Exposure to Potentially Toxic Hydrocarbons and Halocarbons Released From the Dialyzer and Tubing Set During Hemodialysis

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Background: Although much is known about the effect of chronic kidney failure and dialysis on the composition of solutes in plasma, little is known about their impact on the composition of gaseous compounds in exhaled breath. This study was designed to explore the effect of uremia and the hemodialysis (HD) procedure on the composition of exhaled breath. Breath samples were collected from 10 dialysis patients immediately before, during, and after a dialysis session. To determine the potential introduction of gaseous compounds from dialysis components, gasses emitted from dialyzers, tubing set, dialysate, and water supplies were collected.

Study Design: Prospective cohort study.

Participants: 10 HD patients and 10 age-matched healthy individuals.

Predictor: Predictors include the dialyzers, tubing set, dialysate, and water supplies before, during, and after dialysis.

Outcomes: Changes in the composition of exhaled breath.

Measurements: A 5-column/detector gas chromatography system was used to measure hydrocarbon, halocarbon, oxygenate, and alkyl nitrate compounds.

Results: Concentrations of 14 hydrocarbons and halocarbons in patients’ breath rapidly increased after the onset of the HD treatment. All 14 compounds and 5 others not found in patients’ breath were emitted from the dialyzers and tubing sets. Contrary to earlier reports, exhaled breath ethane concentrations in our dialysis patients were virtually unchanged during the HD treatment.

Limitations: Single-center study with a small sample size may limit the generalizability of the findings.

Conclusions: The study documented the release of several potentially toxic hydrocarbons and halocarbons to patients from the dialyzer and tubing sets during the HD procedure. Because long-term exposure to these compounds may contribute to the morbidity and mortality in dialysis population, this issue should be considered in the manufacturing of the new generation of dialyzers and dialysis tubing sets.

INDEX WORDS: Ethane; hydrocarbons; halocarbons; toxic; carcinogens; exogenous sources; dialyzer; dialysis tubing set; PVC; oxidative stress; biomarker; degassing; Baxter; Gambro.
its treatment with the HD procedure on the composition of exhaled breath.

METHODS

Study Design, Setting, and Participants

This prospective cohort study designed to explore the effect of uremia and the HD procedure on the composition of exhaled breath was approved by the Human Subjects Institutional Review Board of the University of California Irvine (HS# 2007-5572) and completed with the assistance of the University of California Institute of Clinical and Translational Science.

Ten stable patients with ESRD (4 men and 6 women) maintained on HD therapy for longer than 3 months were recruited for the study. The underlying cause of ESRD in the study population included diabetic nephropathy (4 patients), hypertensive nephrosclerosis (1), systemic lupus erythematosus (1), polycystic kidney disease (1), cystinosis (1), lithium nephropathy (1), and focal segmental glomerulosclerosis (1). HD blood access consisted of arteriovenous fistulas in 7 patients and polytetrafluoroethylene grafts in 3 individuals. Individuals with evidence of acute or chronic infection, with acute intercurrent illnesses, and receiving antibiotic or immunosuppressive drugs were excluded. Medical history, systolic and diastolic blood pressures, body weight, routine monthly laboratory data, medications, and dietary preferences were recorded. All patients were under dietary fluid, sodium, phosphorus, and potassium restrictions to minimize fluid overload, hyperphosphatemia, and hyperkalemia. Also, all patients used DEHP (bis(2-ethylhexyl)phthalate)-free Cartridge Blood sets, which consist of medical-grade polyvinyl chloride (PVC), DEHP-free, manufactured by Gambro Renal Products (www.gambro.com/en/global). HD therapy was performed thrice weekly using either Baxter (www.baxter.com) EXELTRA High Flux, Single-Use Dialyzers (composed of cellulose triacetate) or the Gambro Polyflux Revaclear Single-Use, High Flux Dialyzers (composed of a blend of polyarylethersulfone and polyvinylpyrrolidine). Systemic anticoagulation with heparin (an initial bolus of 1,500 units of unfractionated heparin followed by 500 units at hours 1 and 2) was used for anticoagulation during HD. All patients received phosphate binders, erythropoiesis-stimulating agents, iron supplements, and a multivitamin preparation.

A group of 10 healthy age-matched individuals (5 men and 5 women) recruited from our local community served as controls. Control individuals showing acute or chronic infection or acute or chronic illnesses were excluded. All participants provided informed consent prior to enrollment in the study.

Outcome Variables

To determine the effects of uremia, we compared exhaled breath composition in dialysis patients with healthy control individuals. To determine the effects of the dialysis procedure, we compared exhaled breath composition pre- and postdialysis.

Data Sources/Measurements

Blood Collections

In all dialysis patients, whole blood was collected from the vascular access before the initiation of dialysis. Blood samples were obtained by a syringe, applying gentle aspiration to minimize shear stress. Blood samples from control individuals were collected from a peripheral vein in the same manner. Standard laboratory methods were used to measure blood hemoglobin and plasma biochemical levels.

Breath Collections

All 10 dialysis patients participated in 2 breath studies that were conducted during the HD procedure at the long (72-hour) and short (48-hour) interdialytic intervals. These 2 studies were conducted at different interdialytic intervals to determine the possible effect of an extra day without the HD procedure. For each study, 18 breath samples were collected from the dialysis patient and 2 breath samples were collected from the control individual. To accurately determine gases exhaled by patients, a room sample was collected simultaneously with each breath sample and used as the background value that was subtracted from the breath sample.

All breath and room samples were collected in the University of California Irvine Dialysis Center in the following sequence: (1) upon the dialysis participant’s arrival, 3 predialysis samples were collected at 5-minute intervals for the first 15 minutes; (2) after cannulation, a breath sample was collected before the initiation of HD; and (3) during dialysis, a sample was collected every 3 minutes for the first 15 minutes, every 5 minutes for the next 15 minutes, and every 30 minutes for the final 2.5 hours. The final postdialysis sample was collected 10 minutes after the end of the procedure. All breath samples had corresponding room samples collected near the patient. Also, along with the very first and last
patient sample, a correlating control sample from a healthy control individual was collected. This sampling procedure is shown in Fig 1.

**Sampling Apparatus**

Evacuated 1.9-L stainless steel canisters, with an attached 12” × ¼”-diameter Teflon tube, were used to obtain exhaled breath samples. Before sampling, all metal components, including the canister, valves, and ultra-torr tee unions, were baked at 150°C for 24 hours and canisters were evacuated to approximately 10⁻² torr. The pressure difference between the evacuated canister and ambient pressure (room or patient’s lung) allowed sample collection.

**Degassing Procedure**

To determine the source of compounds appearing in the exhaled breath after the onset of dialysis, we analyzed the parenteral saline solution, dialysis water supply, dialysate concentrate, dialysate solution, dialysis PVC tubing, and dialyzers (cellulose triacetate or polymer blend) for the presence of dissolved gases. We used helium to purge the gases from these sources using a novel degassing apparatus and glass bulb developed in the Rowland-Blake laboratory at University of California Irvine. The solutions degassed were ultrapure water treated at the University of California Irvine dialysis center, fresh saline solution from the intravenous bag, and dialysate. All solutions were degassed by: (1) connecting the glass bulb between 2 mass flow controllers to control the rate of helium flown in and out of the glass bulb, (2) injecting the solution into the glass bulb with 760 torr (1 atmosphere pressure) of helium, (3) bubbling the solution with helium through a glass frit to produce microsized helium bubbles, and finally (4) collecting the degassed gases. After each degassing experiment, the glass bulb was washed and autoclaved to ensure cleanliness. Chemicals inside the medical apparatus were flushed removed by helium using the following procedure: (1) connecting the apparatuses between the 2 mass flow controllers, (2) flushing the apparatus with a helium flow of 40 mL/min, and finally (3) collecting gases flushed out from the apparatus. The baseline sample for all degassing studies was purified ultrapure helium from Airgas, Inc (www.airgas.com). Approximately 760 torr of all gas samples was collected in an evacuated 1.9-L stainless steel canister. The rate of helium flushed through both solutions and the medical apparatus was 40 mL/min.

All solutions were collected 3 different times and degassed twice using 18 mL of solution. The dialysis PVC tubing was helium flushed for 48 hours, and the dialyzer membranes, for 40 minutes; 2 samples were collected from each dialyzer.

**Table 1. Clinical and Biochemical Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n = 10)</th>
<th>HD Patients (n = 10)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>50 ± 17</td>
<td>51 ± 19</td>
<td>0.8</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dL)</td>
<td>14 ± 3</td>
<td>64.0 ± 8.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.0 ± 0.2</td>
<td>9.4 ± 3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum hemoglobin (g/dL)</td>
<td>14 ± 0.8</td>
<td>11.4 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>4 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Kt/V</td>
<td>NA</td>
<td>1.5 ± 0.3</td>
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</tbody>
</table>

**Box 1. All Compounds Identified in the Room and in Exhaled Breath**

**Hydrocarbons**

- Ethane
- Ethene
- Ethyne
- Propane
- Propene
- n-Butane
- i-Butene
- trans-2-Butene
- cis-2-Butene
- i-Pentane
- n-Pentane
- Isoprene
- n-Hexane
- n-Heptane
- n-Octane
- n-Nonane
- n-Decane
- 2-Methylpentane
- 2,3-Dimethylpentane
- 3-Methylpentane
- 2-Methylhexane
- 3-Methylhexane
- 3-Methylheptane
- Cyclopentane
- Cyclohexane
- Benzene
- Toluene
- Ethylbenzene
- m-Xylene
- o-Xylene
- p-Xylene
- n-Propylbenzene
- 2-Ethyltoluene
- 3-Ethyltoluene
- 4-Ethyltoluene
- α-Pinene
- β-Pinene

**Halocarbons**

- CFC-11
- CFC-12
- CFC-113
- Halon-1211
- HFC-134a
- HCFC-22
- HCFC-141b
- HCFC-142b
- CHCl3
- CH2CCl3

**Alkyl Nitrates**

- Methyl nitrate
- Ethyl nitrate
- 1-Propyl nitrate
- 2-Butyl nitrate
- 2-Pentyl nitrate
- 3-Pentyl nitrate

**Oxygenates**

- Acetaldehyde (CH3CHO)
- Methanol (CH3OH)
- Ethanol (CH3CH2OH)
- Acetone (CH3COCH3)

**Other species**

- DMS
- DMDS

**Abbreviations:** CFC, chlorofluorocarbon; DMDS, dimethyl disulfide; DMS, dimethyl sulfide; HCFC, hydrochlorofluorocarbon; HFC, hydrofluorocarbon; i, iso; m, meta; n, normal; o, ortho; p, para.

**Gas Analysis**

All samples collected were analyzed on a nonmethane hydrocarbon system developed at University of California Irvine by Rowland and Blake as described by Colman et al. Briefly, this system is a 5-column/detector gas chromatography (GC) system capable of quantifying ~100 different hydrocarbon, halocarbon, oxygenate, alkyl nitrate, and sulfur-containing compounds. At standard temperature and pressure, 233 cm³ of sample is cryogenically preconcentrated and injected into the system consisting of 3 Hewlett-Packard 6890 GC system (Agilent Technologies, Life
Sciences/Chemical Analysis; www.chem.agilent.com) equipped with 5 column and detector combinations: a DB-1 (100% dimethylpolysiloxane) column with a flame ionization detector, a Restek 1701 (fused silica; www.restek.com) column with an electron capture detector, an aluminum oxide (Al2O3) PLOT (porous layer open tubular) and DB-1 column with a flame ionization detector, a DB-5 (5% phenyl)-methylpolysiloxane) and Restek 1701 column with an electron capture detector, and a DB-5ms column with a mass selective detector. Carbon dioxide was analyzed using a Carbosphere 80/100 packed column coupled with a thermal conductivity detector.

Mathematical and Statistical Analysis

For the HD breath study, the delta breath concentration is defined as the gas concentration in the breath minus the gas concentration in the same room. When presenting multiple compounds on a table or figure, average delta breath concentration for the 20 studies collected at each sampling time is used. All average data presented in the text and tables are presented in the form of either mean ± standard deviation. For comparative analysis between 2 different types of data sets, P values were calculated using paired t test.29,30

RESULTS

Participant Characteristics

Compared with the control group, the dialysis group showed marked elevations in plasma creatinine and urea nitrogen concentrations and a significant decrease in blood hemoglobin concentrations (Table 1). The Kt/V value in the dialysis group was 1.5 ± 0.3, reflecting adequacy of dialysis therapy. Dietary history showed adequate adherence to the renal diet in all dialysis patients.

Breath Gas Composition Data

Using the nonmethane hydrocarbon analytical system, we quantified a total of 75 compounds in both room and breath samples (Box 1). We report results for exhaled ethane, because it previously has been reported to be a biomarker for oxidative stress, and 14 additional compounds that showed a rapid increase in concentrations after the initiation of HD.

The amount of ethane found in exhaled breath from dialysis patients was similar to that found in controls and virtually identical to that found in room air. As shown in Fig 2, the average breath and room ethane concentration for 16 of the 20 patient studies was close to 0 parts per billion in volume and was unchanged throughout the dialysis treatment. Ethane data from 2 patients using prescribed inhalers were omitted due to extremely high ethane levels coupled with high chlorofluorocarbon 12 (CFC-12) levels; because this compound is used as an aerosolizing agent, we assume the inhaler to be the major source of these gases.

During dialysis, the concentrations in the exhaled breath of 10 hydrocarbons (i-butane, n-butane, n-pentane, n-hexane, n-heptane, n-octane, n-nonane, 3-methylpentane, 3-methylhexane, and 3-methylheptane) and 4 halocarbons (chloroethane [CH3CH2Cl], vinyl chloride [CH2CHCl], dichloromethane [CH2Cl2], and trichloromethane [CHCl3]) rapidly and significantly increased from the start (at time zero) to 3 minutes after the onset of the HD treatment (Fig 3A-C; Table 2). Room air concentrations for most of these compounds were lower than those observed in exhaled breath, thus identifying them as coming from the patient and not originating from room air. Three compounds (3-methylpentane, CH2Cl2, and CHCl3) had very high levels in room air before the start of dialysis and were virtually absent from the patient’s breath. After the initiation of HD, patients’ breath concentrations of these compounds rapidly increased, whereas the

![Figure 2. Breath ethane concentration. Mean ± standard deviation (SD) of breath ethane concentrations measured in all hemodialysis patients and control individuals was comparable to that found in room air and did not change during the dialysis procedure. Abbreviation: ppbv, parts per billion in volume.](image)
Nonane. Values given are based on concentrations measured in room air in the pulmonary system. In these instances, the initial delta values are reported as negative, but subsequent values become positive. This finding suggests that although the compounds in questions initially were found in the room, their presence in the breath truly reflects exhalation of the compound and not simply recirculation of room air in the pulmonary system.

![Graphs A, B, C](image)

**Figure 3.** Exhaled breath concentration of hydrocarbons and halocarbons that rapidly increased at the start of the dialysis session. (A) Highest, (B) next highest, and (C) lowest hydrocarbon and halocarbon concentrations are shown throughout the dialysis procedure. In panel A, in concentrations shown for the healthy controls, the symbol for CHCl₃ superimposes and obscures the symbol for n-nonane. Values given are based on concentration in breath less the concentration in room air; negative numbers thus indicate a higher concentration in room air than exhaled breath. Abbreviation: ppbv, parts per billion in volume; pptv, parts per trillion in volume.
Table 2. Concentrations of Exhaled Breath Compounds That Rapidly Increased With the Start of the Hemodialysis Session

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exhaled Breath Average Concentration</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>i-Butane</td>
<td>818±931</td>
</tr>
<tr>
<td>n-Butane</td>
<td>837±1,950</td>
</tr>
<tr>
<td>n-Pentane</td>
<td>629±1,130</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>144±169</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>782±1,200</td>
</tr>
<tr>
<td>n-Nonane</td>
<td>42±52</td>
</tr>
<tr>
<td>n-Nonane</td>
<td>730±1,910</td>
</tr>
<tr>
<td>3-Methylpentane</td>
<td>32±96</td>
</tr>
<tr>
<td>3-Methylhexane</td>
<td>34±205</td>
</tr>
<tr>
<td>3-Methylheptane</td>
<td>−209±236</td>
</tr>
<tr>
<td>CH₂CHCl₂</td>
<td>15±41</td>
</tr>
<tr>
<td>CH₂CHCl₂</td>
<td>−0.002±0.6</td>
</tr>
<tr>
<td>CH₃Cl₂</td>
<td>−1,320±1,050</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>−136±131</td>
</tr>
</tbody>
</table>

Note: Data from breath samples obtained before (time 0) and 3 minutes after the start of the hemodialysis session are reported as mean ± standard deviation in parts per trillion in volume (pptv) for all hemodialysis patients. Values given are based on concentrations in breath less the concentration in room air; negative numbers thus indicate a higher concentration in room air than exhaled breath.

Abbreviations: i, iso; m, meta; n, normal.

DISCUSSION

Exposure of blood to the surface of a dialyzer membrane and tubing is known to trigger activation of circulating leukocytes and the complement cascade, leading to oxidative and inflammatory stresses marked by the increase in plasma concentrations of proinflammatory cytokines and markers of oxidative stress.5,10,12 Several investigators19,27,31,32 have reported a significant increase in exhaled breath ethane levels during HD treatment and have attributed this phenomenon to HD-induced oxidative stress. In contrast, the exhaled breath ethane concentrations in our dialysis patients were comparable to those found in the control group and were virtually unchanged during the course of HD treatment. Moreover, ethane concentrations in the exhaled breath of patients and controls were nearly identical to that found in room air. This observation excludes endogenous production as a significant source of ethane found in the exhaled breath and diminishes its potential validity as a marker of oxidative stress.

Unlike ethane, concentrations of 10 hydrocarbon and 4 halocarbon compounds markedly increased shortly after the onset of the HD procedure (Fig 3A-C). Two possible explanations for the rapid increase in concentrations of these gases in patients’ breath during the HD procedure are: (1) they were introduced from the exogenous sources, such as dialysis equipment and solutions; or (2) they arise from an endogenous source, such as byproducts of immune activation in response to blood exposure to the dialysis circuit and influx of impurities from the dialysate compartment. To determine whether these compounds were introduced by components of the dialysis system or endogenously produced in response to the dialysis procedure, we undertook careful degassing experiments of the dialysis solution, tubing, and dialyzers used in our facility. None of the target gasses was detected in the deionized water supply, dialysate concentrate, or final dialysate solution. However, all target compounds were abundantly present in the PVC tubing and/or 2 different dialyzers used in our study. The compounds emitted from the PVC tubing included n-pentane, n-octane, 3-methylhexane, 3-methylheptane, CH₂CHCl₂, and CHCl₃. These observations clearly identified PVC tubing as the source of these compounds found in the patients’ exhaled breath.

Dialyzer, Tubing, and Dialysate Degassing Experiments

To determine the source of the 14 targeted compounds, we evaluated the dialysis solutions, blood tubing, and dialyzers. None of the targeted compounds was found in any of the degassed solutions, in other words, parenteral saline solution, dialysis water supply, dialysate concentrate, or dialysate solution. Instead, all targeted compounds were found in both the PVC tubing and dialyzers. Some of the compounds were found in both tubing and dialyzers and some were unique to either tubing or dialyzers. Specifically, i-butane, n-butane, n-hexane, n-heptane, 3-methylpentane, and CH₂CHCl₂ were found in both PVC tubing and dialyzers (Fig 4A), and n-pentane, n-octane, 3-methylhexane, 3-methylheptane, CH₂CHCl₂, and CHCl₃ were found in only PVC tubing (Fig 4B). Although n-nonane and CH₂Cl₂ were found in both dialyzers, the cellulose triacetate membrane emitted significantly higher levels of these compounds (Fig 4C). Interestingly, congruent to the degassing study, all 8 patients who were treated with the cellulose triacetate membrane had a rapid increase in n-nonane and CH₂Cl₂, delta breath concentrations with the onset of the treatment. A patient was dialyzed with both types of membranes and had significantly higher n-nonane and CH₂Cl₂ concentrations with the cellulose triacetate membrane, but not with the polymer blend membrane.

Interestingly, 5 additional compounds that were not found in patients’ exhaled breath during the dialysis study were identified during flushing of the PVC tubing and dialyzers with helium (Table 3).
shortly after the onset of the HD procedure (Fig 4B). In addition, significant amounts of i-butane, n-butane, n-hexane, n-heptane, 3-methylpentane, and CH₃CH₂Cl were emitted from the dialyzers (Fig 4A). These findings point to the dialyzers used in the study as the source of the appearance of these compounds in patients’ exhaled breath shortly after the start of the HD procedure. Interestingly, n-nonane and CH₂Cl₂ appeared in patients’ exhaled breath when dialyzed with the cellulose triacetate dialyzer, but not the polymer blend dialyzer. As shown in Fig 4C, the degassing procedure revealed the presence of these 2 compounds in the tested cellulose triacetate but not the polymer blend dialyzer. Among the compounds released from the HD circuit into the circulation and detected in patients’ breath, CH₂CHCl, CH₂Cl₂, and CHCl₃ are known carcinogens that can damage various organs.³³⁻³⁵

In conclusion, this study documented exposure of patients to several potentially toxic hydrocarbon and halocarbon compounds released to the circulation from the dialyzer membrane and tubing set during the HD procedure. Some of these products, such as CH₂CHCl, CH₂Cl₂, and CHCl₃, are known carcinogens and can be damaging to various tissues.³³⁻³⁵ Therefore, long-term repetitive exposure to these compounds may have adverse consequences and may contribute in part to the high morbidity and mortality in the dialysis population. This issue should be considered in the manufacturing of the new generation of products with long-term dialysis treatments.

### Table 3. Compounds Found Only in Tubing/Dialyzers but Not in Exhaled Breath

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Source</th>
<th>Levels (pptv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrahydrofuran (THF)</td>
<td>Cellulose triacetate membrane</td>
<td>5,170</td>
</tr>
<tr>
<td>Chlorobenzene (CB)</td>
<td>Polymer blend membrane</td>
<td>14,200</td>
</tr>
<tr>
<td>Butanal</td>
<td>PVC tubing</td>
<td>6,750</td>
</tr>
<tr>
<td>2-Butanone (MEK)</td>
<td>PVC tubing</td>
<td>1,280</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>PVC tubing</td>
<td>2,140,000</td>
</tr>
</tbody>
</table>

Abbreviations: pptv, parts per trillion in volume; MEK, methyl ethyl ketone; PVC, polyvinyl chloride.
dialyzers and dialysis tubing sets. Finally, breath ethane does not appear to be a reliable biomarker in this population, suggesting that ethane is not a good indicator of oxidative stress.

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