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Exhaled breath and fecal volatile organic biomarkers of chronic kidney disease

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ABSTRACT

Background: While much is known about the effect of chronic kidney disease (CKD) on composition of body fluids little is known regarding its impact on the gases found in exhaled breath or produced by intestinal microbiome. We have recently shown significant changes in the composition of intestinal microbiome in humans and animals with CKD. This study tested the hypothesis that uremia-induced changes in cellular metabolism and intestinal microbiome may modify the volatile organic metabolites found in the exhaled breath or generated by intestinal flora.
Methods: SD rats were randomized to CKD (5/6 nephrectomy) or control (sham operation) groups. Exhaled breath was collected by enclosing each animal in a glass chamber flushed with clean air, then sealed for 45 min and the trapped air collected. Feces were collected, dissolved in pure water, incubated at 37 °C in glass reactors for 24 h and the trapped air collected. Collected gases were analyzed by gas chromatography.
Results: Over 50 gases were detected in the exhaled breath and 36 in cultured feces. Four gases in exhaled breath and 4 generated by cultured feces were significantly different in the two groups. The exhaled breath in CKD rats showed an early rise in isoprene and a late fall in linear aldehydes. The CKD animals' cultured feces released larger amounts of dimethyldisulfide, dimethyltrisulfide, and two thioesters.
Conclusions: CKD significantly changes the composition of exhaled breath and gaseous products of intestinal flora.
General significance: Analysis of breath and bowel gases may provide useful biomarkers for detection and progression of CKD and its complications.

1. Introduction

A variety of primary renal and systemic diseases result in progressive decline in kidney function which leads to accumulation of diverse uremic retention solutes including nitrogenous waste products and low molecular weight organic compounds. Accumulation of these products contributes to cardiovascular disease and numerous other morbidities by promoting oxidative stress, inflammation and cytotoxicity [1]. Colonic microbial flora is the source of numerous uremic solutes including p-cresol sulfate and indoxyl sulfate which are well known uremic toxins, as well as many as-yet unidentified products [2]. Using a phylogenic microarray to identify microbial species, Vaziri et al. [3] recently showed marked differences in the composition of the intestinal microbial flora between patients with end-stage renal disease (ESRD) and healthy individuals and between the uremic and normal control rats. These observations illustrated the profound impact of chronic renal failure on the composition of the gut microbial flora.

Under normal condition the indigestible complex carbohydrates contained in the diet and mucin, a heavily glycosylated protein secreted by the enterocytes, serve as the primary substrates for the gut microbial flora. However, impaired renal clearance of nitrogenous and other waste products and the rise in their intra- and extracellular fluid concentrations leads to their passive diffusion or secretion in the gastrointestinal tract. In this context massive diffusion of urea into the gastrointestinal tract and its hydrolysis to ammonia and detection in exhaled breath has long been recognized in the uremic patients [4]. In addition earlier studies have demonstrated active secretion of uric acid and oxalate in the colon in chronic kidney disease (CKD) [5–7]. Via introduction of these compounds which can serve as alternative substrates, uremia can modify the structure and function of the gut microbiome [8]. In addition the recently demonstrated CKD-induced disintegration of the colonic epithelial tight junction apparatus [9] may facilitate bidirectional flux of macromolecules across the gut barrier and further expand the array of the available substrates for consumption by the microbial flora. Fermentation of various substrates by microbial flora leads to formation of gaseous compounds the nature of which depends on the given substrates and the biological characteristics of the microbial community. Uremia-induced changes in the structure of intestinal microbiome together with influx of various molecules

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into the gut lumen can lead to generation and absorption of normally-absent or scarce solutes and gaseous products. Such volatile products can then appear in the exhaled breath as exemplified by ammonia which is generated from hydrolysis of urea in the gut of the uremic individuals.

As summarized in elegant reviews by Amann et al. and Hakim et al. [10,11] the potential use of the exhaled breath analysis as a non-invasive tool for the diagnosis and monitoring of disease progression and response to therapeutic interventions in different illnesses has been the focus of extensive investigations. In this context several studies have explored the impact of renal failure on the composition of the exhaled breath in humans and experimental animals. For instance Simenhoff et al. [12] found a significant increase in potentially toxic secondary and tertiary amines (dimethylamine and trimethylamine) in the exhaled breath of ESRD patients immediately before hemodialysis procedure and their fall shortly thereafter. They further showed a significant decline in concentration of these metabolites in patients treated with non-absorbable antibiotics, pointing to the gut microbial flora as a source of these products. Several other studies including that reported by Trovarelli et al. [13] have shown a marked increase in breath isoprene contents in patients with CKD. Marom et al. [14] used gold nanoparticle sensors, to test the expired breath samples for detection and assessment of severity of CKD. They demonstrated that the system could distinguish the difference between healthy individuals and those with early stages of CKD with 79% accuracy and between stage 4 and 5 CKD with 85% accuracy. Monitoring the concentration of ammonia in patients' exhaled breath during hemodialysis procedure Narasimhan and associates [15] found a close correlation between breath ammonia level and serum urea and creatinine concentrations. They concluded that the real-time breath ammonia measurements may be a reliable tool for determining the efficacy of hemodialysis treatment. Finally using a rat model of bilateral nephrectomy, Haick et al. [16] identified 27 volatile organic compounds (VOC) in the breath samples (collected via tracheal tube) of the uremic rats which were absent in the control animals. It should be noted that in some of the reported studies, no adjustment were made for the composition of the inhaled air which is a major variable. Despite the long-standing interest and extensive investigations, adoption of the exhaled breath analysis as a routine tool for the diagnosis and monitoring of diseases has not yet fully materialized. This is primarily due to the technical impediments including standardization of sample collection, pre-concentration, vapor-desorption, and impact of inhaled ambient air impurities, as well as uncertainty about the underlying metabolic pathway(s) [17,18].

While much is known about the effect of CKD on the volume and composition of body fluids, little is known about its impact on the composition the gaseous byproducts of the gut microbiome. Given the emerging evidence for the role of the gut microbial flora as a major source of uremic retention solutes the present study was designed to test the hypothesis that uremia-induced changes in cellular metabolism and intestinal microbiome may modify gaseous metabolites found in the exhaled breath or generated by the intestinal flora. To isolate the effect of uremia per se, we chose to compare rats with CKD induced by 5/6 nephrectomy with their sham-operated control counterparts maintained under identical conditions. Due to differences in ethnic and genetic backgrounds, age, underlying systemic diseases, dietary habits, drug regimens, and others factors that can affect the structure of the intestinal flora and hence their gaseous byproducts it is difficult to isolate the effect of CKD per se in humans. In addition dialysis procedure can lead to marked alteration in exhaled the breath composition [15,19,20]. In contrast to humans, inter-individual variation in composition of microbiome is minimal in genetically identical animals maintained under the same condition and fed the same food. Consequently the differences observed between the CKD and control groups can be attributed to the disease state with certainty.

2. Methods

2.1. Animals

2.1.1. Animals

Male Sprague–Dawley rats, weighing 225–250 g were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). They were housed in a climate-controlled and light-regulated facility with 12:12-h day–night cycles. The animals were fed regular rat chow (Purina Mills, Brentwood, MO) and water ad libitum and randomly assigned to the chronic renal failure (CKD) and normal control groups. The animals assigned to the CKD group underwent 5/6 nephrectomy by surgical resection of the upper and lower thirds of the right kidney followed by left nephrectomy four days later using dorsal incisions [21]. The animals assigned to the control group were subjected to sham operation. All surgical procedures were carried out under general anesthesia using intraperitoneal injection of Ketamine/Xylazine. The animals were observed for 6 weeks at which time they were placed in metabolic cages for a 24-h urine collection. They were then anesthetized and euthanized by exsanguinations using cardiac puncture. The serum creatinine, urea, total cholesterol, triglyceride concentrations, and urinary protein excretion were measured using standard laboratory methods.

2.2. Exhaled breath collection and analysis

Exhaled breath was collected in evacuated stainless steel canisters at weeks 0, 1, 2, 4, and 6. On each occasion the animal was placed in a 10 L glass chamber flushed with ultra clean air (collected at the University of California Research Station in the Sierra Mountains at 10,000 ft. altitude) for 1 h at a flow rate of 1 L/min. This specific time was experimentally determined to be sufficient to completely replace the laboratory air inside the glass chamber with ultra clean air of known composition. After 1 h the chamber was sealed with the animal inside for 45 min. Two liters of air was then collected in a two-liter electropolished stainless steel canister. Prior to sampling, each canister was baked at 150 °C for 12 h, pumped to 10^{-2} Torr, flushed with ultra high purity helium up to 200 Torr, and evacuated again to 10^{-2} Torr.

A schematic of the gas chromatographic system used to analyze the exhaled air samples and the air space of cultured feces is shown in Supplementary Fig. 1. The analytical system used in this study is similar to the system described in Colman et al. [22]. Briefly, 790 mL of the collected air sample is pre-concentrated in a stainless steel loop filled with glass beads and submerged in liquid nitrogen to remove the nitrogen, oxygen and argon present in the sample. The sample is re-vaporized using hot water (at approximately 80 °C) and split into six different column/detector combinations housed in three gas chromatographs (GCs) using UHP helium as the carrier gas: (1) DB-1 column (J&W; 60 m, 0.32 mm I.D., 1 μm film thickness) output to a flame ionization detector (FID) and to a sulfur chemiluminescence detector (SCD); (2) DB-5 column (J&W; 30 m, 0.25 mm I.D., 1 μm film thickness) connected in series to a RESTEK 1701 column (5 m, 0.25 mm I.D., 0.5 μm film thickness) and output to an electron capture detector (ECD); (3) RESTEK 1701 column (60 m, 0.25 mm I.D., 0.50 μm film thickness) output to an ECD; (4) PLOT column (J&W GS-Alumina; 30 m, 0.53 mm I.D.) connected in series to a DB-1 column (J&W; 5 m, 0.53 mm I.D., 1.5 μm film thickness) and output to an FID; (5) DB-5 ms column (J&W; 60 m, 0.25 mm I.D., 0.5 μm film thickness) output to a quadrupole mass spectrometer detector (MSD, HP 5973). The MSD is set to operate simultaneously in SCAN mode (for qualitative identification) and selected ion monitoring (SIM) with one ion chosen to quantify each compound in order to achieve the maximum selectivity and to avoid potential interferences. All gas chromatographs and detectors used in this study are manufactured by Hewlett Packard. Our analytical system allows for