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Profiling of Kidney Injury Biomarkers in Patients Receiving Cisplatin: Time-Dependent Changes in the Absence of Clinical Nephrotoxicity

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Abstract

The success of cisplatin-containing regimens to treat solid tumors is limited, in part, by nephrotoxicity. In rodents, several urinary proteins have emerged as more sensitive indicators of cisplatin-induced kidney injury. We sought to characterize time-dependent changes in the urinary concentrations of 12 proteins including KIM-1, calbindin, β 2M, and TFF3 after cisplatin therapy. Urine was collected at baseline, 3 (range: 2-5), and 10 (range: 9-11) days from 57 patients with solid tumors receiving outpatient cisplatin therapy (≤ 25 mg/m²). Serum creatinine was largely unchanged after cisplatin infusion. However, compared to baseline values, several novel biomarkers were significantly increased in the urine including β 2M, which was 3-fold higher by

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day 3 ($p<0.0001$). Urinary KIM-1 and TFF3 were elevated 2-fold by day 10 ($p=0.002$ and $p=0.002$, respectively) whereas calbindin levels were increased 8-fold ($p<0.0001$). We report novel time-dependent changes in the urinary excretion of noninvasive markers of subclinical kidney injury after cisplatin treatment.

Keywords

biomarker; cisplatin; nephrotoxicity; noninvasive; subclinical

Introduction

Cisplatin is a chemotherapeutic agent widely used for the treatment of solid tumors. Despite the ability of hydration and electrolyte management to reduce the incidence of acute kidney injury (AKI), up to one-third of patients receiving cisplatin therapy at doses greater than 50 mg/m² still experience nephrotoxicity (1). The current diagnostic criteria for AKI, as established by the Kidney Disease Improving Global Outcomes (KDIGO) and the Acute Kidney Injury Network (AKIN), are largely based on serum creatinine (SCr), which is a surrogate marker of glomerular filtration (2). The estimated glomerular filtration rate GFR (eGFR), which approximates a reduction in the functional capacity of the kidney, is highly insensitive. Even with a 50% loss of functioning renal mass, eGFR values remain within the normal range. Furthermore, mounting research has revealed multiple limitations of SCr-based diagnostics including competition for tubular secretion, fluctuations during acute injury, and variation due to muscle mass and meat intake (3).

Timely detection of kidney injury may allow for earlier interventions to reduce morbidity and mortality. Several novel urinary proteins have been investigated for their ability to detect AKI in a more sensitive and specific manner. In rats, kidney injury molecule-1 (KIM-1) was elevated in the urine 24 h after a single dose of cisplatin and outperformed traditional markers of toxicity including SCr and blood urea nitrogen (BUN) (4, 5). Biomarkers such as urinary albumin, lipocalin 2 (also known as neutrophil gelatinase-associated lipocalin (NGAL) in humans), KIM-1, and osteopontin exhibited time-dependent elevations in cisplatin-treated rats that mirrored findings from histopathological analysis (6). In 2008, the Food and Drug Administration (FDA) approved the preclinical use of seven novel urinary proteins (KIM-1, clusterin, albumin, total protein, beta 2-microglobulin (β 2M), cystatin C and trefoil factor 3 (TFF3)) for regulatory decision-making alongside traditional markers (7). Other novel urinary biomarkers (tissue inhibitor of metalloproteinase 2, insulin-like growth factor binding protein 7) have been approved by the FDA for clinical detection of AKI through a point-of-care device, NephrocheckTM (8); however, its utility, specifically for the detection of cisplatin-induced AKI, has yet to be determined. Previous studies have largely compared biomarker performance based on the clinical diagnosis of AKI or resulting from a combination of etiologies including cardiac surgery, sepsis, transplant, chronic kidney disease, renal cancer and nephrotoxic medications (9-13). Few studies have prospectively examined concentrations of novel biomarkers following administration of a nephrotoxic agent in the setting of cancer or in patients that do not exhibit AKI as defined by KDIGO or AKIN (often termed subclinical nephrotoxicity). Studies in patients treated with cisplatin

suggest that KIM-1 and monocyte chemoattractant protein-1 (MCP-1) can detect AKI with high sensitivity (AUROC values: 0.858 and 0.850, respectively) (14, 15). Furthermore, urinary calbindin and β 2M have been shown to be elevated 1468% and 903%, respectively in patients with subclinical cisplatin nephrotoxicity (n=14) (16).

In this study, we sought to prospectively characterize time-dependent changes in twelve urinary proteins (KIM-1, calbindin, clusterin, glutathione *S*-transferase-pi (GST-pi), interleukin-18 (IL-18), MCP-1, albumin, β 2M, cystatin C, NGAL, osteopontin, and TFF3) in patients receiving outpatient cisplatin therapy for various solid tumors. For this effort, two commercial ELISA-based multiplex panels were used to simultaneously quantify urinary protein concentrations prior to and at two time points after cisplatin administration. Early detection of clinical and subclinical changes in renal function would strengthen a clinician's ability to prevent further damage and identify patients at-risk for cisplatin nephrotoxicity.

Results

Patient Characteristics

A total of sixty patients were enrolled in the study. Three patients were excluded from analysis because they were either lacking a baseline urine collection (0 h or 0-2 h) or only had one time-point collected. Characteristics for the remaining subjects (n=57) are shown in Table 1. The majority of patients were Caucasian (89%) with a similar number of male and females. The mean and range of patient ages were 56.6 (26-80) years and body mass index (BMI) values were 27 (18-46) kg/m². The average prescribed cisplatin dose and range were 63.4 (25-120) mg/m² and varied based on tumor type (Table 2).

Clinical Laboratory Parameters in Patients Receiving Cisplatin

Using the KDIGO criteria, AKI was defined by increases in SCr more than 0.3 mg/dL over 48 h or 1.5 times baseline after one dose of cisplatin (17). Only one patient developed AKI based on the criteria with a 2.3-fold increase in SCr from baseline. Mean SCr, BUN, and eGFR were not changed significantly 13 $\pm$ 7 days after cisplatin infusion (Table 3). Similarly, the ratio of urinary albumin-to-creatinine was also not changed after cisplatin. Several electrolytes, including Na⁺, Ca²⁺, and Cl⁻, were significantly decreased after cisplatin therapy although they remained within normal concentration ranges (Table 3). The concentrations of other electrolytes, Mg²⁺, K⁺, and HCO³⁻, did not differ in patients before and after cisplatin treatment.

Time-Dependent Changes in Urinary Protein Biomarker Concentrations following Cisplatin Infusion

Urinary concentrations of proteins, including β 2M (3.3-fold), cystatin C (1.9-fold), TFF3 (1.7-fold), KIM-1 (1.6-fold), and albumin (1.5-fold) were significantly elevated by day 3 (range 2-5 days) compared to baseline (Figure 1, Table 4). A slight reduction in clusterin levels was also observed on day 3 (p=0.043). By 10 days (range 9-11 days), calbindin (8.3-fold), KIM-1 (2.8-fold), albumin (2-fold), TFF3 (2-fold), clusterin (1.9-fold), cystatin C (1.8-fold), MCP-1 (1.7-fold), and GST-pi (1.6-fold) were significantly increased (Figure 1, Table 4). β 2M (1.4-fold), although still significantly increased from baseline, trended

downward on day 10 compared to day 3. No change in the mean concentrations of IL-18, NGAL or osteopontin concentrations were observed at either time point. While the changes in mean concentrations of some biomarkers may not have changed, individual patients exhibited increases at Day 3 or 10 (Supplemental Table 1). The normalization of biomarker concentrations to urinary creatinine also reflected similar time-dependent changes (Supplemental Table 2). β 2M (5-fold), albumin (2.1-fold), KIM-1 (1.9-fold), and TFF3 (1.8-fold) were significantly increased at day 3 after normalizing to urinary creatinine. Likewise, biomarker concentrations for calbindin (8.7-fold) and KIM-1 (2.1-fold) following normalization were also significantly higher around day 10 after cisplatin.

Correlation of Clinical Laboratory Parameters to Urinary Protein Biomarker Concentrations

The concentrations of urinary proteins at day 10 showed little correlation with changes in traditional measures of AKI, including SCr, BUN and eGFR, that were measured 13 ± 7 days after cisplatin infusion (Supplemental Table 3). The only relationship that was revealed was a positive correlation between urinary calbindin levels and changes in BUN concentrations ($r=0.312$, $p=0.032$) at day 10. The results were similar when the clinical laboratory parameters were expressed as percent changes (data not shown). Strong relationships between the various protein biomarkers and urinary albumin concentrations at days 3 and 10 were observed (Table 5). At day 3, the strongest correlations with urinary albumin were for clusterin ($r=0.671$) and TFF3 ($r=0.646$). At day 10, the strongest correlations with urinary albumin were for KIM-1 ($r=0.765$) and MCP-1 ($r=0.761$).

Association of Patient-Specific Factors with Urinary Concentrations of Protein Biomarkers

Several patient-specific factors were assessed for relationships with concentrations of urinary protein biomarkers including: age, sex, BMI, presence of concomitant disease, use of intravenous (IV) contrast dyes, number of cisplatin cycles, prescribed dose, and body surface area (BSA)-based dosing. There were no correlations between any of the 12 biomarkers and age or BMI (data not shown). Urinary clusterin levels at day 3 were 5-fold lower in females than males. There were no other significant sex differences at other time-points (baseline, 3 or 10 day) or for other biomarkers (Supplemental Table 4). There were also no consistent correlative relationships between biomarkers and prescribed dose, BSA-based dose, or total cumulative dose received (data not shown).

We also assessed relationships between protein biomarker concentrations and medical record documented concomitant diseases (type 2 diabetes mellitus, hyperlipidemia, Hashimoto's thyroiditis, Human Immunodeficiency Virus, hypertension) at each time-point (Supplemental Table 5). Urinary albumin concentrations at baseline and at day 3 were significantly elevated in patients noted to have any of these concomitant diseases. Patients who had received IV contrast dye at least 30 days prior to cisplatin infusion had higher urinary IL-18 protein levels at baseline as well as elevated urinary GST-pi protein levels at day 3 (Supplemental Table 6).

Discussion

We report novel time-dependent changes in the urinary excretion of protein biomarkers in the absence of clinical AKI in patients receiving cisplatin. Compared to baseline values, several proteins were significantly increased in urine including KIM-1, clusterin, albumin, cystatin C and TFF3, which were elevated 2-fold by day 10. Urinary β 2M concentrations were elevated 3-fold by day 3 whereas calbindin levels were increased 8-fold on day 10. More moderate increases (less than 2-fold difference in means) were observed for GST-pi and MCP-1 on day 10. Overall, traditional biomarkers, eGFR, BUN, and SCr did not change significantly from before to 13 ± 7 days after administration of cisplatin.

The incidence of AKI varies between 8-40% depending on cisplatin dose, frequency of administration, and peak free platinum concentrations in plasma (18). Based on these estimates, we expected a higher number of AKI episodes than resulted in this study, where only one patient met the KDIGO criteria for Stage 2 AKI (2). Other studies have used different AKI criteria based on arbitrary cut-offs in SCr or eGFR increase from baseline (19, 20). The lower incidence of AKI in this study could also be due to several factors including a limited sample size, the timing of blood collection for biomarkers, and the low to moderate cisplatin dose ranges prescribed (as low as 25 mg/m²). Patients were largely treatment-naïve and varied in age (26-80 years). Patients over 50 years of age tend to have a higher incidence of cisplatin nephrotoxicity (21). Additionally, African American patients are more susceptible than other races to nephrotoxicity and were not well-represented in the current study, which was composed of largely Caucasian patients (Table 1) (1). AKI is normally detected within 48 h to 7 days after cisplatin administration. Due to the non-interventional nature of the study, the measurement of traditional serum biomarkers such as SCr and BUN occurred over a range wider than desired (13 ± 7 days), as clinically determined. It is possible that earlier assessment of SCr and BUN may have captured AKI in a greater number of patients.

Normal urinary protein biomarker ranges have been recently defined for healthy volunteers (Table 6) (22). The baseline protein concentrations for KIM-1, calbindin, clusterin, cystatin C, albumin, NGAL, osteopontin, and TFF3 in our cancer patients fell within or were close to the ranges reported. However, the values at the upper range of baseline in cancer patients tended to be higher than healthy volunteers, in particular for clusterin, β 2M, cystatin C, NGAL, osteopontin and TFF3 (Table 6). β 2M is an exception where the mean baseline concentration was elevated by 1.5-fold from the reported range in healthy volunteers (22). The mean concentrations of cystatin C, albumin, NGAL, and TFF3 post-cisplatin infusion were also within the ranges reported for healthy volunteers. Several of the top performing biomarkers had elevated levels above healthy volunteer ranges on day 3 (for β 2M) and day 10 (for KIM-1 and calbindin). These findings support previous studies showing elevations in urinary β 2M and calbindin in subclinical AKI and urinary KIM-1 increases in clinical AKI in patients treated with cisplatin (14, 16).

We have reported both absolute and normalized biomarker values in this study to determine whether normalization altered the ability to detect changes in concentrations after cisplatin infusion. Overall, the top performing biomarkers were consistent whether expressed as

normalized to creatinine or as absolute concentrations. Biomarker values normalized to creatinine are often reported, due to variability in urine output between patients. The underlying assumption is that urinary creatinine excretion is constant within an individual and that biomarker excretion is linear with urinary creatinine excretion, neither of which may be the case (3, 23, 24). Studies have also reported that absolute concentrations were more informative in distinguishing AKI on hospital admission; however, normalized concentrations better reflected long-term outcomes including death and dialysis (14, 25).

Investigating the biological roles of top biomarkers could inform our understanding of the sequence of injury, functional loss, and repair processes that occur after cisplatin exposure. Biomarkers reflective of functional proximal tubule (PT) injury (β 2M and cystatin C) peaked at day 3. β 2M is a low-molecular weight protein filtered through glomeruli and extensively reabsorbed by PTs (26). β 2M appears in the urine when plasma concentrations exceed the renal reabsorptive threshold (5 mg/L) or with PT damage. In this study, we observed an increase in β 2M levels at day 3 that was diminished by day 10. This pattern of urinary β 2M was similar in another study of cisplatin-treated patients at days 4 and 8 without overt AKI post treatment (16). Albumin is also normally reabsorbed by the PTs. Therefore, albuminuria is often observed during direct tubular toxicity (27). In this study, urinary albumin exhibited a time-dependent increase at both days 3 and 10. Furthermore, many of the biomarkers, both the top performers as well as those that did not reflect cisplatin exposure, exhibited high correlations with urinary albumin. However, as shown in this study and by others, albuminuria also occurs in the presence of concomitant diseases such as hypertension and diabetes mellitus. Thus, finding an accurate cut-off value for predicting tubular toxicity using urinary albumin may be complicated (reviewed in 28).

Biomarkers with high fold changes from baseline on day 10 (KIM-1 and TFF3) have been associated with tubule repair. KIM-1 is a transmembrane protein found on the apical membrane of PTs following injury (29, 30). The ectodomain of KIM-1 is cleaved and shed into the tubular lumen. It can be detected in the urine of multiple species including humans. Immunohistochemistry has shown that KIM-1 is expressed in de-differentiated and regenerating PT cells (31) and is difficult to detect in completely atrophic cells. Functionally, KIM-1 can confer phagocytic capabilities to PT cells, allowing enhanced clearance of apoptotic cell debris. Similar protective functions have been described for TFF3. TFF3 is a small peptide hormone that is secreted by mucus-producing and epithelial cells. Although its role in the kidney is largely unknown, TFF3 has been shown to play a role in mucosal and surface maintenance, inhibition of apoptosis, promotion of cell survival and migration in the lung and intestine (32). Cisplatin-treated rats showed markedly decreased levels of TFF3 in the kidneys and urine compared to control rats across two dose groups (3.5 and 7 mg/kg) at 3 and 8 days (reviewed in (33)). In patients, higher circulating and urinary TFF3 has been associated with the severity of chronic kidney disease and certain cancers (34, 35). In our study, urinary TFF3 exhibited a time-dependent *increase* post cisplatin, which may reflect species differences in toxicity responses.

Calbindin is a calcium-binding protein found primarily on distal tubules (DT) and collecting ducts of the kidney (36). Although calbindin is associated with DT damage, it has also been shown to be up-regulated *in vitro* in an immortalized human proximal tubule cell line, HK-2,

after exposure to cisplatin (16, 37). In a clinical study, urinary calbindin peaked in response to cisplatin (n=14, 70 mg/m²) after 8 days but in the absence of changes in SCr, BUN, and creatinine clearance (16). Additionally, calbindin concentrations did not change in response to administration of other chemotherapy drugs in the same patients. These findings support our data and holds promise for calbindin as a specific indicator of cisplatin induced injury even in the absence of overt functional damage.

NGAL, IL-18 and osteopontin did not show significant time-dependent changes in our study. There have been several reports suggesting these proteins are early biomarkers of AKI in cancer patients and rodents treated with cisplatin (38, 39). Although identified roles for these proteins in AKI are limited, they are most often associated with inflammation. Both NGAL and osteopontin are associated with the thick ascending Loop of Henle, DT and/or collecting ducts (40, 41). Therefore, NGAL and osteopontin may not be sensitive for PT injury alone and may require a threshold of necrosis and inflammation that involves multiple nephron regions for them to be up-regulated and/or secreted. Additionally, as mentioned by Shinke et. al. (2015), it is possible that the selection of time points account for differences in the ability of these three biomarkers to detect cisplatin nephrotoxicity (14). Previous studies in cisplatin treated patients have reported that early time points (12 h – 3 days) showed increased NGAL and IL-18 levels compared to baseline (13, 14).

Given the lack of overt nephrotoxicity, it was surprising to see statistically significant, but clinically insignificant, decreases in the serum electrolyte panel. Depletion of electrolytes including magnesium, calcium, potassium, sodium, and phosphate has been associated with cisplatin nephrotoxicity (42). The reabsorption of many electrolytes including sodium, chloride, and calcium occurs primarily in the PT and cisplatin-induced damage may explain reduced serum levels. However, total serum calcium is also affected by serum protein loss (46% protein bound) (reviewed in 43). The lack of significant changes in magnesium is not surprising since hypomagnesemia is more commonly associated with DT injury (44). Although PT injury may contribute, it is difficult to attribute these minor electrolyte changes to kidney toxicity as they fluctuate with volumes administered and can be affected by many factors including diet, protein binding, hormones, and/or underlying disease processes such as cancer.

Elevations in urinary biomarker concentrations did not correlate with patient-specific factors including age, sex, or BMI. Although age is a factor affecting cisplatin nephrotoxicity, the age range tested in this study was wide. Furthermore, it may be possible that without overt AKI these factors may not impart as large of a role. Predictably, urinary albumin was elevated in patients with concomitant diseases at baseline and day 3. Many chronic conditions, including diabetes mellitus and hypertension, result in glomerular damage that increases urinary albumin excretion (28). IL-18 was significantly elevated at baseline in patients that had previously received IV contrast dye. Studies have shown that IL-18 was able to predict contrast-induced nephropathy earlier than SCr in patients undergoing percutaneous coronary intervention after 12 h (AUROC: 0.811) (45). The up-regulation of inflammatory cytokines, such as IL-18 and the antioxidant enzyme GST-pi, could be explained by the fact that many contrast agents induce free radicals and oxidative stress (46). Across the different cisplatin dose parameters (prescribed dose, BSA-based dose and

cumulative dose) there were no consistent biomarker changes. Studies have shown that BSA-based dosing does not increase the accuracy of predicting cisplatin exposure (47). Other factors not assessed in this study, including race, tumor type, and cancer stage, may contribute to cisplatin injury. The sample size of the study limited the number of covariates that could be assessed for their influence on cisplatin injury.

In conclusion, the present study has shown that certain urinary biomarkers might be particularly sensitive for detecting cisplatin-induced subclinical AKI. Multiple urinary biomarkers (KIM-1, calbindin, β 2M, clusterin, MCP-1, cystatin C, GST-pi, albumin, and TFF3) showed time-dependent elevations for detecting cisplatin exposure in the absence of clinically detectable AKI. Additionally, commonly studied biomarkers in various settings of AKI (NGAL, IL-18, osteopontin) did not show any significant changes. It should be recognized that these conclusions are based on aggregate data and thus, individual fold changes for each subject have been provided (Supplemental Table 1). Further understanding of the pathophysiological role of these proteins and whether they are involved in tubular injury or repair is necessary to utilize them as specific indicators of cisplatin-induced AKI and for selecting the correct time points for quantification. Likewise, greater investigation is needed to understand the utility of profiling multiple biomarkers, rather than one or two candidates, for detecting clinical or subclinical AKI.

Methods

Selection of Participants

A prospective study of patients receiving outpatient chemotherapy for various solid tumors at the University of Colorado Cancer Center, Aurora, CO, a National Cancer Institute-Designated Consortium Comprehensive Cancer Center was conducted. Fifty-six patients received cisplatin 25 mg/m² intravenously while one patient was administered cisplatin intraperitoneally. Patients were hydrated pre- and post-treatment with saline (1-2 L). Study inclusion criteria included: 1) Age $\geq$ 18 years, 2) hemoglobin $\geq$ 10 g/dL, 3) no consumption of grapefruit juice or alcohol within 7 days, 4) no history of alcohol consumption of >14 drinks/week, 5) no history of organ transplantation or kidney dialysis, 6) willingness to comply with study, 7) not pregnant or lactating, 8) no changes in medications within previous 4 weeks, 9) normal liver function (alanine aminotransferase and aspartate aminotransferase $<2\times$ upper limit of normal) and 10) baseline eGFR > 60 mL/min/m² (using the 4-variable Modification of Diet in Renal Disease equation) (48). Exclusion criteria included: 1) diagnosis of kidney cancer, 2) previous exposure to platinum-based chemotherapy (other than the currently prescribed regimen), 3) herbal supplement use, 4) exposure to other known nephrotoxins (including contrast agents) within the previous 30 days, and 5) concurrent use of inhibitors of transport proteins involved in cisplatin secretion into urine. The Institutional Review Boards at the University of Colorado (Protocol 12-1510) and Rutgers University (Protocol E13-716) approved the protocols for recruitment and sample collection. Twenty-eight patients were recruited before the first cycle of cisplatin therapy and twenty-nine patients were recruited before the second cycle of cisplatin therapy. The average length of time between the first and second cycles of cisplatin was 17 days (range of 6 – 34 days).

Urine Samples

Urine was collected from spontaneous voids at baseline (pre-cisplatin infusion), between 2-5 days (designated as day 3) and 9-11 days (designated as day 10) post-cisplatin infusion. For patients missing baseline urine collection, urine in the 0-2 hour timed collection after cisplatin infusion was used for analysis. Urine was centrifuged at 3000xg and supernatant was aliquoted into 2 mL collection tubes and frozen within 30-60 minutes of collection at -80°C. At the time of analysis, samples were thawed and placed on ice and centrifuged at 1500 rpm for 5 minutes. Ten µl of supernatant were pipetted for biomarker analysis.

Quantification of Urinary Protein Biomarkers

Calbindin, clusterin, KIM-1, GST-pi, IL-18, MCP-1, albumin, β2M, cystatin C, NGAL, osteopontin, and TFF3 were measured using Bio-Plex Pro RBM human kidney toxicity assay panels 1 and 2 (Bio-Rad, Life Science, Hercules, CA). Washing steps were conducted using the Bio-Plex Pro II wash station (Bio-Rad). Samples were analyzed using a Bio-Plex, MagPix Multiplex Reader (Bio-Rad), which reports the mean fluorescence intensity (MFI) proportional to the concentration of analyte bound to each bead. Concentrations were extrapolated from a known standard curve using a five-parameter logistic curve. Recommended dilutions of urine samples in dilution buffer provided in the assay kit were followed (1:10 for panel 1 and 1:50 for panel 2). Values that were above the detection limit for the Bio-Plex assays were diluted and re-analyzed. Concentrations below the limit of detection were substituted with the lower limit of quantification divided by 2 (Supplemental Table 7). Data are presented as absolute concentrations and normalized to urinary creatinine concentrations quantified using the DCA Vantage Analyzer (Siemens, Princeton, NJ).

Data and Statistical Analysis

Data are presented for individual patients together with the corresponding group mean ± SD (baseline, days 3 and 10). Data were tested for normality using the D'Agostino-Pearson omnibus test. Time-dependent differences among individual biomarkers were evaluated by Wilcoxon rank-sum tests for absolute concentrations and ANOVA to test normalized biomarker concentrations. Differences in patient-specific factors (categorical) were assessed by ANOVA. Clinical laboratory values for pre- and post-cisplatin treatment were compared using paired t-tests. Differences were considered statistically significant at p<0.05. Pearson or Spearman correlation coefficients were used to measure the strength of association between biomarker and urinary albumin concentrations, kidney function measures or patient-specific factors (continuous). All statistical analyses and plots were done by GraphPad Prism V6 (GraphPad Software, La Jolla, CA), Partek Genomics Suite (Partek GS 6.4, St Louis, CA) or SAS 9.4 (SAS Institute Inc. Cary, North Carolina).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Highlights

- What is the current knowledge on the topic? Current clinical measures of cisplatin-induced nephrotoxicity are inadequate at detecting low levels of injury. Emerging data in rodents and humans suggest that proteins excreted into the urine after cisplatin administration may provide more sensitive, non-invasive detection of toxicity.
- What question did this study address? The current study assesses the time-dependent urinary excretion of 12 protein biomarkers in 57 patients receiving cisplatin-containing chemotherapy and explores relationships with traditional clinical markers, patient sex, concomitant diseases and the use of intravenous contrast dye.
- What this study adds to our knowledge - Multiple biomarkers (notably, β 2M, KIM-1, TFF3, and Calbindin) exhibited significant time-dependent elevations in urine in the absence of clinical nephrotoxicity. Urinary β 2M concentrations were elevated 3.3-fold by day 3 in patients receiving cisplatin whereas greater increases in KIM-1 (2.8-fold), TFF3 (2-fold) and Calbindin (8.3-fold) were observed on day 10.
- How this might change clinical pharmacology or translational science – Early detection of clinical and subclinical changes in renal function would strengthen a clinician's ability to prevent further damage and identify patients at-risk for cisplatin nephrotoxicity.

Figure 1. Time-Dependent Changes in Absolute Urinary Concentrations of Protein Biomarkers following Cisplatin Infusion

Urinary protein concentrations were measured using a Bio-Plex assay at baseline (n=57), 3 (range of 2-5, n=50) days and 10 (range of 9-11, n=47) days post cisplatin-infusion during the first or second cycle of chemotherapy. Concentrations were measured in urine supernatants and presented as ng/mL. Scatter plots show individual levels (n=57) and mean $\pm$ SD concentration at each time point. Solid lines represent statistically significant differences ($p < 0.05$) compared to baseline protein levels.

Table 1

Demographic Information for Patients (n=57) Receiving Cisplatin

Age (mean ± SD)	56.6 ± 13.2 years
Gender	Male = 29
	Female = 28
Body Mass Index (mean ± SD)	27.0 ± 6.0 kg/m ²
Cisplatin Dose (mean ± SD)	63.4 ± 22.8 mg/m ²
Race	Caucasian = 51
	Hispanic = 4
	African American = 1
	Not Reported = 1

Table 2

Dose of Cisplatin According to Tumor Type

Tumor Type	Patients (n, %)	Dose (mg/m²) mean $\pm$ SD^a
Head/Neck	14, 25%	52.1 $\pm$ 22.5
Lung	10, 18%	66.6 $\pm$ 18.2
Genital	9, 16%	51.1 $\pm$ 22.6
Digestive	9, 16%	61.1 $\pm$ 20.1
Melanoma	6, 11%	76.0 $\pm$ 9.8
Bladder/Pelvis	4, 7%	70.0 $\pm$ 0.0
Bone/Blood	2, 4%	97.5 $\pm$ 31.8
Lymphoma/Sarcoma	2, 4%	60.0 $\pm$ 56.6
Breast	1, 2%	75.0

^aThe average length of cisplatin infusion was 1-2 h and fractioned cisplatin doses were administered to 9 out of 57 patients.

Table 3
Clinical Laboratory Findings in Patients (n=57) Before and After Cisplatin Infusion

Clinical Parameter	Before Mean $\pm$ SD	After ^a Mean $\pm$ SD	P	Normal Reference Range
Mg ²⁺ (mEq/L)	1.7 $\pm$ 0.2	1.6 $\pm$ 0.3	0.220	1.3 – 2.1
Na ⁺ (mEq/L)	137 $\pm$ 2.4	135 $\pm$ 3.5	0.001	133 – 145
K ⁺ (mEq/L)	4 $\pm$ 0.5	4 $\pm$ 0.7	0.712	3.5 – 5.1
Ca ²⁺ (mEq/L)	9.6 $\pm$ 2.3	9.2 $\pm$ 2.3	0.0002	8.6 – 10.3
HCO ₃ ⁻ (mEq/L)	25 $\pm$ 2.1	25 $\pm$ 2.5	0.196	21 – 31
Cl ⁻ (mEq/L)	104 $\pm$ 3.3	103 $\pm$ 5.1	0.026	90 – 108
SCr (mg/dL)	0.82 $\pm$ 0.2	0.86 $\pm$ 0.2	0.330	0.70 – 1.30
BUN (mg/dL)	14.1 $\pm$ 5.4	14.5 $\pm$ 6.1	0.529	7 – 25
eGFR (mL/min/1.73m ²)	89.4 $\pm$ 24.8	90.6 $\pm$ 24.7	0.878	60
Urine Albumin/Cr (mg/mg) ^b	29.9 $\pm$ 40.6	50.7 $\pm$ 95.2	0.092	NA

^aThe time frame for assessment of clinical parameters was 13 $\pm$ 7 days post cisplatin infusion.

^bThese data were assessed on day 3 post-cisplatin infusion.

NA: not applicable

Table 4
Time-Dependent Changes from Baseline in Urinary Protein Biomarkers following Cisplatin Infusion^a

Biomarker (ng/mL)	Baseline (mean ± SD)	Day 3 (mean ± SD)	P	Day 10 (mean ± SD)	P
KIM-1	0.203 ± 0.293	0.332 ± 0.524	0.024	0.575 ± 0.711	0.002
Calbindin	52.50 ± 83.59	60.63 ± 65.96	0.094	434.4 ± 856.4	<0.0001
Clusterin	57.44 ± 211.9	40.39 ± 101.0	0.043	108.2 ± 422.7	0.005
GST-pi	27.40 ± 74.07	33.43 ± 93.49	0.221	43.76 ± 88.96	0.011
IL-18	0.089 ± 0.223	0.042 ± 0.067	0.158	0.126 ± 0.189	0.061
MCP-1	0.507 ± 1.154	0.381 ± 0.549	0.330	0.838 ± 1.124	0.003
Albumin ^b	10.84 ± 13.86	15.92 ± 15.28	0.028	21.68 ± 24.08	0.008
β2M	136.9 ± 245.0	452.1 ± 422.2	<0.0001	188.1 ± 262.3	0.026
Cystatin C	32.91 ± 45.72	60.91 ± 119.1	0.015	57.71 ± 102.1	0.024
NGAL	43.45 ± 80.59	42.03 ± 59.39	0.346	51.77 ± 75.99	0.168
Osteopontin	1,235 ± 1,504	1,240 ± 1,597	0.784	2,630 ± 3,854	0.055
TFF3	755.9 ± 931.4	1,282 ± 1,187	0.001	1,531 ± 1,505	0.002

^aUrinary biomarker concentrations were not normalized to creatinine levels for this analysis.

^bThe units for urinary albumin concentrations are µg/mL.

Table 5Correlation Coefficient (r) of Biomarker and Urinary Albumin Concentrations^a

Absolute Biomarker	Albumin 3-Day Absolute		Albumin 10-Day Absolute	
	r	P	r	P
KIM-1	0.547	<0.0001	0.765	<0.0001
Calbindin	0.459	0.00079	0.714	<0.0001
Clusterin	0.671	<0.0001	0.731	<0.0001
GST-pi	0.512	0.00015	0.557	<0.0001
IL-18	0.377	0.00762	0.731	<0.0001
MCP-1	0.553	<0.0001	0.761	<0.0001
β2M	0.424	0.00215	0.417	0.004
Cystatin C	0.539	<0.0001	0.693	<0.0001
NGAL	0.546	<0.0001	0.696	<0.0001
Osteopontin	0.435	0.00158	0.574	<0.0001
TFF3	0.646	<0.0001	0.701	0.000

^aUrinary biomarker concentrations were not normalized to creatinine levels for this analysis.

Table 6

Baseline Reference Ranges for Absolute Urinary Biomarkers in Treatment Naïve Solid Tumor Patients Compared to Healthy Volunteers

	Solid Tumor (N=28) ^a	Healthy (N=39) (Brett et. al.)
Age (mean ± SD)	60.7 ± 12.2	43.9 ± 12.4
Male/Female	13/15	20/19
BMI (mean ± SD)	28.2 ± 7.2	24.4 ± 2.7
Race (% Caucasian)	93	97
Biomarkers (ng/mL)		
KIM-1	0.005 – 0.92	0.05 – 0.48
Calbindin	1.1 – 220.1	0.31 – 114.0
Clusterin	0.15 – 911.5	0 – 70
GST-pi	0.25 – 91.7	NR ^c
IL-18	0.01 – 1.6	NR
MCP-1	0.002 – 1.8	NR
Albumin ^b	0.32 – 53.6	0.4 – 62.0
β2M	0.99 – 1275.7	10 – 90
Cystatin C	0.08 – 277.7	3.2 – 75.0
NGAL	0.4 – 512	3 – 254
Osteopontin	1.1 – 7508	66 – 1230
TFF3	25.1 – 5485	30 – 3100

^aThese values are the baseline concentrations in cisplatin-naïve patients enrolled during cycle 1.

^bThe units for urinary albumin concentrations are µg/mL.

^cNR: Not reported.