Table 1. Participant characteristics.

Number	26
Age	
Average [range]	63.8 [51-78]
Gender	
Male/female	21/5
Histology	
Epi/Sar/Bi	21/4/1
Stage	
I/II/III/IV	1/5/8/12
Regimen	
CDDP + Pemetrexed	18
CDDP + Gemcitabin	2
CBDCA + Gemcitabin	5
Pemetrexed	1
Response	
PR	5
SD	10
PD	11
Response rate	19.2%
N-ERC [ng/mL]	
Average [range]	21.19 [1.58-97.54]

Abbreviation: CDDP, cisplatin; CBDCA, carboplatin; Epi, epitheloid; Sar, sarcomatoid; Bi, biphasic; PR, partial response; SD, stable disease; PD, progressive disease.

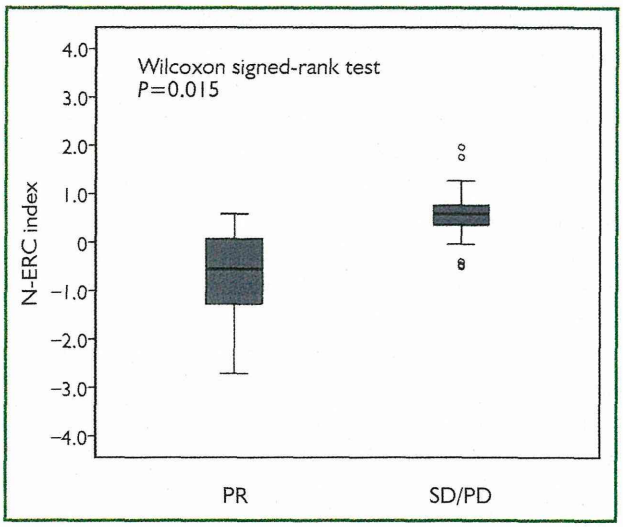

Figure 1. The comparison of the N-ERC index between PR patients and SD/PD patients. The N-ERC index was calculated by Log_2 (N-ERC level after 2 courses of chemotherapy level /N-ERC level prior to chemotherapy) P=0.015.

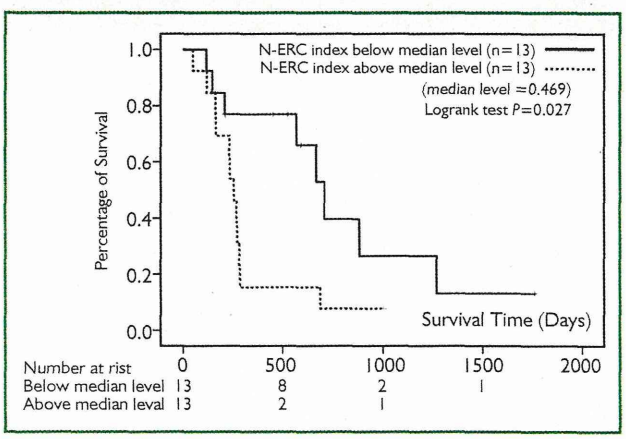

Figure 2. The comparison of the overall survival between the patients whose N-ERC index were below the median level and those whose N-ERC index were above the median level. The horizontal bar indicates the survival time (days), while the vertical bar indicates the percentage of survival. The median value of N-ERC index is 0.469. P=0.027.

marker (4) and useful monitoring marker for MPM (12). In this study, we employed a new index, "N-ERC index", which is calculated by Log_2 (N-ERC value after 2 courses of chemotherapy/N-ERC value prior chemotherapy). The reason why logarithmic transformation was applied in our study is due to the fact that a wide variance in the N-ERC baseline level can be adjusted and also the ratio of N-ERC change before and after chemotherapy can be more accurately evaluated by logarithmic transformation. This mathematical method was adopted from the previous report by Vollmer RT *et al.* (14). We demonstrated that the N-ERC index in patients with PR is significantly lower than that in patients with SD/PD. This result is consistent with our previous report (12). In addition, we also showed that patients whose N-ERC index is below 0.469 (median N-ERC value) survived significantly longer than those whose N-ERC index is over 0.469 (median N-ERC value). These results indicated that N-ERC could be a novel and useful marker for predicting not only the chemotherapeutic response, but also the survival at the time that chemotherapy is evaluated. Interestingly, the low N-ERC level group included 4 SD patients and 5 PD patients. One of the possible reasons for this could be due to difficulties in evaluating tumor reduction based on the Modified RECIST criteria. This finding may also suggest that there could be a deviation between the therapeutic response and prognosis. However, further validation of our findings by a large scale study is needed because our sample size is too small to make any definitive conclusions.

In general, patients with MPM who are subjected to chemotherapy tend to be elderly and fragile because of age-related comorbidities. Therefore, predicting the patients' prognosis after 2 courses of chemotherapy is extremely important. Although the performance status after 2 courses

of chemotherapy deteriorates in certain patients, the patients whose N-ERC index is quite low were found to be able to survive longer than those with a high N-ERC index.

There are several limitations associated with our study. First, our study was a kind of pilot study comprising 26 patients. Secondly, it included patients with a variety of stages and chemotherapeutic regimens. Therefore our small study could not lead to any definitive conclusions, and further validation is therefore required in order to establish the N-ERC index as a valid biomarker for MPM.

In conclusion, we herein demonstrated the serum N-ERC level to correlate with the therapeutic effect of chemotherapy and that the N-ERC index could be associated with the overall survival. We designated the relative N-ERC change ratio as the "N-ERC index". Our novel biomarker could therefore be an innovative tool for determining disease management. Our results suggest that the "N-ERC index" may therefore accurately reflect the therapeutic effect. It may therefore serve as a useful guide for predicting the patient prognosis in MPM after treatment with chemotherapy.

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ORIGINAL ARTICLE

Hydration with magnesium and mannitol without furosemide prevents the nephrotoxicity induced by cisplatin and pemetrexed in patients with advanced non-small cell lung cancer

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ABSTRACT

Background: The aim of this study was to examine the effect of hydration with magnesium and mannitol without furosemide on the nephrotoxocity accompanying combination chemotherapy using cisplatin and pemetrexed in patients with advanced non-small cell lung cancer (NSCLC).

Methods: Fifty patients with NSCLC who received cisplatin plus pemetrexed, using either old hydration protocol including normal saline with mannitol and furosemide, or a new one including normal saline with magnesium and mannitol without furosemide were retrospectively analyzed. Nephrotoxicity was compared between patients treated using the old protocol and those treated with the new protocol. Univariate and multivariate analyses were performed to identify the independent factors associated with protection against nephrotoxicity in patients with NSCLC who received cisplatin plus pemetrexed.

Results: Thirty patients received the old hydration protocol, while 20 patients were treated using the new hydration protocol. The patients treated using the new hydration protocol showed a significantly greater increase in creatinine clearance (P=0.0004) and a decrease in the serum creatinine level (P=0.0148) after one course of chemotherapy compared with those treated using the old hydration protocol. There were no differences in the chemotherapeutic response or overall survival between the groups (P=0.572). The new hydration protocol with supplemented magnesium with mannitol without furosemide was an independent factor for the protection against nephrotoxicity induced by cisplatin and pemetrexed in patients with advanced NSCLC [HR 0.232 (95% CI: 0.055-0.986), P=0.039].

Conclusions: These results demonstrate that the new hydration protocol comprising supplementation with magnesium without furosemide could prevent the nephrotoxicity induced by cisplatin and pemetrexed without affecting the treatment outcome.

KEY WORDS Lung cancer; cisplatin; magnesium; nephrotoxicity; pemetrexed

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Introduction

Lung cancer is a leading cause of death in Japan and other developed countries (1). More than 60% of patients with lung cancer, especially those with non-small cell lung cancer (NSCLC), are inoperable at the time of diagnosis and need to receive chemotherapy containing cisplatin (2). Recently, the percentage of adenocarcinomas among cases of NSCLC has been increasing (3), therefore, regimens containing cisplatin and pemetrexed are expected to be more frequently used (4). Cisplatin is one of the most active and widely used drugs, and still remains a standard component of combination chemotherapy for lung cancer (5). However, nephrotoxicity is a well-known side effect of cisplatin treatment (6). The logistic regression analyses have shown that the risk factors for the development of nephrotoxicity include older age, female gender, current smoking, and hypoalbuminemia (7). The nephrotoxic damage appears to be a clinical problem in 28-42% of patients who receive cisplatin (8). Therefore, many researchers have sought less toxic methods for administering cisplatin. It has been reported that hydration with magnesium supplementation can reduce the nephrotoxocity induced by cisplatin (8,9). In contrast,

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	Old regimen		time		New regimen		time
Pre hydration	①	Normal saline 500 mL		①	ST1® 500 mL		2 hr
		metoclopramide 10 mg	2 hr	②	ST3® 500 mL		
	②	ST3®* 500 mL	2 hr		$MgSO_4$ 8 mEq		2 hr
	③	Normal saline 100 mL		③	Normal saline 50 mL		
		5HT3 receptor antagonist			5HT3 receptor antagonist		
			30 min		dexamethasone 12 mg		
					H_2 blocker		15 min
PEM	④	Normal saline 100 mL		④	Normal saline 100 mL		
		Pemetrexed	10 min		Pemetrexed		10 min
	⑤	Normal saline 100 mL	30 min	⑤	Mannitol 200 mL		30 min
CDDP	⑥	Normal saline 300 mL		⑥	Normal saline 300 mL		
		Cisplatin	3 hr		cisplatin		1 hr
Post hydration	⑦	ST3® 500 mL		⑦	ST3® 500 mL		2 hr
		metoclopramide 10 mg	2 hr	⑧	ST1® 500 mL		2 hr
	⑧	Mannitol 300 mL	1 hr				
	⑨	furosemide 20 mg	iv				
	⑩	Normal saline 500 mL					
		5HT3 receptor antagonist	2 hr				
total		3,100 mL	13 hr		2,700 mL		9 hr

Figure 1. The chemotherapy hydration regimens used in this study. ST3 (Solita T3®) is a solution containing Na (35 m Eq/L), K (20 mEq/L), Cl (35 mEq/L), L-lactate (20 mEq/L) and glucose (4.3 g/dL). ST1 (Solita T1®) is a solution containing Na (90 mEq/L), Cl (70 mEq/L), L-lactate (20 mEq/L) and glucose (13 g/dL). PEM, pemetrexed; CDDP, cisplatin.

the protective effect of furosemide against nephrotoxicity is still controversial (10). In fact, Lehane *et al.* reported that high doses of furosemide cause nephrotoxicity, and it has been suggested that its use with cisplatin may aggravate the nephrotoxicity (11). To the best of our knowledge, there have been no studies that have examined the protective effect of hydration using saline lacking furosemide supplemented with magnesium and mannitol on the nephrotoxicity induced by cisplatin and pemetrexed in patients with advanced non-squamous NSCLC.

We recently established a new hydration method using 2,700 mL of saline supplemented with magnesium and mannitol without furosemide, and examined the effect of this hydration protocol on the protection against the nephrotoxicity induced by cisplatin and pemetrexed in non-squamous NSCLC patients.

Subjects and methods

Fifty patients with NSCLC who received cisplatin (75 mg/m^2) plus pemetrexed (500 mg/m^2) from May 2009 to March 2012 were retrospectively analyzed in this study. Chemotherapy was repeated every three weeks unless otherwise noted. Until July 2010, the old hydration protocol, comprising 3,100 mL of normal saline with mannitol (300 mL) and furosemide (20 mg), was used for 30 patients (old group). Because of the relatively high incidence of renal toxicity for old hydration group, the old hydration protocol was replaced with a new protocol, which contained 2,700 mL of normal saline lacking furosemide with mannitol (200 mL) and magnesium sulfate (8 mEq), and was given to 20 patients (new group). Both regimens are shown in Figure 1. Nephrotoxicity was evaluated by the both serum creatinine level and creatinine clearance (Ccr) and were compared between patients treated with the old and new protocols. The serum creatinine level was measured by an enzymatic method. The Ccr was calculated with Cockcroft & Gault's formula (12). Hematological toxicities and non-hematological toxicities except renal toxicity were defined according to the Common Terminology Criteria for Adverse Event (CTCAE) version 4.0. The efficacy of chemotherapy was evaluated based on the response rate, disease control rate and overall survival according to the RECIST criteria, version 1.1. Comprehensive informed consent was obtained from all patients.

Statistical analyses

To evaluate the differences in the patients' characteristics, toxicities, and chemotherapeutic responses, the chi-square test was used. The differences in the nephrotoxicity (evidenced by

Table 1. The characteristics of all patients, the patients in the old regimen group and those in the new regimen group.

		All (n=50)	Old regimen (n=30)	New regimen (n=20)	P value
Age	Median (range)	61 (38-74)	60 (38-74)	63 (41-72)	0.4513
Sex	Male	30 (60)	20 (66.7)	10 (50.0)	0.2386
	Female	20 (40)	10 (33.3)	10 (50.0)	
Histology	Adenocarcinoma	46 (92)	28 (93.3)	18 (90.0)	0.6704
	Others	4 (8)	2 (6.7)	2 (10.0)	
Stage	I, II	2 (4)	2 (6.7)	0 (0.0)	0.0337
	III	7 (14)	6 (20.0)	1 (5.0)	
	IV	33 (66)	15 (50.0)	18 (90.0)	
	Postoperative recurrence	8 (16)	7 (23.3)	1 (5.0)	
line	1st	41 (82)	24 (80.0)	17 (85.0)	0.6521
	2nd	9 (18)	6 (20.0)	3 (15.0)	
PS	0	32 (64)	19 (63.3)	13 (65.0)	0.9043
	1	18 (36)	11 (36.7)	7 (35.0)	
HT	+	12 (24)	10 (33.3)	2 (10.0)	0.0584
	–	38 (76)	20 (66.7)	18 (90.0)	
DM	+	7 (14)	4 (13.3)	3 (15.0)	0.8679
	–	43 (86)	26 (76.9)	17 (85.0)	

PS, performance status; HT, hypertension; DM, diabetis mellitus. Patients characteristics according to the chemotherapy hydration regimens is also shown.

the ⊿serum creatinine and ⊿Ccr) between the old regimen and new regimen were analyzed with Mann-Whitney's U test. To analyze the overall survival (OS), survival curves were drawn by the Kaplan-Meier method. The OS was calculated from the date of initiation of chemotherapy to the date of death. The OS rates were compared using the log-rank test according to the regimen (old regimen vs. new regimen). The univariate and multivariate analyses using a multiple regression analysis were performed to identify the independent factors associated with protection against nephrotoxicity in patients with non-squamous NSCLC who received cisplatin plus pemetrexed using the SPSS (IBM, USA, New York), software program, version 19. P values <0.05 were considered to be statistically significant.

Results

The characteristics of all patients, the patients in the old regimen group and those in the new regimen group are shown in Table 1. The median age of all patients was 61 years old (range, 38-74 years old). Of the total of 50 patients, 30 patients were males (60%), 47 patients had adenocarcinoma (94%). The clinical stage was I and II in two, III in seven, IV in 33 and postoperative recurrence in eight patients. The ECOG performance status was 0 in 32 patients and 1 in 18 patients. As a co-morbidity related to the renal toxicity, 12 patients had hypertension (HT) and seven had diabetes mellitus (DM). There were no significant differences in the patient age, gender, performance status, histology, administered lines of treatment or incidence of DM between the groups. With regard to HT, patients in the new regimen group tended to less frequently have HT than those in the old group. However, there were no significant differences between the groups. In contrast, the old regimen group included more advanced stage patients compared to the new regimen group.

The completion rate of four cycles was 43.5% and 46.7% for the old and new regimen groups, respectively (data not shown). This difference was not statistically significant. The change in the serum creatinine level (maximum serum creatinine during one course of chemotherapy - serum creatinine before chemotherapy) and the change in the Ccr (Ccr before chemotherapy - nadir Ccr during one course of chemotherapy) were calculated. Figure 2A and B show the comparison of the change in the serum creatinine and the change in the Ccr in both groups. The nephrotoxicity in the old regimen group was more severe than that in the new regimen group.

We also compared other toxicities, besides renal dysfunction, between the groups. Both groups demonstrated similar toxicity profiles, and there were no significant differences in any of the other toxicities between the groups (data not shown). Of note, the response rate and disease control rate in the two groups were also not significantly different (Table 2). Even though the overall survival in the new regimen group did not reach the median survival time, the overall survival in the new regimen group appears to be

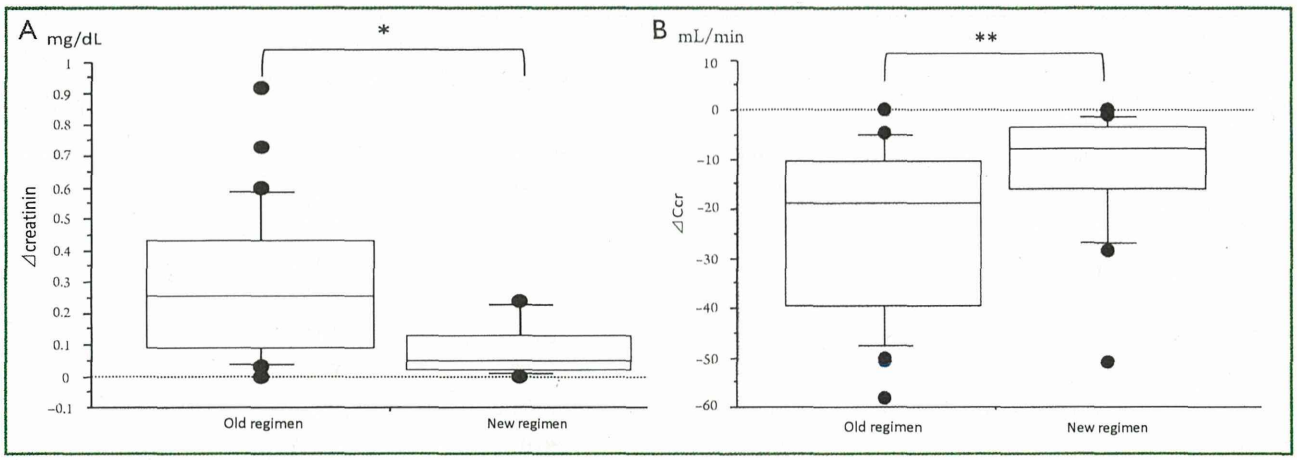

Figure 2. Differences in nephrotoxicity between patients treated using the old and new regimens. The ⊿serum creatinine and ⊿Ccr were calculated as described in the Subjects and Methods. The ⊿serum creatinine and ⊿Ccr in both groups were compared. The ⊿serum creatinine and ⊿Ccr are shown by vertical bars in A and B, respectively. *P=0.0004, **P=0.0148 *vs.* the new regimen.

Table 2. Treatment response (n=50).

Response	n	Old Regimen n=30	New Regimen n=20
CR	0	0	0
PR	10	7	3
SD	21	11	10
PD	8	4	4
NE	11*	8	3
Response rate	25%	23.3%	15.0%
Disease control rate	62%	60.0%	65.0%
			P=0.5723

CR. complete response, PR. partial response, SD. stable disease, PD. progressive disease, NE. not evaluable, *. Eleven patients with NE contain 6 patients with discontinuation of treatment due to toxicity, 4 patients with no evaluable lesion, and 1 patient with hospital tranceference.

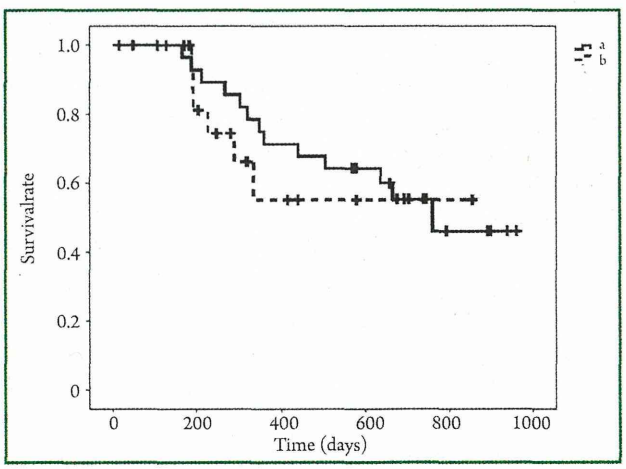

Figure 3. Overall survival. A comparison of the overall survival of patients with advanced non-squamous NSCLC between those treated using the old regimen group (a) and new regimen (b). There were no significant differences between the groups (P=0.4722).

equivalent to that in the old regimen group (Figure 3).

We next performed a univariate analysis to identify the factors influencing renal toxicity. As shown in Table 3, renal toxicity was more frequently observed in males and in the old regimen group than in females and in the new regimen group. Interestingly, there were no differences in nephrotoxicity based on the patient age, histology, stage, performance status, or co-morbidities (HT and DM) in the two groups. According to the multivariate analysis (Table 4), only the new chemotherapy regimen was an independent factor predicting the protection of renal toxicity caused by cisplatin and pemetrexed.

Discussion

In this study, we clearly demonstrated that a new regimen utilizing 2,700 mL of saline lacking furosemide supplemented with magnesium and mannitol with rapid cisplatin infusion (300 mL of saline containing 75 mg/m^2 cisplatin in 1 hour)

Table 3. Patient characteristics according to nephrotoxicity.

		All (n=50)	Nephrotoxicity+, (n=21)	Nephortoxicity-, (n=29)	P value
Age	Median (range)	61 (38-74)	62 (38-74)	60 (39-72)	0.4551
Sex	Male	30 (60)	16 (76.2)	14 (48.3)	0.0467
	Female	20 (40)	5 (23.8)	15 (41.7)	
Histology	Adenocarcinoma	46 (94)	19 (90.5)	27 (93.1)	0.5232
	Others	4 (6)	2 (9.5)	2 (6.9)	
Stage	I	33 (66)	11 (52.4)	22 (75.9)	0.0836
	Other stage	17 (34)	10 (47.6)	7 (17.2)	
line	1st	40 (80)	17 (81.0)	24 (82.8)	0.8697
	2nd	10 (20)	4 (19.0)	5 (17.2)	
PS	0	32 (64)	11 (52.4)	21 (72.4)	0.1452
	1	18 (36)	10 (47.6)	8 (17.6)	
Regimen	old	30 (60)	17 (81.0)	13 (44.8)	0.0101
	new	20 (40)	4 (19.0)	16 (55.2)	
HT	+	12 (24)	6 (28.6)	6 (20.7)	0.3814
	–	38 (76)	15 (71.4)	23 (79.3)	
DM	+	7 (14)	4 (19.0)	3 (10.3)	0.5195
	–	43 (86)	17 (81.0)	26 (89.7)	

Nephrotoxicity+ is defined with the grade 1 and greater of serum creatinin after 1 course of pemetrexed and cisplatin. PS, performance status; HT, hypertention; DM, diabetes mellitus, Parenthesis indicates the percentage.

Table 4. Predictive factors of nephrotoxicity caused by pemetrexed and cisplatin according to the Multivariate analysis.

Factor	95% CI	HR	P value
Sex (male/female)	0.761-11.041	2.899	0.6882
Stage (other stage/4)	0.327-5.452	1.335	0.1109
Chemotherapy regimen (new/old)	0.055-0.986	0.232	0.0393

CI, confidential interval; HR, hazard ratio.

would be useful to avoid the renal toxicity caused by cisplatin and pemetrexed, without any reduction in the efficacy of the regimen in patients with advanced non-squamous NSCLC. To exclude the possibility that the patient background, such as age, gender, stage and co-morbidities, would affect the renal toxicity induced by cisplatin and pemetrexed, a multivariate analysis using a multiple regression method was performed, and revealed that the new regimen was an independent predictive factor for the protection against the nephrotoxicity induced by cisplatin and pemetrexed.

There has been speculation about what ingredients (factors) in the new regimen contributed to protecting against the nephrotoxicity induced by cisplatin and pemetrexed. Several researchers had previously reported that magnesium supplementation protected against cisplatin-induced nephrotoxicity (8,9). Willox *et al.* performed a randomized trial to evaluate the effect of magnesium supplementation in testicular cancer patients receiving cisplatin, and demonstrated its effect on renal protection (9). Bodnar *et al.* have revealed the nephroprotective effect of magnesium supplementation during chemotherapy with cisplatin in patients with epithelial ovarian cancer (8). In addition, Lajer *et al.* reported that magnesium depletion enhances cisplatin-induced nephrotoxicity (13). Based on these previous reports, the magnesium supplementation in the new regimen appears to have been at least partly responsible for the reduced incidence of nephrotoxicity in this study.

Mannitol causes osmotic diuresis. Hayes and Frick revealed that mannitol decreased cisplatin nephrotoxicity (14,15). Clinically, mannitol reduces the urine concentration of cisplatin, and this effect is considered to be the mechanism underlying the amelioration of renal toxicity. Since most of the previous reports supported its effect on nephroprotection, except one controversial paper in which hydration with saline + mannitol was not nephroprotective compared to saline alone, mannitol was included in our new regimen (16).

Although other researchers have already reported the effect of furosemide on reducing the renal toxicity, its effect on the prevention of nephrotoxicity is still controversial (10). In fact, it has been reported that furosemide protects renal function, while

it worsens renal histopathology (11). Moreover, McMurtry *et al.* reported that furosemide enhances rodent nephrotoxicity (11). Therefore, furosemide was not included in the new regimen, resulting in amelioration of the nephrotoxicity induced by cisplatin and pemetrexed. We also employed the rapid infusion of cisplatin (75 mg/m^2/300 mL/1 hour) in the new regimen. The nephrotoxicity induced by cisplatin is related to the contact time of free cisplatin to the proximal tubules in the kidneys (17). Therefore, the rapid cisplatin infusion method used in the new regimen might have contributed to the reduction of cisplatin-induced nephrotoxicity. The new regimen involving the supplementation with magnesium and mannitol, in concert with rapid cisplatin infusion, could prevent cisplatin-induced nephrotoxicity.

It has been reported that supplementation with magnesium would affect *in vitro* and *in vivo* tumor growth (18). In fact, Parsons *et al.* have suggested that magnesium depletion decreases tumor growth (19). However, there were no statistically significant differences in the chemotherapeutic response or overall survival between the groups in our study, although the clinical outcomes appeared to be a little bit better in patients treated using the old regimen compared to those treated with the new regimen. Our results are supported by the report by Willox *et al.*, in which the supplementation with magnesium did not modify the chemotherapeutic response or prognosis for patients with testicular cancer (9).

There are several limitations in this study, which need to be addressed. Our study population was relatively small and retrospective, large scale and prospective studies are needed to confirm the utility of this protocol. In addition, there are several differences between the old regimen and the new regimen besides magnesium and furosemide. For instance, dexamethasone is contained in the new regimen, while it is not contained in the old regimen. We could not exclude the possibility that addition of dexamethanose in the new regimen was useful to prevent cisplatin-induced nehrotoxicity.

In conclusion, we clearly demonstrated that saline hydration without furosemide, supplemented with magnesium and mannitol, with rapid cisplatin infusion ameliorated the nephrotoxicity caused by cisplatin and pemetrexed in patients with advanced non-squamous NSCLC.

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Magnitude of influenza virus replication and cell damage is associated with interleukin-6 production in primary cultures of human tracheal epithelium

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ABSTRACT

Primary cultures of human tracheal epithelium were infected with influenza viruses to examine the relationships between the magnitude of viral replication and infection-induced cell damage and cytokine production in airway epithelial cells. Infection with four strains of the type A influenza virus increased the detached cell number and lactate dehydrogenase (LDH) levels in the supernatants. The detached cell number and LDH levels were related to the viral titers and interleukin (IL)-6 levels and the nuclear factor kappa B (NF-κB) p65 activation. Treatment of the cells with an anti-IL-6 receptor antibody and an NF-κB inhibitor, caffeic acid phenethyl ester, reduced the detached cell number, viral titers and the LDH levels and improved cell viability after infection with the pandemic influenza virus [A/Sendai-H/N0633/2009 (H1N1) pdm09]. A caspase-3 inhibitor, benzyloxycarbonyl-DEVD-fluoromethyl ketone, reduced the detached cell number and viral titers. Influenza viral infection-induced cell damage may be partly related to the magnitude of viral replication, NF-κB-p65-mediated IL-6 production and caspase-3 activation.

1. Introduction

The high virulence of the influenza virus causes severe illness and increases the fatality rate in patients. Precise information regarding the pathogenic magnitude of the influenza viruses is needed for the effective treatment of influenza viral infection and for the prevention of confusion and fear during a pandemic infection. The pathogenic magnitude of the influenza virus has been reported to be associated with the non-structural (NS) gene segment of the influenza A (H5N1) virus (Cheung et al., 2002), contributing to the increase in inflammatory cytokine production. Furthermore, several studies have reported mechanisms of the high pathogenicity, which include the elevation in pulmonary concentrations of inflammatory cytokines, including interleukin (IL)-1, IL-6 and interferon (IFN)-γ, the decrease in anti-inflammatory cytokine production and the elevation in viral replication (de Jong et al., 2006; Lipatov et al., 2005). Influenza viral infection induces viral replication, cytokine production and cell damage in the airway epithelium, which is the first target of the infection. However, the mechanisms and relationship between viral replication, cytokine production and cell damage in the human airway epithelium have not been well studied.

Influenza viral infection-induced production of inflammatory cytokines, including IL-6 and tumor necrosis factor (TNF)-α, and proteases may cause damage to airway and alveolar epithelial cells and vascular endothelial cells (Mauad et al., 2010; Ruwanpura et al., 2011; Wang et al., 2010) and may subsequently exacerbate bronchial asthma and chronic obstructive pulmonary disease, and develop acute respiratory distress syndrome (Nicholson et al., 1993; Perez-Padilla et al., 2009; Rohde et al., 2003). IL-6 and TNF-α are associated with cell death and the activation of caspases in swine macrophages after pandemic A/H1N1 viral infection (Gao et al., 2012). However, the mechanisms for cytokine production-induced airway cell damage resulting from influenza viral infection have not been well studied.

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