory drugs.
[a]These risk factors were compared with lung cancer.
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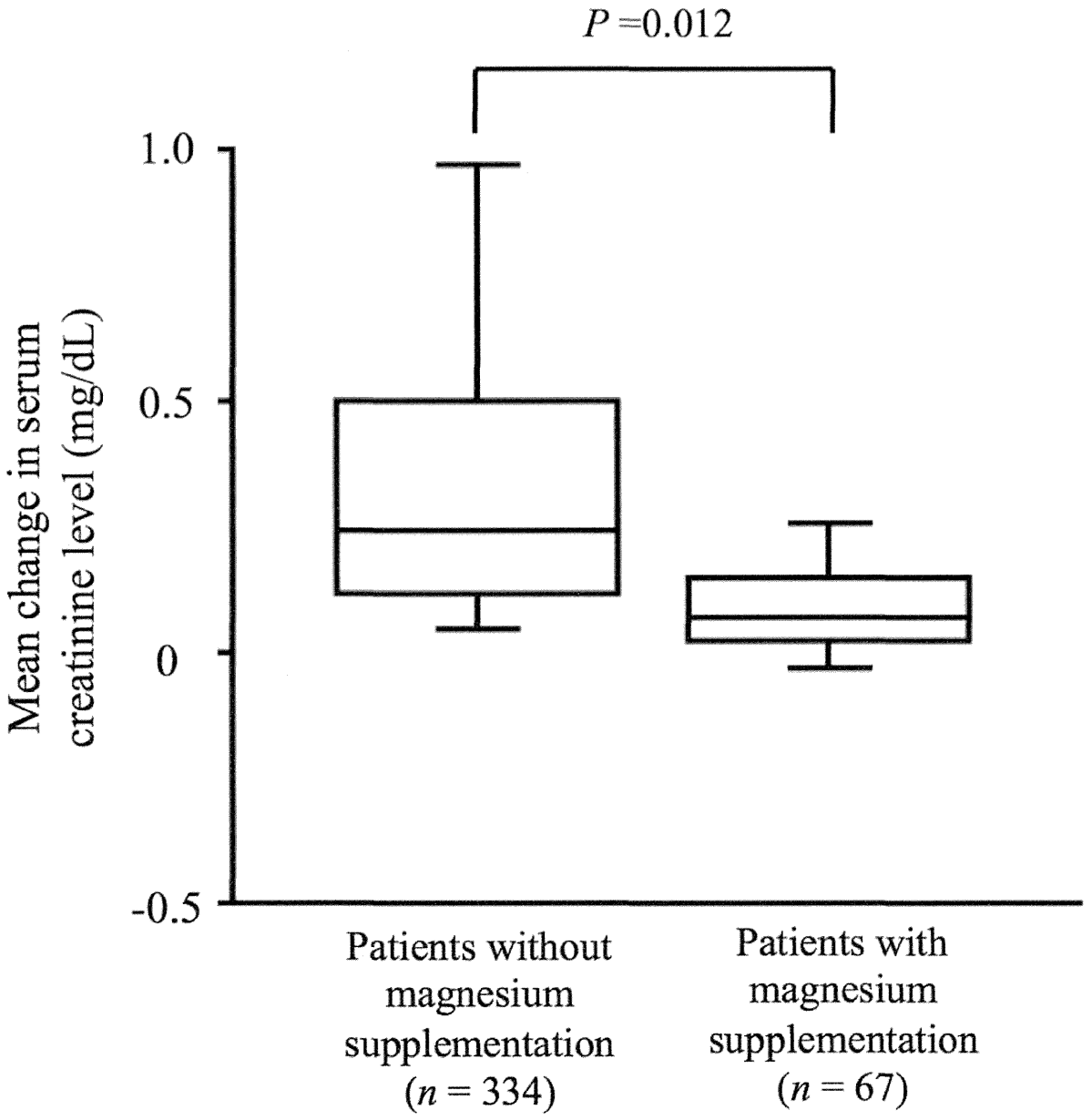

Figure 1. Box-and-whisker plot for the relation between intravenous magnesium supplementation and the mean change in serum creatinine concentration during the first course of cisplatin chemotherapy. The difference between the two groups was analyzed with the unpaired Student's *t* test.
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1.937; 95% CI, 1.277–2.940; $P=0.002$). Exploratory analysis revealed no significant interaction between intravenous magnesium supplementation and other covariates (data not shown).

Effect of magnesium supplementation on serum creatinine levels

As shown in Table 2, we found that the prevalence of cisplatin-induced nephrotoxicity was substantially lower in patients who received intravenous magnesium supplementation than in those who did not (6% vs. 37%). To investigate the effect of magnesium supplementation on cisplatin-induced nephrotoxicity, we evaluated the mean change from baseline in the serum creatinine concentration during the first course of high-dose cisplatin therapy. Patients who received magnesium supplementation therapy ($n=67$) showed a mean change in serum creatinine level of 0.188 ± 0.081 mg/dL (mean ± SE), whereas those who did not receive the treatment ($n=334$) showed a mean change of 0.444 ± 0.043 mg/dL ($P=0.012$), suggesting that magnesium supplementation therapy limited the elevation of serum creatinine level induced by cisplatin (Figure 1). We further examined how magnesium supplementation might prevent cisplatin-induced nephrotoxicity. Data on the serum magnesium concentration

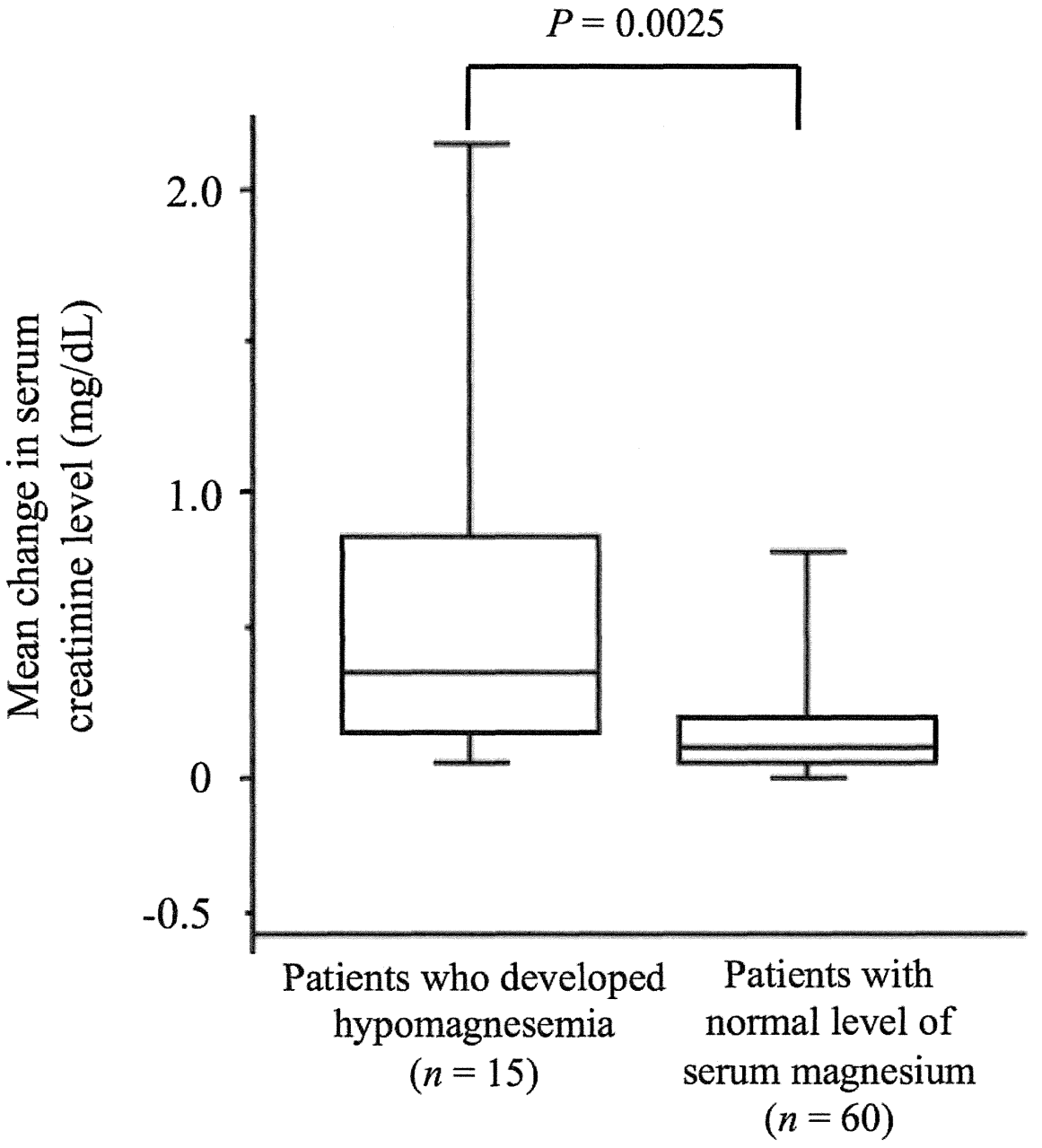

Figure 2. Box-and-whisker plot for the relation between the development of hypomagnesemia and the mean change in serum creatinine concentration during the first course of cisplatin chemotherapy. The difference between the two groups was analyzed with the unpaired Student's *t* test.
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during the first course of cisplatin chemotherapy were available for 75 of the 401 study patients. No patient showed hypomagnesemia at baseline. Among the 52 patients who received magnesium supplementation, 6 individuals (12%) developed hypomagnesemia of grade 1 or worse, whereas 9 (39%) of the 23 patients who did not receive magnesium supplementation developed this condition ($P = 0.040$), indicating that magnesium supplementation significantly reduced the proportion of patients who developed hypomagnesemia. Furthermore, the 15 patients who developed hypomagnesemia during cisplatin treatment showed a significantly greater mean increase in the serum creatinine concentration from baseline compared with those who maintained a normal level of serum magnesium ($P = 0.0025$) (Figure 2). These results suggest that intravenous magnesium supplementation protects against cisplatin-induced nephrotoxicity by preventing hypomagnesemia.

Discussion

Nephrotoxicity remains a clinical problem for 25 to 42% of patients treated with cisplatin [16–18]. In the present study, we found that 32% (127/401) of individuals who received cisplatin at a dose of at least 60 mg/m^2 developed acute nephrotoxicity despite the adoption of conventional measures of hydration and osmotic diuresis. Although the nephrotoxicity was transient and reversible in most cases, 43% (55/127) of the patients with acute nephrotoxicity went on to develop irreversible renal failure. These results indicate that the conventional prophylactic procedures were not sufficient to prevent cisplatin-induced nephrotoxicity in a subset of patients.

We found that magnesium supplementation therapy was significantly associated with both a reduced frequency and reduced severity of renal toxicity, consistent with previous observations [11,12]. Cisplatin treatment results in a substantial increase in magnesium excretion [19–21], with this effect being apparent even before the onset of overt renal toxicity [22], and hypomagnesemia is associated with cisplatin-induced nephrotoxicity [23]. In the present study, a decrease in the serum magnesium concentration was observed in 20% of patients and was significantly associated with renal toxicity during the first course of cisplatin treatment. Organic cation transporter 2 (OCT2) has been implicated in cisplatin nephrotoxicity in a study with isolated human proximal tubules [24], and hypomagnesemia results in up-regulation of OCT2 and thereby increases the renal accumulation of cisplatin and exacerbates acute kidney injury in an animal model [25]. These various findings suggest that magnesium supplementation protects against cisplatin-induced nephrotoxicity, likely by preventing hypomagnesemia, a notion that warrants validation in a prospective study. The dosage of magnesium sulfate for such supplementation therapy has varied widely in previous studies, ranging from 8 to 60 mEq [9–11,13,26,27], and it therefore remains to be standardized in future trials.

To assess the potential risk factors for cisplatin-induced nephrotoxicity, we performed multivariable analyses. Consistent with previous results [14,28], we found that a poor PS was associated with an increased risk for cisplatin nephrotoxicity. This finding underscores the notion that patients with a PS of 2, which is characterized by an increased risk for severe toxicity in general, need special attention with regard to the potential development of nephrotoxicity during high-dose cisplatin chemotherapy, especially given that such treatment in these patients is controversial [29]. We also found that the regular use of NSAIDs was associated with cisplatin-induced nephrotoxicity. Nonselective inhibition of cyclooxygenases 1 and 2 by NSAIDs attenuates prostaglandin-dependent renal function, including modulation of renal vascular tone and electrolyte and water excretion, in particular during renal stress, as manifested by a reduction in the rate of renal perfusion [30,31]. Such effects of NSAIDs might thus enhance cisplatin-induced nephrotoxicity. Although the significance of the association between the regular use of NSAIDs and cisplatin-induced nephrotoxicity was marginal ($P=0.047$) in our analysis, it is of concern because NSAIDs are commonly administered to manage cancer-related pain [32]. Further investigations are thus warranted to evaluate the potential risk of regular NSAID use during high-dose cisplatin chemotherapy.

With regard to tumor type, we found that individuals with esophageal cancer were at a significantly higher risk for cisplatin-induced nephrotoxicity than were those with lung cancer. To our knowledge, such an association has not previously been described. The median dosage of cisplatin in patients with esophageal cancer was 70 mg/m^2, which was not higher than that overall (78 mg/m^2). Moreover, whereas most patients with esophageal cancer in our analysis were treated with cisplatin together with 5-fluorouracil as the standard care, this regimen was also administered to patients with gastric or head and neck cancer. A difference in dosage or in the combination of chemotherapeutic agents thus could not account for the difference in nephrotoxicity among the malignancies. Caution is necessary in the interpretation of this finding, however, with further study being warranted to determine the mechanism of renal toxicity apparent selectively in patients with esophageal cancer.

Limitations of the present study include possible selection bias of treatment, which is inevitable in a retrospective analysis, and a small sample size for patients with a known serum magnesium concentration and for those who received intravenous magnesium supplementation. Even though all patients treated after July 2011 received magnesium sulfate regardless of their characteristics, cohort effects may still be present that influence the association between magnesium supplementation and nephrotoxicity. In addition, we could not fully assess the incidence and intensity of nonhematologic toxicities in our study as a result of its retrospective nature. Such toxicities, including nausea, vomiting, and diarrhea, might be associated with an increased risk for cisplatin-induced nephrotoxicity. Furthermore, comorbidities relevant to inherent nephrotoxicity, such as proteinuria, hypocalcemia, and renal tubular acidosis, were not assessed in the present study.

In conclusion, our data have revealed a significant association of cisplatin-induced nephrotoxicity with a relatively poor PS and, to a lesser extent, with the regular use of NSAIDs. Our findings also suggest that magnesium supplementation might be effective for protection against the renal toxicity of cisplatin, a conclusion that should be further addressed in a prospective trial.

Supporting Information

Table S1 Chemotherapy regimens according to tumor type.
(DOCX)

Table S2 Association between concurrent chemotherapy agents and the occurrence of nephrotoxicity.
(DOCX)

Author Contributions

Conceived and designed the experiments: H. Kawakami. Contributed reagents/materials/analysis tools: H. Kawakami YC. Wrote the paper: YK H. Kawakami JT YC. Collected the data: YK. Contributed study materials or patients: YK H. Kawakami TS KO KT MT S. Nishina JT KF MN YY S. Nishida TT KN. Analyzed and interpreted the data: YK H. Kawakami YC. Administrative support: S. Nishida TT KN. Contributed in critical revision of the manuscript for important intellectual content: YK H. Kawakami TS KO KT MT H. Kaneda S. Nishina JT KF MN YY YC S. Nishida TT KN.

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