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Differences in Urinary Renal Failure Biomarkers in Cancer Patients Initially Treated with Cisplatin

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Abstract. *Background/Aim: We investigated whether measuring the excretion of each acute kidney injury (AKI) biomarker after cisplatin (CDDP) administration is useful for predicting AKI and evaluated the most appropriate AKI marker in patients treated with CDDP. Patients and Methods: We measured NAG, Kim-1, and NGAL in urinary samples of 40 cancer patients treated with chemotherapy on day 1 (before chemotherapy), day 2, and day 5 after treatment; serum creatinine (sCr) was compared on days 7 and 28 after CDDP administration vs. baseline. Results: NAG, Kim-1, and NGAL excretion (creatinine corrected) were not significantly elevated 5 days after receiving chemotherapy in the non-CDDP chemotherapy group. Conversely, all markers were significantly higher 5 days after receiving chemotherapy in the CDDP group when compared to baseline. Conclusion: Urinary NAG, Kim-1, and NGAL can detect renal injury more sensitively than sCr.*

Cisplatin (CDDP) is an antineoplastic agent that has been shown to provide survival benefits in patients with various types of cancer (1, 2). However, this compound has been shown to induce kidney injury (3). Therefore, early diagnosis of acute kidney injury (AKI) in patients receiving CDDP is important to lower the risk of severe renal failure. Common predictors of kidney injury are serum creatinine (sCr) and glomerular filtration rate (GFR) measured *via* urine analysis. However, these biomarkers are not sensitive nor specific enough to predict AKI in the early stages of renal failure (4). Treatment by CDDP tends to be administered over long-term hospitalization; however, recently, this chemotherapy has been administered over short-term hospitalization or in ambulatory care facilities because of developments in antiemetic drugs (5). Hence, if early discrimination of the risk of acute renal failure is made possible, there can be earlier detection and treatment of AKI supported by long-term monitoring of renal status.

Kidney injury by CDDP is caused by renal tubular damage (6, 7), and a previous study showed that some enzymes excreted in the urine can predict renal tubular damage (8). One of these enzymes is *N*-acetyl-β-D-glucosaminidase (NAG), that is a lysosomal enzyme that is abundant in the cells of the proximal kidney tubule, and is excreted in the urine at the time of cell damage (9). NAG has been shown in previous studies to be increased in the early stages of AKI (9-11). However, endogenous urea can reportedly decrease the activity of this enzyme (12). Furthermore, its levels can increase as a result of diseases other than AKI such as rheumatoid arthritis and abnormal glucose tolerance, and its specificity to AKI is low (13, 14). Here, an excellent AKI biomarker may detect AKI earlier than sCr and GFR fluctuations would; further, this detection would not be influenced by diseases other than AKI.

Kidney injury molecule-1 (Kim-1) and neutrophil gelatinase-associated lipocalin (NGAL) may be effective for the early diagnosis of AKI in patients receiving CDDP (8, 15). However, Kim-1 is known to increase in pre-renal AKI with accompanying dehydration, and it has an unsuitable profile in hydration therapy during the course of CDDP treatment (8). On the other hand, NGAL has little fluctuation in pre-renal AKI, and may be effective in the evaluation of

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Key Words: N-acetyl-β-D-glucosaminidase, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, cisplatin, acute kidney injury.

Table I. *Comparison of the characteristics of patients in the CDDP and non-CDDP chemotherapy groups.*

N=40	CDDP (+) N=27	CDDP (–) N=13	*p*-Value	Healthy volunteer N=6
Cancer diagnosis				
Esophagus	16	0		
Stomach	7	0		
Lung	4	2		
Colon	0	8		
Soft tissue tumor	0	2		
Anal canal	0	1		
CDDP dose (mg/m^2)	69±7.8	0		
Age (years)	63.8±8.6	63.1±10.9	0.83	54.3±4.6
Male (n, %)	21 (78)	7 (54)	0.12	3 (50)
BSA (/m^2)	1.6±0.2	1.6±0.16	0.70	1.6±0.17
Diabetes (n, %)	3 (11)	0 (0)	0.21	0 (0)
Hypertension (n, %)	7 (26)	5 (38)	0.42	1 (17)
Serum Cr (mg/dl)	0.71±0.16	0.70±0.22	0.95	0.74±0.18
eCCr (mL/min)	83.0±24	84.5±25	0.85	91.1±25
NAG (U/L)	8.84±10.2	12.9±21.0	0.41	1.82±1.1
NAG/Cr (U/g Cr)	6.60±4.4	11.1±14.2	0.14	2.75±0.9
Kim-1 (ng/ml)	1.41±1.1	1.71±2.3	0.58	0.40±0.3
Kim-1/Cr (ng/mg Cr)	1.62±2.0	1.48±1.0	0.81	0.54±0.4
NGAL (ng/ml)	8.79±7.2	13.0±14.4	0.22	5.11±4.5
NGAL/Cr (ng/mg Cr)	8.93±6.2	13.0±9.4	0.12	6.93±5.2

Data are presented as means±standard deviations (S.D.). BSA: Body surface area; eCCr: estimated creatinine clearance (Cockcroft–Gault); NAG: *N*-acetyl-β-D-glucosaminidase; NGAL: neutrophil gelatinase-associated lipocalin; Kim-1: kidney injury molecule-1; Cr: creatinine.

acute renal injury after CDDP therapy (16). However, only a few subjects of studies have assessed the fluctuation of NGAL after CDDP administration, and there is insufficient data on this topic. In addition, few reports have directly compared the levels of novel markers such as Kim-1 and NGAL in patients treated with CDDP, and measurement of the fluctuations of each tubule biomarker in these patients may be useful in biomarker research. We investigated the existence of fluctuations in NAG, Kim-1, and NGAL caused by cancer, as well as variations in these biomarkers after CDDP administration to clarify the most appropriate AKI marker in patients treated with CDDP.

Patients and Methods

Patients. This study was a prospective, observational study conducted at Aichi Cancer Center Hospital, Nagoya, Japan, from October 2012 to August 2013. Patients were recruited based on the following inclusion criteria: (1) at least 20 years old and (2) naive to treatment with CDDP. Patients who were initially administered anti-cancer drugs other than CDDP and healthy volunteers were enrolled as controls. The exclusion criteria included patients with an estimated creatinine (Cr) clearance of less than 50 ml/min as calculated by the Cockcroft–Gault formula. All patients were of Japanese descent and provided written informed consent. This study was approved by the ethics committee of Aichi Cancer Center Hospital, and was conducted in accordance with the declaration of Helsinki.

Methods and measurements. We collected midstream urinary samples on waking up in the morning from all participants before the administration of chemotherapy (day 1) and on days 2 and 5 after treatment. Furthermore, blood specimens were collected the day before starting chemotherapy and on days 7 (6-8) and 28 (21-35) after treatment of sCr measurement. Blood specimens and urinary samples were collected at one point from healthy volunteers. We also measured NAG, NGAL, and Kim-1 in the urine specimens. Urine Cr was measured for correcting each tubule biomarker concentration.

NGAL and Kim-1 concentrations were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, Inc. Minneapolis, MN, USA). NAG and Cr were measured using a colorimetric method and enzyme assay (SRL, Inc. Tokyo, Japan).

Statistical analysis. The characteristic data were analyzed using Student's *t*-test and the Mann–Whitney *U*-test. Differences in data were analyzed using one-way analysis of variance followed by Dunnett's test. p-Values<0.05 were considered statistically significant. All calculations were performed using EZR version 1.27 (17).

Results

The characteristics of the 46 subjects enrolled in this study are summarized in Table I. Twenty-seven patients received CDDP. The majority of patients had esophageal cancer (n=16, 60%), and followed a 5-fluorouracil-based regimen

Table II. *Comparison of baseline characteristics for all cancer patients included in this study.*

		Cancer patients						
	Healthy volunteer	Esophagus	Stomach	Colon	Lung	Soft tissue tumor	Anal canal	All
n	6	16	7	8	6	2	1	40
Age (years)	54.3±4.6	65.3±6.4*	61.6±12	64.8±9.4	62.8±8.1	52±22	66	63.6±1.5*
Male (n, %)	3 (50)	14 (88)	3 (43)	3 (38)	6 (100)	1 (50)	1	28 (70)
BSA (/m^2)	1.6±0.17	1.7±0.17	1.6±0.18	1.6±0.16	1.7±0.13			1.6±0.17
Diabetes (n, %)	0 (0)	2 (13)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	3 (8)
Hypertension (n, %)	1 (17)	7 (44)	0 (0)	2 (25)	1 (17)	1 (50)	1 (100)	12 (30)
Serum Cr (mg/dl)	0.74±0.18	0.74±0.17	0.63±0.13	0.71±0.25	0.72±0.10			0.71±0.18
eCCr (mL/min)	93.7±13	79.5±20	82.9±23	77.7±17	93.8±30			83.5±23
NAG (U/l)	1.8±1.1	6.2±4.9	15.4±17	9.2±13	6.0±5.9			10.2±14
NAG/Cr (U/g Cr)	2.8±0.9	6.3±4.9	7.2±4.0	11.5±15	5.3±4.5			8.0±9.0
Kim-1 (ng/ml)	0.40±0.3	1.5±1.4	1.6±0.9	1.2±0.8	0.80±0.5			1.5±1.6
Kim-1/Cr (ng/mg Cr)	0.54±0.4	1.4±0.6	2.7±3.8	1.4±0.6	0.77±0.4			15±1.7
NGAL (ng/ml)	5.1±4.5	7.5±7.4	11.6±4.4	8.8±7.4	7.6±8.7			10.2±10
NGAL/Cr (ng/mg Cr)	6.9±5.2	7.9±5.4	11±4.1	13±9.5	8.0±9.4			10.3±7.6

Data were analyzed statistically using one-way analysis of variance followed by the Dunnett's test. *$p<0.05$ *vs*. healthy volunteers. Data are presented as means±standard deviations (S.D.). BSA: Body surface area; eCCr: estimated creatinine clearance (Cockcroft–Gault); NAG: *N*-acetyl-β-D-glucosaminidase; NGAL: neutrophil gelatinase-associated lipocalin; Kim-1: kidney injury molecule-1; Cr: creatinine.

(*e.g*. DCF: docetaxel, CDDP, and 5-fluorouracil or FP: CDDP and 5-fluorouracil); the median CDDP dose was 69±7.8 mg/m^2. Thirteen patients were treated with non-CDDP chemotherapy, and most had colon cancer. There was no significant difference in the age, diabetes history, hypertension history, or renal function between the two groups. In addition, when comparing the levels of each AKI biomarker before administering chemotherapy in the patients with esophageal cancer, stomach cancer, colon cancer, and lung cancer, which were many enrolled types of cancer subjects in the study, there was no significant difference in any of the markers compared to healthy volunteers (Table II).

There were no patients with stage 1 or higher AKI (sCr level elevated by ≥1.5 fold or ≥0.3 mg/dl) as assessed by the AKI Network criteria in the CDDP group at 7 days after administration nor before the second course of chemotherapy (Figure 1). Hence, it was not possible to investigate the variations in each AKI biomarker in the presence or absence of AKI.

When comparing the CDDP and non-CDDP chemotherapy groups, no significant increase in any of these markers was observed on days 2 and 5 after treatment compared to baseline in the non-CDDP chemotherapy patients. Conversely, in the CDDP patients, although no change was observed on day 2 after treatment in the levels of any of these markers, the levels of all markers were significantly higher on day 5 than at baseline (Figure 2), despite the absence of elevation in the sCr level.

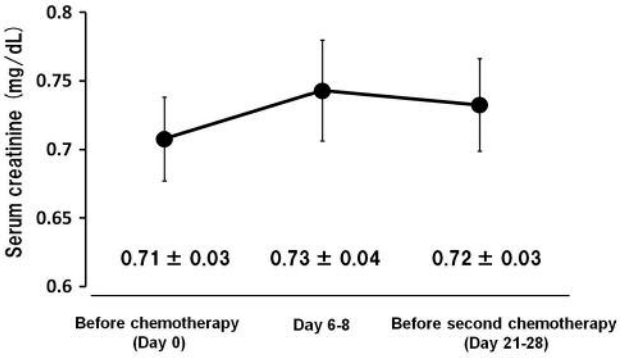

Figure 1. *Time course of sCr levels after CDDP administration. The results of each plot are presented as the means±standard error (S.E.); (n=27).*

Discussion

The aim of the present study was to establish effective biomarkers for the early prediction of AKI caused by the disturbance of the proximal renal tubule after CDDP administration. For accurate AKI prediction, the biomarker levels should not vary with diseases other than AKI. There were no significant differences between cancer patients before the administration of anticancer drugs and the healthy controls in the values of NAG, Kim-1, or NGAL, and these markers seemed to be less likely to change in cancer patients (Table II). In this study, we aimed to compare the levels of

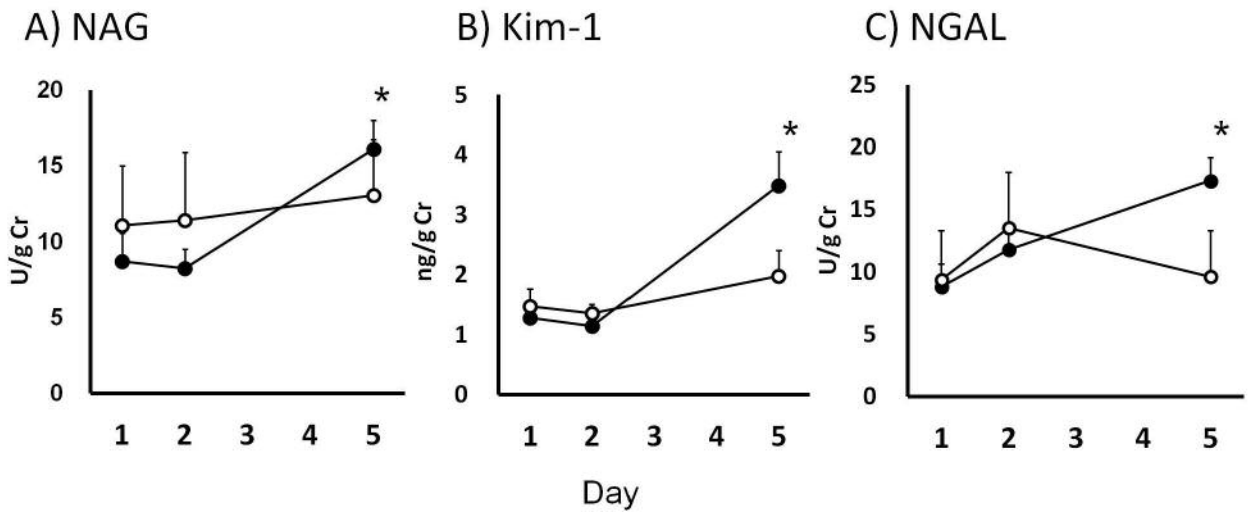

Figure 2. *Time course of the urinary biomarker levels after chemotherapy. Data were analyzed statistically by one-way analysis of variance followed by the Dunnett's test. *p<0.05 vs. before chemotherapy (day 1). The results of each plot are presented as the means±standard error (S.E.); CDDP chemotherapy group (●), non-CDDP chemotherapy group (○).*

each biomarker between AKI cases and non-AKI cases; however, there were no AKI patients in our study groups. Therefore, we compared the levels of each biomarker between the CDDP and non-CDDP chemotherapy patients.

In a previous study, NGAL increased to more than 1,000% of the baseline value at 24 h after the administration of CDDP chemotherapy in AKI onset patients (18). In our study, the maximum increase of NGAL at 24 h after chemotherapy treatment (day 2) was 541%, and the NGAL levels were not significantly higher than those previously reported in patients with AKI onset. Therefore, it seems that the prediction of AKI using urinary NGAL has good reproducibility. On the other hand, urinary Kim-1 (Kim-1/urinary Cr) reportedly increased to above 1,300 pg/mL [500 (pg/ml)/(Cr mmol/l)] at 24 h after the administration of CDDP chemotherapy in AKI onset patients (15). There were 10 patients in this study with Kim-1 levels higher than 1,300 pg/ml, but no cases were found with Kim-1/urinary Cr levels more than 500 (pg/ml)/(Cr mmol/l) (4.4 ng/Cr mg) when the levels were corrected with urine Cr on day 2 after treatment. Urinary Kim-1 levels are known to increase in pre-renal AKI with accompanying dehydration, and therefore, this marker is unsuitable for predicting AKI when CDDP chemotherapy is combined with a diuretic agent. However, it may be possible to solve this problem by correcting the levels with urine Cr.

Although the incidence of renal disorder after CDDP administration has been reported to be about 10% in a previous report (19), in recent years, the incidence of acute renal disorder has decreased to 2-4.6% as a result of improvements in hydration and antiemetic therapies (20-22). Therefore, it is necessary to conduct a larger-scale study on whether each biomarker can predict the onset of AKI and to evaluate the clinical significance of measuring each biomarker early after CDDP administration.

In the present study, the levels of NAG, Kim-1, and NGAL excretion (Cr corrected) were significantly increased on day 5 after the administration of anticancer drugs compared with baseline in the CDDP group, despite the absence of sCr elevation. The elevation of these biomarkers is not considered to be clinically problematic; however, these biomarkers could be detected in early renal injury that could not be detected by sCr. Because these biomarkers were not increased 24 h after the administration of chemotherapy, it is assumed that the time course of the increase associated with renal injury is comparable.

In conclusion, our results showed that urinary NAG, Kim-1, and NGAL can detect renal injury more sensitively than sCr. Each biomarker has similarly superior sensitivity and is elevated with similar time course; therefore, we did not find any differences in the levels of each biomarker. We believe that NAG, Kim-1, and NGAL can reflect potential renal injury following CDDP administration, and these biomarkers may be useful in the early assessment of renal injury.

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