Urinary Metabolomic Analysis to Detect Changes After Intravenous, Non-ionic, Low Osmolar Iodinated Radiocontrast for Computerized Tomographic Imaging

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Introduction: Contrast-induced nephropathy is a result of injury to the proximal tubules caused by oxidative stress and ischemia. Metabolomics is a novel technique that has been used to identify renal damage from drug toxicities. The objective of this study is to analyze the metabolic changes in the urine after dosing with intravenous (IV) contrast for computed tomograph (CT) of the chest

Methods: A convenience sample of patients undergoing a chest CT with IV contrast who had at least one of the following: age ≥50 years, diabetes, heart failure, chronic kidney disease, coronary artery disease, or diastolic blood pressure >90 mmHg -- were eligible for enrollment. Urine samples were collected prior to imaging and 4-6 hours post imaging. Samples underwent gas chromography/ mass spectrometry profiling. We measured peak metabolite values and log transformed data. Paired T tests were calculated. We used significance analysis of microarrays (SAM) to determine the most significant metabolites.

Results: The cohort comprised 14 patients with matched samples; 9 /14 (64.3) were males, and the median age was 61 years (IQR 50-68). A total of 158 metabolites were identified. Using SAM we identified 9 metabolites that were identified as significant using a delta of 1.6.

Conclusion: Changes in urinary metabolites are present soon after contrast administration. This change in urinary metabolites may be potential early identifiers of contrast-induced nephropathy and could identify patients at high-risk for developing this condition. [West J Emerg Med. 2014;15(2):152–157.]

INTRODUCTION

Contrast-induced nephropathy (CIN) is the third leading cause of acute renal failure in hospitalized patients. Although the exact mechanism for the development of CIN is unknown, data support that factors involved in the process may include direct toxicity to the renal tubular epithelium, oxidative stress, ischemic injury and tubular obstruction.[1] The increasing number of indications for the use of computed tomography (CT) with contrast in the emergency department (ED) has led to concerns that the incidence of contrast- induced nephropathy may increase. It has been reported that the use of contrast-enhanced CT has increased over 200% in the last decade. [2] Studies have reported that the risk for CIN is highest is patients with heart failure, diabetes, prior renal failure, hypertension, and increasing age.[3] Unfortunately, there are currently no methods to identify subjects at risk for CIN; therefore, these patients are only identified after they develop signs of acute kidney injury, which in many cases (such as

in chronic kidney disease patients) leads to the progression toward end stage renal disease.

Recent advances in the use of metabolomics have allowed the identification of specific metabolite patterns or profiles associated with acute kidney injury. Metabolic profiling refers to the measurement of a group of small molecule metabolites that reflect cellular responses to organ injury. In particular, using high performance liquid chromatography (HPLC) and gas chromatography (GC) coupled to mass spectrometry has allowed the quantification and identification of these metabolites.

We hypothesize that patients who are prone to CIN have a metabolic change that predisposes them to CIN soon after contrast administration; if this is the case, metabolomics is an ideal mechanism for the early identification of patients at risk of developing CIN as it allows the identification of the multiple markers of injury. However, the first step in proving this hypothesis is to determine that a metabolomic change occurs after intravenous contrast administration. We therefore completed a pilot study evaluating changes in urinary metabolites before and after intravenous contrast administration in a high-risk group of patients for developing contrast-induced nephropathy. The specific aim was to identify urine metabolites that change after IV contrast administration.

METHODS

This is a pilot study of prospectively identified patients undergoing a CT of the chest with intravenous contrast during their ED evaluation. The institutional review boards at all participating centers approved this study.

Study Setting and Selection of Participants

A convenience sample of patients was enrolled. To be eligible for the study, patients had to be >18 years old, undergoing CT angiography of the chest and have at least 1 of the following high-risk features for CIN: Diabetes,[4-5] coronary artery disease,[3] congestive heart failure,[4,6] chronic kidney disease (baseline creatinine >1.5mg/dl or GFR <60 ml/min/1.73 m^2.[7] Additionally, patients must have been given a physician assessment of >75% likelihood of hospital admission. All patients received between 110 and 130 mL of non-ionic radiocontrast (Omnipaque 350, GE Healthcare Inc., Princeton, NJ), delivered via automated timing injector into an arm vein.

We excluded patients from the study if they had an estimated GFR <15 mls/min/1.73m^2, a history of organ transplantation, were currently on immunosuppressive medications, were septic or on antibiotic therapy, had a history of, or were currently receiving dialysis of any type, or had an exposure to iodinated radiocontrast within 3 days prior to the study.

Data Collection and Processing

After a patient was identified as fulfilling inclusion and exclusion criteria, and informed consent was obtained, we collected data prospectively. This included race, demographics, dietary history, medical history, and physical examination. Additionally, electrocardiogram (ECG) findings as documented by the treating emergency physician were recorded. Medical history was confirmed through patient self-report and review of the medical record when available. Medications administered in the ED and before arrival were also recorded. The final ED diagnosis was recorded and based on the treating physician's impression. No laboratory tests were mandated as part of the trial study. Laboratory tests and chest radiography findings, as documented by a board-certified radiologist, were obtained from the medical record.

Urine samples were collected as a midstream sample or via a foley bag if one was already in place at the time of study enrollment. Urine samples were collected prior to imaging and 4-6 hours post imaging. Samples were divided into 2 ml aliquots and frozen at -80°C. We compared each patient's pre-CT urine peak metabolite levels to their own individual post-CT urine peak metabolite levels.

We followed all patients by chart review throughout their index stay to document in-hospital events. Serum creatinine levels were recorded at presentation, 24 and 48 hours.

GC-MS Analysis

Urine samples require no preparation prior to freezing. Neat urine samples were lyophilized without further pretreatment. To the dried samples, we added 20 µl of 40 mg/ml methoxylamine hydrochloride in pyridine, and samples were agitated at 30°C for 30 min. Subsequently, 180 µl of trimethylsilylating agent N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was added, and samples were agitated at 37°C for 30 min. GC–MS analysis was performed using a Agilent 6890 N gas chromatograph (Atlanta, GA, USA) interfaced to a time-of-flight (TOF) Pegasus III mass spectrometer (Leco, St. Joseph, MI, USA).[8-10] Automated injections were performed with a programmable robotic Gerstel MPS2 multipurpose sampler (Mülheim an der Ruhr, Germany). We performed initial peak detection and mass spectrum deconvolution with ChromaTOF software (version 2.25, Leco), and later exported samples to the netCDF format for further data evaluation with MZmine and XCMS. We identified samples with >50% certainty. If this degree of certainty was not met they were given the name (unknown) followed by a numeric number.

Statistical analysis

We presented continuous data as medians and interquartile range (IQR). The statistical analysis was performed on (natural) log-transformed peak concentration data to account for increases in the data variance that can occur. We performed paired T-test to determine differences in the metabolites with a p-value threshold of <0.05 mg. We used SAM, which assigns a score to each metabolite on the basis of change in metabolite expression relative to the standard

Table 1. Delta values and significant metabolites.

Delta	Significant metabolites	False discovery rate
0.07	80	0.086
1.3	22	0.045
1.6	11	0.016
2.0	4	0.004

Table 2. Demographics and patient characteristics.

Variable	N (%) or mean (% or SD)
Age (median, IQR)	61 (50,68)
Male	9 (64.3)
Caucasian race	7 (50.0)
Smoker	8 (57.1)
Heart failure	5 (35.7)
Diabetes	6 (42.9)
Hypertension	11 (78.6)
Coronary artery disease	5 (35.7)
Heart rate bpm (median, IQR)	92.5 (79,112)
Systolic blood pressure mm/Hg	140 (119,157)
Serum creatinine mg/dL (median, IQR)	0.94 (0.83,1.14)
Serum BUN mg/dL (median, IQR)	13.5 (9,22)
48 hour change in creatinine mg/dL (median, IQR)	0.02 (-0.04, 0.03)
Time since last meal hours (median, IQR)	11.2 (6.5, 19.2)
ED treatment	
Diuretics	3 (23.1)
Steroids	2 (15.3)
Final diagnosis	
Chest pain-not otherwise specified	6 (42.9)
Asthma/COPD	2 (15.4)
Heart failure	1 (7.7)
Other (pulmonary emboli, pneumonia, effusion)	5 (35.7)

BPM, beats per minute; *IQR,* interquartile range; *BUN,* blood urea nitrogen, *ED*, emergency department; *COPD*, chronic obstructive pulmonary disease

deviation of repeated measurements, to determine the most significant metabolites. For metabolites with scores greater than an adjustable threshold, SAM uses permutations of the repeated measurements to estimate the percentage of metabolites identified by chance, the false discovery rate (FDR). This adjustable threshold is determined by a tuning parameter (delta). The optimal delta was selected based on the target identification of 10 significant metabolites. (Table 1) We performed statistical analysis using MetaboAnalyst (Canada).[11]

RESULTS

The cohort comprised 14 patients with matched samples pre and post CT; 9 were males (64.3%) and the median age was 61 years (IQR 50-68). A majority of the patients had a history of hypertension 11 (78.6). There was no difference between the creatinine values at pre-CT compared to 24 or 48 hours. The most common final diagnosis was chest pain not otherwise specified. The median time from the last food intake in these patients was 11.2 (IQR 6.5, 19.2) (Table 2).

We identified a total of 158 metabolites. In the univariate analysis we identified 20 metabolites with significant difference between pre- and post-peak concentrations (Table 3).

Using SAM we identified 9 metabolites that were identified as significant using a delta of 0.6. The false discovery rate was 1.6%. All 9 covariates had a significant decrease in peak concentration after CT. Of the covariates, 2 were not identified as known metabolites. In addition, 3 were noted to be amino acids, 2 were heterocylcic compounds, and 1 was a breakdown product of carbohydrates.

DISCUSSION

Alteration in renal function has been reported to occur in 4-20% of patients undergoing CT angiograms of the chest and head. Contrast-induced nephropathy (defined as an increase of serum creatinine of 25% or higher within 2 days of receiving contrast or as a rise in plasma Cr of 0.5 mg/dL above baseline) is a leading cause of acute renal failure in this hospitalized patient population.[12] Identification in the alteration in renal function is delayed when using serum creatinine as a marker of injury. In this study we were able to detect changes in urinary metabolites within 6 hours after the administration of intravenous contrast.

This study provides the foundation to further evaluate the association of change in urinary metabolites and the development of CIN after CT imaging of the chest with contrast and identify specific metabolism pathways associated within the kidney after contrast administration. Intravenous contrast has been associated with renal toxicity. The mechanisms behind the development of CIN are complex. It has been suggested that the development of CIN is a result of the interplay of vasoconstriction, oxidative stress, and direct tubular toxicity leading to hypoxia of the outer medulla.[13,14-15] Numerous studies have reported on the effects of contrast media on various urinary enzymes and markers of glomerular and tubular function.[16-20] The complex pathophysiology behind the renal effects of intravenous contrast provides the opportunity to identify multiple markers of potential renal injury.[21]

After contrast dye administration there is a transient increase in renal blood flow followed by a decrease in renal blood flow due to vasoconstriction. This vasoconstriction

Table 3. Paired T test of log adjusted peak concentrations.

Peaks(mz/rt)*	p.value	-log10(p)
Furoylglycine	0.00123	2.90872
Unknown31&	0.00182	2.73956
Creatinine	0.00213	2.67232
Unknown28&	0.00548	2.26161
5-hydroxymethyl-2-furoic -acid	0.00586	2.23236
Levoglucosan	0.00599	2.2227
Cystine	0.01051	1.97832
Adenosine	0.01106	1.95643
Sucrose	0.01361	1.86613
Unknown34&	0.01609	1.79347
Glycerol-3-galactoside	0.01646	1.78365
Quinic acid	0.02317	1.63509
Palmitic acid	0.0237	1.62519
Taurine	0.02783	1.55552
Succinic acid	0.03123	1.50543
Idonic acid	0.04061	1.39138
hydroxypyridine	0.0421	1.37569
Deoxyribonic acid	0.04327	1.36383
Lactobionic acid	0.0449	1.34779
Glutamine	0.04787	1.31997

* mz/rt: specific mass-charge ratios (mz) /LC column retention times (RT)
&metabolites that were not identified were labelled "Unknown" followed by a numeric value

may be mediated by adenosine, Angiotensin II or calcium. In addition, there appears to be direct damage to the kidney as a result of morphological alterations including proximal tubularvacuolar transformation, interstitial edema and tubular degeneration.[22]

Studies have reported that ischemia from renal vasoconstriction causes cell sodium and chloride concentrations to rise, and cell potassium and phosphorus concentrations as well as cell dry weights to fall.[21] These changes are most pronounced in the proximal straight tubule (PST) cells. PST show deranged electrolyte homeostasis for a prolonged period after injury.[23] Amino acids such as glycine, alanine, and taurine have been shown to be protective to renal tubular cells when studied in vitro. In one study, the majority of amino acid derangements noted in the were PST cells were reversed except for low glycine contents in the cortex, whereas in the outer medulla aspartate, glycine and taurine contents were diminished.[24] These results indicate increasing manifestation of PST cell injury in the reflow period. Studies have shown that as PST are injured there is a depletion of intracellular pools of amino acids as a result of injury to Na+ pump function. In injury there is also an alteration of catabolism of alanine and glutamate formation.[25-26] We noted a decrease in glycine, n-(2-furanylcarbonyl)-in the urine, during our study. This raises the potential of amino acid supplementation as a potential nephrotoxicity protectant.

We also reported a decrease in 5-hydroxymethlfurfural (HMF) levels after CT of the chest. HMF is largely formed by breakdown of hexoses such as glucose and fructose. It is bioactivated through sulfonation of its acyclic hydroxyl functional group to 5-sulfooxymethylfurfural (SMF). It can also be metabolized in the kidneys to 5-hydroxy-methyl-2-furoic acid and other compounds such as glycine compounds in the kidney which are then secreted in the urine. The presence of HMF has been associated with nephrotoxicity in rats although never directly studied in humans.[27] HMF is produced by the oxidation of sugars through decarboxylation, oxidation, dehydration and reduction. The exact mechanism of decreased elimination in the urine is unknown.

In the kidney, adenosine locally activates adenosine A2 receptor and adjusts blood flow to meet demand. It is an intra-renal metabolite that accumulates in the kidney during renal ischemia. Elevated serum adenosine levels have been associated with a reduction in glomerular filtration rate (GFR). As GFR decreases there is an increase in sodium absorption further reducing GFR. In addition, adenosine is directly toxic to the renal cells. Our decline in urine adenosine may be due to the increase vasospasm and therefore renal ischemia associated with contrast administration.[28] We also noted a decrease in the urinary metabolites of creatinine and cystine. Both of these metabolites are associated with the breakdown products of amino acids and are reabsorbed in the proximal tubule. It is possible that the reduction in GFR as a result of contrast dye leads to increased reabsorption of these metabolites.

Prior studies have shown that the alteration of urinary concentration of metabolites that occurs as a result of toxic exposure is somewhat dependent on the toxic agent. For example, a study of nephrotoxicity as a result of $HgCl_2$ treatment resulted in a decreased concentration of citrate and 2-oxoglutarate while acetate, succinate, and lactate were increased.[29] These results are thought to be secondary to damage in the proximal tubule caused by this agent and the resulting uncoupling of oxidative phosphorylation. The injury to this area may result in alterations in loss of absorption capacity of amino acids. We also noted a decrease in urinary succinate levels in our study. However, unlike HgCl2-induced changes we also saw a reduction in alanine concentration. It has been suggested urinary changes in metabolites may be specific to different types of toxin-induced renal injury. It may be the overall pattern of the changes in urinary metabolites that can be used to identify early injury,rather than a single metabolite change.[29]

LIMITATIONS

This is a pilot trial that was underpowered to detect an association of specific metabolites with CIN. The aim of the study was to evaluate urinary metabolite changes before and

after intravenous contrast. Given our sample size for this pilot project, we were not powered to detect changes in creatinine. Also related to the small sample size, we were unable to adjust for underlying medical illnesses, time since last meal, or baseline renal function.

CONCLUSION

Diagnostic testing using CT is becoming routine in the ED. As the indications for this diagnostic modality increase, so does the potential increase in adverse events associated with this test procedure. CIN is a well-described complication of this diagnostic modality; however, current methods of identifying this disease are insensitive and require lengthy observation. In this study we have shown that there are changes in urinary metabolites within 6 hours of CT imaging. It is therefore possible that urinary metabolites may be potential novel markers of renal injury.

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REFERENCES

1. Messana JM, Cieslinski DA, Humes HD. Comparison of toxicity of radiocontrast agents to renal tubule cells in vitro. *Ren Fail.*1990;12(2):75-82.
2. Mitchell AM, Jones AE, Tumlin JA, et al. Incidence of contrast-induced nephropathy after contrast-enhanced computed tomography in the outpatient setting. *Clin J Am Soc Nephrol.* 2010;5(1):4-9.
3. Mitchell AM, Kline JA. Contrast nephropathy following computed tomography angiography of the chest for pulmonary embolism in the emergency department. *J Thromb Haemost.* 2007;5(1):50-54.
4. Mehran R, Nikolsky E. Contrast-induced nephropathy: definition, epidemiology, and patients at risk. *Kidney Int Suppl.* 2006;100:S11-15.
5. Bartholomew BA, Harjai KJ, Dukkipati S, et al. Impact of nephropathy after percutaneous coronary intervention and a method for risk stratification. *Am J Cardiol.* 2004;93(12):1515-1519.
6. Freeman RV, O'Donnell M, Share D, et al. Nephropathy requiring dialysis after percutaneous coronary intervention and the critical role of an adjusted contrast dose. *Am J Cardiol.* 2002;90(10):1068-1073
7. Mehran R, Aymong ED, Nikolsky E, et al. A simple risk score for prediction of contrast-induced nephropathy after percutaneous coronary intervention: development and initial validation. *J Am Coll Cardiol.* 2004;44(7):1393-1399.
8. Weckwerth W, Loureiro ME, Wenzel K, et al. Differential metabolic networks unravel the effects of silent plant phenotypes. *Proc Natl Acad Sci U S A.* 2004;101(20):7809-7814.
9. Nikiforova VJ, Kopka J, Tolstikov V, et al. Systems rebalancing of metabolism in response to sulfur deprivation, as revealed by metabolome analysis of Arabidopsis plants. *Plant Physiol.* 2005;138(1):304-318.
10. Schad M, Mungur R, Fiehn O, et al. Metabolic profiling of laser microdissected vascular bundles of Arabidopsis thaliana. *Plant Methods.*2005;1(1):2.
11. Xia J, Psychogios N, Young N, et al. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. *Nucleic Acids Res.* 2009;37(Web Server issue):W652-660.
12. McCullough PA, Wolyn R, Rocher LL, et al. Acute renal failure after coronary intervention: incidence, risk factors, and relationship to mortality. *Am J Med.* 1997;103(5):368-375.
13. Rauch D, Drescher P, Pereira FJ, et al. Comparison of iodinated contrast media-induced renal vasoconstriction in human, rabbit, dog, and pig arteries. *Invest Radiol.* 1997;32(6):315-319.
14. Liss P, Nygren A, Olsson U, et al. Effects of contrast media and mannitol on renal medullary blood flow and red cell aggregation in the rat kidney. *Kidney Int.* 1996;49(5):1268-1275.
15. Heyman SN, Brezis M, Epstein FH, et al. Early renal medullary hypoxic injury from radiocontrast and indomethacin. *Kidney Int.* 1991;40(4):632-642.
16. Parvez Z, Patel NB, Nelson JE. Urinary adenosine deaminase binding protein, a marker of contrast media induced acute renal damage. *Clin Chim Acta.* 1990;190(1-2):111-113.
17. Jakobsen JA. Renal effects of iodixanol in healthy volunteers and patients with severe renal failure. *Acta Radiol Suppl.* 1995;399:191-195.
18. Jakobsen JA, Berg KJ, Brodahl U, et al. Renal effects of nonionic contrast media after cardioangiography. *Acta Radiol.* 1994;35(2):191-196.
19. Jakobsen JA, Nossen JO, Jorgensen NP, et al. Renal tubular effects of diuretics and X-ray contrast media. A comparative study of equimolar doses in healthy volunteers. *Invest Radiol.* 1993;28(4):319-324.
20. Kaneko K, Ikebe A, Shimazaki S, et al. Differences in renal tubular toxicity of high- and low-osmolality contrast media. *Nephron.* 1989;51(4):579-580.
21. Tumlin J, Stacul F, Adam A, et al. Pathophysiology of contrast-induced nephropathy. *Am J Cardiol.* 2006;98(6A):14K-20K.
22. Thomsen HS, Dorph S, Larsen S, et al. Urine profiles and kidney histology after ionic and nonionic radiologic and magnetic resonance contrast media in rats with cisplatin nephropathy. *Acad Radiol.* 1995;2(8):675-682.
23. Wilson PD, Hartz PA. Mechanisms of cyclosporine A toxicity in defined cultures of renal tubule epithelia: a role for cysteine proteases. *Cell Biol Int Rep.* 1991;15(12):1243-1258.
24. Beck FX, Ohno A, Dorge A, et al. Ischemia-induced changes in cell element composition and osmolyte contents of outer medulla. *Kidney Int.* 1995;48(2):449-457.
25. Weinberg JM, Buchanan DN, Davis JA, et al. Metabolic aspects of protection by glycine against hypoxic injury to isolated proximal tubules. *J Am Soc Nephrol.* 1991;1(7):949-958.

26. Weinberg JM, Nissim I, Roeser NF, et al. Relationships between intracellular amino acid levels and protection against injury to isolated proximal tubules. *Am J Physiol.* 1991;260(3 Pt 2):F410-419.
27. Bakhiya N, Monien B, Frank H, et al. Renal organic anion transporters OAT1 and OAT3 mediate the cellular accumulation of 5-sulfooxymethylfurfural, a reactive, nephrotoxic metabolite of the Maillard product 5-hydroxymethylfurfural. *Biochem Pharmacol.* 2009;78(4):414-419.
28. Vallon V, Osswald H. Adenosine receptors and the kidney. *Handb Exp Pharmacol.* 2009;(193):443-470.
29. Kim KB, Um SY, Chung MW, et al. Toxicometabolomics approach to urinary biomarkers for mercuric chloride (HgCl)-induced nephrotoxicity using proton nuclear magnetic resonance ((1)H NMR) in rats. *Toxicol Appl Pharmacol.* 2010;249(2):114-126.