Urinary neutrophil gelatinase-associated lipocalin levels predict cisplatin-induced acute kidney injury better than albuminuria or urinary cystatin C levels


Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Taiwan
Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Taiwan
Division of Hematology and Oncology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Taiwan
Faculty of Renal Care, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Received 10 February 2012; accepted 30 March 2012
Available online 11 January 2013

KEYWORDS
Acute kidney injury; Cisplatin; Neutrophil gelatinase-associated lipocalin

Abstract Cisplatin-induced acute kidney injury (AKI) is a major concern among clinicians in prescribing cisplatin-based chemotherapy. This study evaluated and compared the ability of urinary biomarkers, including urinary neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, and the urinary albumin to creatinine ratio (ACR) to predict cisplatin-induced AKI. Thirty-three cancer patients receiving cisplatin-based chemotherapy were prospectively studied, including 10 (30%) who developed AKI (the study group). Changes of urinary biomarkers were compared at 4 hours, 8 hours, and 12 hours, and 1 day, 2 days, 3 days, and 4 days after cisplatin intravenous infusions (75 mg/m²) versus the baseline. There was a significant increase in urinary NGAL levels from 12 hours to 4 days (p < 0.05) compared to baseline after cisplatin infusion in the AKI group. The magnitude of these changes over time differed significantly by group (p < 0.001). The area under the receiver operating curve describing the relationship between urinary NGAL levels and AKI within 12 hours was 0.865 (95% confidence interval 0.808–0.922) compared to baseline.
Introduction

Cisplatin, found in the 1960s to inhibit cell division, is currently one of the most widely used anticancer chemotherapy drugs [1,2]. Among the current chemotherapy medications, cisplatin and platinum-related medications are mainly used for head and neck cancer, esophageal cancer, genital cancer (i.e., testicular and ovarian cancer), cervical cancer, and non-small-cell lung cancer [3,4]. Current studies show that the mechanisms for tumor suppression of cisplatin are to crosslink DNA and interfere with its replication and synthesis [5,6].

Although cisplatin is used widely, its adverse clinical effects, including neurotoxicity, ototoxicity, nephrotoxicity, nausea, and vomiting, remain challenging for clinicians [7]. Researchers and clinicians have exerted efforts to remove or circumvent these side effects, such as developing new platinum analogs with less toxicity. The most well known is carboplatin, which has less nephrotoxicity [8]. Other ways of preventing cisplatin-induced kidney injury include giving large amounts of intravenous fluids and mannitol [9,10]. However, increasing numbers of studies are revealing that, even with these management methods, around one-third of the patients receiving cisplatin-based chemotherapy suffer acute kidney injury (AKI) [2,11]. Clinically, cisplatin-induced AKI occurs about 10 days after cisplatin treatment, and it is marked by a decline in glomerular filtration rates, increased serum creatinine levels, hypomagnesemia, and hypokalemia [2,7]. The pathologic changes in cisplatin-induced AKI involve renal tubular cell damage [7], and the mechanisms of cisplatin-induced renal tubular cell damage include apoptosis, which is induced by activated caspase-8 and which causes cellular stress and the release of apoptogenic factors [12], endoplasmic reticulum stress [13], p53 activation [7], mitogen-activated protein kinase activation [14], oxidative stress [15], and inflammation by induced tumor necrosis factor-α [16].

From the risk, injury, and failure; and loss; and end-stage kidney disease (RIFLE) criteria [17] and the AKI Network criteria [18], the Acute Dialysis Quality Initiative Group has tried to redefine and prevent AKI. These criteria are based on serum creatinine levels and calculations of the estimated glomerular filtration rate (eGFR), but many studies have revealed that serum creatinine levels do not parallel acute kidney damage and using these as criteria may delay clinical diagnosis [19,20]. New AKI biomarkers use therefore necessary. Recent studies have investigated some biomarkers for AKI [21], including neutrophil gelatinase-associated lipocalin (NGAL), which is a 25-kDa protein. NGAL has a very low concentration in most human tissues but spikes when kidney, liver, colon, and lung epithelial cells are damaged [22]. In animal models, NGAL levels increase after ischemic kidney injury or exposure to nephrotoxic drugs [23]. From the results of several human prospective studies, NGAL may be a more sensitive biomarker than creatinine in predicting AKI [24,25].

Cystatin C (Cys C), which is a 13-kD cysteine proteinase inhibitor protein, filters through the glomerulus and is reabsorbed by proximal tubule cells without secretion [26]. In animal studies, urinary Cys C predicts AKI earlier than other biomarkers [27]. In a prospective study of 123 adults who underwent cardiac surgery and who suffered AKI according to the AKI Network criteria, urinary Cys C levels predicted AKI best when patients were admitted to the intensive care unit [area under the curve (AUC) = 0.70, p < 0.001]. Urinary NGAL levels best predicted AKI 6 hours after intensive care unit admission (AUC = 0.88; p < 0.001) [28].

Proteinuria is a marker of chronic kidney disease. A cohort study observed that, in patients who had coronary artery bypass graft surgeries, the severity of preoperative proteinuria reflected the stage of acute ischemic kidney injury after the operation [29]. The Atherosclerosis Risk in Communities study enrolled 11,200 patients in order to evaluate the relationship between proteinuria and AKI. The results showed that the risk of AKI increased with the severity of albuminuria [30].

Although clinicians have exerted efforts to prevent cisplatin-induced AKI, the results are not satisfactory [7]. A biomarker that predicts AKI earlier than serum creatinine levels may provide clinicians with more time to apply the appropriate intervention. In an animal study, urinary NGAL levels that were measured by western blots were increased within 3 hours after cisplatin treatment in an AKI study group [31]. In a human prospective study, 12 patients (26%) had serum creatinine levels that were increased by over 25% 5.8 ± 2.3 days after cisplatin as compared to baseline. Urinary NGAL levels increased significantly more in cases than in controls on Days 1, 2, 3, and 15, whereas serum creatinine levels were increased significantly on Days 3, 7, and 15 [32]. Serum Cys C levels have also been shown to be a more sensitive marker than creatinine levels in cisplatin chemotherapy, with a 21% increase without a significant creatinine change [33]. However, no studies have so far evaluated the role of urinary Cys C levels in cisplatin-induced AKI.

This study measured and compared urinary biomarkers in order to determine which can predict cisplatin-induced AKI earlier. This prospective cohort study compared NGAL levels, Cys C levels, and the urinary albumin to creatinine ratio (ACR) at several time points in cancer patients who suffered from cisplatin-induced AKI.
Methods

Patients

This study enrolled cancer patients older than 18 years with pathologically diagnosed head and neck cancer, esophageal cancer, or thymic cancer who were indicated for cisplatin-based chemotherapy and admitted to the Kaohsiung Medical University Hospital Hematology and Oncology ward from August 2009 to August 2010. Eastern Cooperative Oncology Group Performance Statuses of 0–1 were another inclusion criterion. The exclusion criteria consisted of the use of any nephrotoxic drugs before chemotherapy, eGFR < 60 mL/min/1.73 m², and confounding factors that could interfere with renal function, including sepsis and the use of contrast medium examination. The hospital’s Institutional Review Board (KMUH-IRB-980216) approved this study.

Study design

Baseline serum and urinary biochemistry examinations were performed on all patients before cisplatin-based chemotherapy. After baseline evaluation of renal function and urinary profile, 500 mL of 0.9% isotonic saline containing 1 g of magnesium sulfate and 75 mg/m² of cisplatin in 750 mL of 0.9% normal saline with 250 mL mannitol for 4 hours were infused. From the start to the end of cisplatin infusion, 500 mL of 0.9% isotonic saline and 1 L 10% dextrose solution were infused from another venous line. Large amounts of hydration, including 1 L of 0.9% isotonic saline and 1 L 10% dextrose solution/day, were given to patients from Day 1 to Day 6 in order to prevent cisplatin-induced AKI. Betamethasone (4 mg) was administered every 6 hours from Day 1 to Day 5, and tropisetron hydrochloride (5 mg) was prescribed before chemotherapy as an antiemetic.

Urinary samples were collected for biochemical studies at 4 hours, 8 hours, and 12 hours and 1 day, 2 days, 3 days, and 4 days after cisplatin infusion. The urinary samples were separated in order to measure creatinine and albumin immediately and then stored at −80 °C until the NGAL and Cys C analysis. In order to evaluate if patients had AKI, serum creatinine levels on Days 4 and 10 after cisplatin infusion were collected and measured. All serum biochemical parameters were measured on Day 10 after cisplatin infusion in order to evaluate body changes.

Assay

Urinary NGAL concentrations were measured by a NGAL enzyme-linked immunosorbent assay kit (BioPorto, Gentofte, Denmark). The inter- and intra-assay coefficients of variance for NGAL were 3.4% and 4.7%, respectively. Urinary Cys C levels were measured by particle-enhanced immunonephelometry assay with an automatic device (N Latex Cystatin C, BN ProSpec; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). The inter- and intra-assay coefficients of variance were 1.7% and 2.9%, respectively. Serum and urinary creatinine levels were measured by the enzyme method, while urinary albumin levels were measured by an immunoturbidimetric assay. These biochemical concentrations were all measured by an automatic device (Cobas Integra 400; Roche Diagnostics, Basel, Switzerland).

The eGFRs were estimated by the following isotope dilution mass spectrometry-traceable modification diet of renal disease 4-variable equation [34]:

\[
\text{eGFR} = 175 \times (\text{SCr})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female})
\]

where SCr is serum creatinine levels in mg/dL and age is in years.

Statistical analysis

The baseline characteristics and biochemical examinations of the patients were compared by two-tailed unpaired t tests, and \( p < 0.05 \) was considered statistically significant. Data from the eight time points, including urinary NGAL, Cys C, and U-ACR, were analyzed by two-way repeated measures ANOVA in order to evaluate time and time-by-group interactions. The predictive value of urinary NGAL levels for AKI after cisplatin infusion over baseline and of other baseline covariates (i.e., age, sex, body mass index, serum creatinine levels, and urinary total protein at baseline) were analyzed by multivariate logistic regression. All statistical analyses were performed with SPSS software (version 18.0; 2009; IBM Corporation, Armonk, NY, USA). The receiver operating characteristic (ROC) curves were constructed and compared using STATA software (StataCorp LP, College Station, TX, USA).

Results

Baseline characteristics

Forty-seven cancer patients were screened for study enrollment, and 13 were excluded based on the exclusion criteria. Of the 34 patients who provided consent, 33 completed the protocol. The reasons for failures to complete the protocol included an unanticipated septic shock within 2 days of cisplatin infusion. Twenty-four of these patients (73%) had head and neck cancer and 21 (64%) had Stage IV cancer. Other baseline characteristics are listed in Table 1. There was no hematuria or proteinuria as measured by urine dipstick at the precisplatin infusion baseline, and the urinary protein-to-creatinine ratio between groups was not significantly different (AKI, 0.2 ± 0.3 vs. no AKI, 0.1 ± 0.1 mg/mg; \( p = 0.08 \)).

Of the 33 study patients, 10 (30%) were in the cisplatin-induced AKI risk class, which was defined by a decline in eGFR levels of over 25% on Day 4 compared to baseline, according to the RIFLE criteria [17]. In the AKI group, the eGFRs progressively declined after cisplatin infusion and were significantly lower than at baseline (121.5 ± 31.4 mL/min/1.73 m²) on Day 4 (95.1 ± 22.1 mL/min/1.73 m², \( p = 0.04 \)) and Day 10 (87.9 ± 20.8 mL/min/1.73 m², \( p = 0.01 \)). While the baseline eGFR was 95.1 ± 26.0 mL/min/1.73 m², there were no discernable changes in the no AKI group on Day 4 (94.9 ± 24.1 mL/min/1.73 m²; \( p = 0.95 \)) or Day 10 (96.0 ± 26.5 mL/min/1.73 m²; \( p = 0.97 \)).

On Day 17, the eGFR (98.3 ± 27.1 mL/min/1.73 m²) tended to recover towards baseline in eight cases (80%) of the AKI group. In the other two cases, serum creatinine
levels on Day 17 were still >25% higher compared to baseline.

Urinary NGAL

There was no significant difference between baseline urinary NGAL values in the AKI and no AKI groups without creatinine correction (17.2 ± 14.1 vs. 7.3 ± 11.1 ng/mL; p = 0.061). Urinary NGAL values are usually reported as urinary concentrations indexed to urinary creatinine excretion [subsequently referred to as urinary NGAL levels and expressed in ng NGAL/mg of creatinine (ng/mg)]. There was no significant difference in the baseline urinary NGAL concentrations between groups (AKI vs. no AKI, p = 0.28; Fig. 1A). The urinary concentrations of NGAL increased in all individuals following cisplatin infusion despite the administration of large amounts of hydration. Compared to baseline, urinary NGAL values were significantly increased at 12 hours (64.6 ± 54.6 ng/mg, p = 0.01) and on Day 1 (75.7 ± 68.3 ng/mg, p = 0.01), Day 2 (81.9 ± 83.5 ng/mg, p = 0.02), and Day 3 (43.8 ± 33.0 ng/mg, p = 0.04) in the AKI group. In the no AKI group, urinary NGAL values were not significantly increased at 12 hours (14.0 ± 12.8 ng/mg, p = 0.26) or on Day 1 (11.0 ± 16.3 ng/mg, p = 0.77), Day 2 (16.1 ± 19.8 ng/mg, p = 0.025), or Day 3 (12.5 ± 14.8 ng/mg, p = 0.50) compared to baseline. The magnitude of these changes over time differed significantly by group (time-by-group interaction, p < 0.001, by repeated-measures ANOVA; Fig. 2A).

Urinary Cys C

Urinary Cys C values are reported as urinary concentrations indexed to urinary creatinine excretion [referred to as urinary Cys C and expressed in mg Cys C/g of creatinine (mg/g)]. There was no significant difference in baseline urinary Cys C concentrations between the groups (AKI vs. no AKI, p = 0.94; Fig. 1B). Urinary Cys C was not significantly increased compared to baseline in the AKI group. In the no AKI group, although urinary Cys C values were significantly increased compared to baseline on Day 1 (9.3 ± 6.9 mg/g, p = 0.005) and Day 2 (9.7 ± 9.9 mg/g, p = 0.02), the magnitude of these changes over time did not differ significantly by group (p = 0.913, by repeated-measures ANOVA; Fig. 2B).

Albumin to creatinine ratio

The ACR is expressed in μg albumin/mg of creatinine (μg/mg). There was no significant difference in the baseline ACRs between the two groups (AKI vs. no AKI, p = 0.78; Fig. 1C). The ACR was increased in all individuals following cisplatin infusion despite large amounts of hydration. Compared to baseline, the ACR values were increased significantly at 8 hours (29.6 ± 19.2 μg/mg, p = 0.03) and on Day 4 (55.6 ± 51.9 μg/mg, p = 0.02) in the AKI group. In the no AKI group, although the ACR values were significantly increased at 4 hours (29.6 ± 23.7 μg/mg, p = 0.001) and 8 hours (25.3 ± 16.7 μg/mg, p = 0.001), and on Day 2...
compared to baseline, the magnitude of changes over time did not differ significantly by group ($p = 0.183$, by repeated-measures ANOVA; Fig. 2C).

ROC curves

The ROC curves were constructed in order to test the ability of urinary biomarkers to predict AKI (Table 2). The AUC of urinary NGAL levels at 8 hours postcisplatin was 0.76 [95% confidence interval (CI) = 0.58–0.94; $p = 0.021$], and it was 0.87 (95% CI = 0.69–1; $p = 0.001$) at 12 hours (Fig. 3), compared to baseline, the magnitude of changes over time did not differ significantly by group ($p = 0.183$, by repeated-measures ANOVA; Fig. 2C).

Discussion

The present study compared the roles of urinary NGAL, Cys C, and ACR in cisplatin-induced AKI. The results showed that urinary NGAL levels, which had values that were customarily indexed to urine creatinine, were increased significantly at 12 hours and on Days 1, 2, and 3 post-cisplatin infusion compared to baseline in the AKI group. After statistical analysis, the urinary NGAL values at 12 hours postcisplatin significantly predicted the risk of AKI based on the RIFLE criteria. Changes in urinary NGAL values occurred earlier than those in previous reports [32]. Other urinary biomarkers such as Cys C and ACR may change at several time points after cisplatin infusion, but their values were not predictive of cisplatin-induced AKI. Other findings were similar to those in previous reports where one-third of patients still suffered AKI despite treatment with mannitol and large amounts of hydration before cisplatin [2,11]. The use of NGAL levels as an AKI biomarker successfully passed through the preclinical, assay development, and initial clinical testing stages of biomarker development. It has now entered the prospective screening stage for many kinds of AKI [22,31]. The NGAL levels may be the answer in the search for a troponin-like biomarker for AKI. Nonetheless, a persistent question is whether or not a single marker like NGAL will be sufficient for cisplatin-induced AKI, which has multiple targets of injury. In order to diagnose and prevent cisplatin-induced AKI early, it is necessary to identify the injured renal part and monitor the disease progression. NGAL is an injury marker of the renal tubules and other renal part injury markers, such as Cys C and albuminuria, may compromise the defect [35].

The present study showed that, even with large amounts of hydration, urinary NGAL values were significantly increased at 12 hours postcisplatin in a group of patients who fulfilled the risk classification of the RIFLE criteria. The magnitude of the urinary NGAL level increase was not as high as those previously reported in ischemic AKI studies [21,31]. This may indicate that urinary NGAL is a very sensitive biomarker in detecting subclinical renal damage, which is hard to detect using previous renal function parameters.

Cisplatin-induced AKI is a major side effect in the clinical use of cisplatin. Numerous approaches have been reported to explore renal protection strategies, including hydration, combined with mannitol or furosemide [9,10]. The unsatisfactory results may be due to multiple targets in cisplatin-induced AKI. How to approach the balance
between anticancer and renal protection is a clinical dilemma when using cisplatin. This may be the reason why renal protection strategies only offer partial effects, especially if the renal protective approach interferes with the anticancer effects, which is unacceptable. The development of AKI biomarkers is a new direction in the prevention of cisplatin-induced AKI. The earlier timing of diagnosis allows for more renal protective strategies. The
increase in urinary NGAL suggests that the presence of tubular injury is the major target of cisplatin-induced AKI. The definition of the risk classification in the RIFLE criteria is that a 25% decrease in eGFR indicates subclinical renal damage that is usually neglected by clinicians. This study demonstrates that urinary NGAL is significantly increased earlier than other urinary biomarkers and is a useful predictive marker of cisplatin-induced AKI.

In this study, urinary NGAL levels at baseline were elevated in both the AKI (16.4 ± 21.5 ng/mg) and no AKI (9.9 ± 12.7 ng/mg) groups. The increased urinary NGAL levels may be due to the advanced stage of cancer in these patients. Previous studies have proven that NGAL is increased in cancer patients [36,37]. Most patients in this study had Stage IV cancer (mean, 64%; AKI, 50% vs. no AKI, 69%). Recent findings suggest that NGAL actively participates in the processes underlying the growth, development, and differentiation of different human tissues, including tumors [38]. Disease characteristics may explain the differences in baseline urinary NGAL. The difference between the two study groups was not significant, but, due to the small patient number in each group, a larger study is warranted for the clinical validation of this difference.

Although a previous report showed that serum Cys C may be a biomarker for the assessment of renal function in cisplatin chemotherapy [33], urinary Cys C was not a useful marker for detecting cisplatin-induced AKI in the present study. While the U-ACR increased in both the AKI and no AKI groups in this study, there was no statistically significant difference. This result may have been due to the small number of patients, and a larger study may be needed to evaluate the role of U-ACR in cisplatin-induced AKI.

This study had some limitations. First, this was a single-center study of limited size, and, thus, the power was not sufficient to draw definitive conclusions. Larger studies are necessary for clinical validation. Second, the follow-up time was short and did not reveal if the increase in urinary NGAL correlated with a long-term decline in renal function, renal replacement therapy, or mortality.

In conclusion, our study found that increased urinary NGAL was a useful biomarker of AKI in adults following cisplatin treatment, and it was better than albuminuria or urinary Cystatin C. The "window of opportunity" is narrow in cisplatin-induced AKI, and time is limited for introducing proper treatment after the initiating insult. Therefore, the search for a new biomarker of renal dysfunction continues. Due to its sensitivity and specificity, NGAL could be valuable in the detection of AKI.

Acknowledgments

This study was supported by a grant from Kaohsiung Municipal Ta-Tung Hospital (KMTTH-99-024).

References


Table 2  Area under the curve AUC for receiver operating characteristic curves of urinary biomarkers for predicting acute kidney infection.

<table>
<thead>
<tr>
<th>Time</th>
<th>Urinary NGAL</th>
<th>Urinary Cystatin C</th>
<th>ACR</th>
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<tr>
<td></td>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
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<tr>
<td>0 h</td>
<td>0.67 0.47–0.87 0.48 0.23–0.73 0.43 0.19–0.68</td>
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<tr>
<td>4 h</td>
<td>0.68 0.47–0.88 0.64 0.43–0.85 0.52 0.29–0.75</td>
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<tr>
<td>8 h</td>
<td>0.76 0.58–0.94 0.62 0.41–0.83 0.53 0.28–0.79</td>
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<tr>
<td>12 h</td>
<td>0.87 0.69–1.0 0.53* 0.31–0.76 0.52* 0.27–0.76</td>
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<tr>
<td>24 h</td>
<td>0.87 0.7–1.0 0.63 0.4–0.85 0.52* 0.28–0.77</td>
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<td>48 h</td>
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<tr>
<td>72 h</td>
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<tr>
<td>96 h</td>
<td>0.81 0.64–0.97 0.51 0.3–0.72 0.7 0.49–0.91</td>
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*p < 0.05 versus urinary neutrophil gelatinase-associated lipocalin (NGAL).

CI = confidence interval.

Figure 3. The receiver operating characteristic curves for urinary biomarkers at 12 hours after postcisplatin infusion. The area under the curve was greatest for urinary NGAL [0.87; 95% confidence interval (CI): 0.69–1.0], which was followed by urinary Cys C (0.53; 95% CI: 0.31–0.76), and then urinary ACR (0.52; 95% CI: 0.27–0.76). Urinary NGAL was the only biomarker to significantly predict the future development of AKI.


