EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Metabolomics and Gene Expression Analysis Reveal Down-regulation of the Citric Acid (TCA) Cycle in Non-diabetic CKD Patients

Stein Hallan [a,b,c,*,1,2], Maryam Afkarian [d,e,1], Leila R. Zelnick [d,f], Bryan Kestenbaum [d,f], Shoba Sharma [g], Rintaro Saito [a,2], Manjula Darshi [a,2], Gregory Barding [h], Daniel Raftery [h], Wenjun Ju [i,j], Matthias Kretzler [i,j], Kumar Sharma [a,k,2,3], Ian H. de Boer [d,f,3]

[a] Center for Renal Translational Medicine/Institute for Metabolomic Medicine, University of California San Diego, San Diego, CA, United States
[b] Department of Clinical and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway
[c] Department of Nephrology, St. Olav Hospital, Trondheim, Norway
[d] Kidney Research Institute, University of Washington, Seattle, WA, United States
[e] Division of Nephrology, Department of Medicine, University of California, Davis, CA, United States
[f] Division of Nephrology, Department of Medicine, University of Washington, Seattle, WA, United States
[g] University of Texas Health San Antonio, San Antonio, TX, United States
[h] Northwest Metabolomics Research Center, University of Washington, Seattle, WA, United States
[i] Division of Nephrology, Department of Medicine, University of Michigan, Ann Arbor, MI, United States
[j] Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, United States
[k] Department of Nephrology and Hypertension, Veterans Administration San Diego HealthCare System, San Diego, CA, United States

ARTICLE INFO

Article history:
Received 21 September 2017
Received in revised form 27 October 2017
Accepted 28 October 2017
Available online xxxx

Keywords:
Citric acid cycle
Chronic kidney disease
Metabolomics
Gene expression
Mitochondria

ABSTRACT

Chronic kidney disease (CKD) is a public health problem with very high prevalence and mortality. Yet, there is a paucity of effective treatment options, partly due to insufficient knowledge of underlying pathophysiology. We combined metabolomics (GCMS) with kidney gene expression studies to identify metabolic pathways that are altered in adults with non-diabetic stage 3–4 CKD versus healthy adults. Urinary excretion rate of 27 metabolites and plasma concentration of 33 metabolites differed significantly in CKD patients versus controls (estimate range − 68% to + 113%). Pathway analysis revealed that the citric acid cycle was the most significantly affected, with urinary excretion of citrate, cis-aconitate, isocitrate, 2-oxoglutarate and succinate reduced by 40–68%. Reduction of the citric acid cycle metabolites in urine was replicated in an independent cohort. Expression of genes regulating aconitate, isocitrate, 2-oxoglutarate and succinate were significantly reduced in kidney biopsies. We observed increased urine citrate excretion (+74%, p = 0.00009) and plasma 2-oxoglutarate concentrations (+12%, p = 0.002) in CKD patients during treatment with a vitamin-D receptor agonist in a randomized trial. In conclusion, urinary excretion of citric acid cycle metabolites and renal expression of genes regulating these metabolites were reduced in non-diabetic CKD. This supports the emerging view of CKD as a state of mitochondrial dysfunction.

1. Introduction

Chronic kidney disease (CKD) is an important public health problem with a high prevalence world-wide (10–14% of the general population) and strongly increased age-standardized death rate (GBD_Collaborators 2015). Yet, there is a paucity of effective treatment options with no major breakthroughs to reduce kidney damage since the introduction of renin-angiotensin system blockers 30 years ago. Lack of relevant animal models is a major limitation to identify novel targets for therapy, and the tradition of CKD patients being excluded from many clinical trials makes drug repurposing from other indications difficult (Ramos et al. 2015; Strippoli et al. 2004). Therefore, studies with patient based pathophysiology research and clinical testing of mechanisms may be highly beneficial.

For decades, the main focus has been on glomerular dysfunction and pathology, but recently it has been suggested that the proximal tubule is important for initiation and progression of CKD (Bonventre 2014; Takaori et al. 2016). This nephron segment has a very high content of mitochondria and is highly dependent on oxidative phosphorylation (Chevalier 2016). Likewise, muscle weakness and atrophy, fatigue, and non-renal organ dysfunction are major CKD symptoms indicating a

* Corresponding author at: Department of Clinical and Molecular Medicine, Gastrosenteret, Prinsesse Kristinas gt. 1, 7000 Trondheim, Norway.
E-mail address: stein.hallan@ntnu.no (S. Hallan).
[1] Drs. Hallan and Afkarian jointly led this work.
[2] Now at University of Texas Health San Antonio.
[3] Drs. Sharma and de Boer jointly supervised this work.

https://doi.org/10.1016/j.ebiom.2017.10.027

basal defect in cell metabolism. Mitochondrial dysfunction has turned out to be an important mechanism in diabetic kidney disease (DKD) (Hallan and Sharma 2016). A recent metabolomics comparison of 24-h urine samples from subjects with DKD and healthy controls identified 13 metabolites that were reduced in DKD, 12 of which were intermediates in mitochondrial metabolic pathways, suggesting a global suppression of mitochondrial activity (Sharma et al. 2013). Interestingly, 12 of the 13 metabolites were not reduced in diabetes without CKD, suggesting that presence of CKD, and not diabetes alone, was necessary for the observed urine metabolite pattern. Independent studies of kidney biopsy tissue demonstrated reduction of mitochondrial proteins and mitochondrial biogenesis (Sharma et al. 2013).

Metabolomics, the quantitative analysis of small molecules in biological samples, has uncovered numerous abnormalities in the blood of uremic patients. These include accumulation of renally excreted gut metabolites such as p-cresol sulfate (Aronov et al. 2011; Yu et al. 2014), indoxyl sulfate (Aronov et al. 2011; Kobayashi et al. 2014; Yu et al. 2014), and trimethylamine-N-oxide (Mutsaers et al. 2013); altered metabolism of amino acids such as tryptophan (Duranton et al. 2014; Goek et al. 2013; Kobayashi et al. 2014; Rhee et al. 2013; Toyohara et al. 2010), arginine (Duranton et al. 2014; Goek et al. 2013; Nkuipou-Kenfack et al. 2014; Shah et al. 2013; Yu et al. 2014), tyrosine/phenylalanine (Duranton et al. 2014), and glycine (Yu et al. 2014); impaired organic anion transport (Shah et al. 2013; Sharma et al. 2013); and increased anaerobic metabolism(Qi et al. 2012). Although urine is an important bio-sample for metabolomic studies in CKD, there have been relatively few studies evaluating metabolomics in 24-h urine. Furthermore, metabolomics may be a useful tool to identify novel therapeutic targets for CKD and to evaluate the effects of promising interventions. Vitamin D receptor agonists (VDRAs) such as paricalcitol are associated with reduced all-cause and cardiovascular mortality (Duranton et al. 2013; Zheng et al. 2013), reduced urine albumin excretion, and may have other renoprotective and cardioprotective effects (de Borst et al. 2013). However, the mechanisms underlying these effects, as well as other metabolic effects of VDRAs in CKD, remain incompletely understood, limiting full translation to clinical care.

To further investigate the metabolic abnormalities associated with non-diabetic CKD and to explore the effects of VDRAs, we quantified plasma and urine metabolites among participants in a clinical trial of paricalcitol (de Boer et al. 2013). We used an established wide-ranging panel of metabolites that are dysregulated in human inborn errors of metabolism and compared results to healthy controls. Key differences at baseline were replicated in an independent cohort, and expression of genes relevant to the significantly altered metabolites was analyzed in kidney tissue. Finally, we examined the effect of interventional treatment with paricalcitol vs. placebo on blood and urine metabolite profiles in CKD.

2. Methods

2.1. Study Populations

First, we compared 22 non-diabetic CKD stage 3–4 patients and 10 healthy controls to identify abnormalities in plasma and urine present in CKD patients. CKD participants were recruited from nephrology clinics at three medical centers associated with the University of Washington and enrolled in a clinical trial designed to test the effects of paricalcitol on glucose metabolism (NCT01003275) (de Boer et al. 2013). Inclusion criteria for the Paricalcitol study included age ≥ 18 years; estimated GFR 15–59 mL/min/1.73m^2; and fasting serum glucose 100–125 mg/dL. Exclusion criteria included a clinical diagnosis of diabetes or use of glucose-lowering medications; history of maintenance dialysis or kidney transplantation; use within past 8 weeks of prednisone, immunosuppressive medications, or other medications known to strongly affect blood glucose; change in dose of any medication within 8 weeks; and serum calcium >10.1 mg/dL. Healthy control participants were University of Washington employees required to be ≥18 years of age, and the same exclusion criteria were applied. In addition, healthy control participants were required to have an estimated GFR ≥ 60 mL/min/1.73m^2.

Second, major findings were replicated in the Study of Glucose and Insulin in Renal Disease (SUGAR), which included 45 non-diabetic CKD patients and 15 controls matched for age, sex and race (de Boer et al. 2016). SUGAR is a cross-sectional study of insulin and glucose metabolism in moderate to severe non-diabetic CKD. From 2011 to 2014, participants were recruited from nephrology and primary care clinics associated with the University of Washington and the neighboring institutions in Seattle, WA. Inclusion criteria included age ≥ 18 years and estimated GFR < 60 mL/min/1.73m^2. Healthy control participants were individuals with GFR ≥ 60 mL/min/1.73m^2, spot urine albumin-creatinine ratio < 30 mg/g, with the same distribution of age, sex and race as the enrolled participants with CKD. Exclusion criteria included a clinical diagnosis of diabetes, end-stage renal disease (ongoing or imminent maintenance hemodialysis or kidney transplantation) or use of medications known to affect glucose metabolism (e.g. corticosteroids), fasting serum glucose ≥ 126 mg/dL, and hemoglobin < 10 g/dL. All study procedures were approved by the University of Washington Institutional Review Board, and all participants provided written informed consent.

We also studied gene expression in kidney biopsies from 155 patients from the European Renal cDNA Bank cohort with biopsy-proven, non-diabetic CKD (FSGS or minimal change n = 24, hypertensive nephropathy n = 15, IgA nephropathy n = 27, minimal change disease n = 14, membranous glomerulonephritis n = 21, rapidly progressive glomerulonephritis n = 22, lupus nephritis n = 32). The control kidney biopsies were obtained from healthy kidney transplant donors (n = 31) prior to kidney donation, following the usual clinical protocols.

2.2. Paricalcitol Treatment

CKD participants enrolled in the paricalcitol cross-over intervention trial were allocated to paricalcitol for 8 weeks and placebo for 8 weeks, separated by an 8-week washout period (de Boer et al. 2013). The order of paricalcitol and placebo treatment periods was randomly assigned by the University of Washington Investigational Drug Services and was blinded to both participants and investigators. The active intervention was paricalcitol (19-nor-1,25-(OH)2-vitamin D2) 2 micrograms daily by mouth. Participants were encouraged not to change their use of non-study medications during the course of the study. Healthy control participants were not treated with paricalcitol (de Boer et al. 2013).

2.3. Covariate Data

Demographic data and comorbidities were assessed by questionnaire. Weight and height were measured while wearing light clothing. Blood pressure was measured after resting in a seated position for at least 5 min. Serum creatinine was measured in fasting plasma using a method traceable to isotope dilution mass spectrometer and GFR was estimated with the 2009 CKD-EPI equation (Levey et al. 2009). Timed urine samples (approximately 24-hour) were collected on ice, the urine volume registered, and 10 mL samples frozen at −80 for later analysis; albumin was measured in fresh urine immediately after collection using a turbidimetric assay and used to calculate daily albumin excretion rate.

2.4. Metabolomics

For CKD participants and healthy controls in the paricalcitol trial, plasma and urine samples were analyzed on a gas chromatography mass spectroscopy (GC–MS) platform at ClinMet, Inc. (San Diego, CA). This panel of metabolites has been established over the years to detect inborn errors of metabolism, and the focus on organic acids is also well suited for CKD since the handling of these compounds are central

Please cite this article as: Hallan, S., et al., Metabolomics and Gene Expression Analysis Reveal Down-regulation of the Citric Acid (TCA) Cycle in Non-diabetic CKD Patients, EBioMedicine (2017), https://doi.org/10.1016/j.ebiom.2017.10.027