

Original Article

Elective haemodialysis increases exhaled isoprene

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Abstract

Background. Uraemic odour is a characteristic feature of patients with end-stage renal disease (ESRD). However, few investigations have been carried out into the composition of exhaled air in ESRD patients undergoing haemodialysis (HD). Increases of exhaled isoprene levels by a factor of up to 2.7 following HD have been reported.

Methods. We attempted to confirm these findings in 50 patients undergoing HD using haemophan ($n=23$) or polysulphone ($n=27$) dialysis membranes. Parallel evaluation of ambient air, calorie intake, medication and haemodynamic variables was performed. Samples were analysed using proton transfer reaction–mass spectrometry (PTR–MS).

Results. Significant changes in breath isoprene concentration were observed when comparing patients before [39.14 ± 14.96 parts per billion (ppbv)] and after [63.54 ± 27.59 ppbv] dialysis ($P < 0.001$). The quotient of values before and after dialysis was 1.84 (SD 1.41). No significant differences in isoprene kinetics were found between the use of haemophan and polysulphone membranes. No significant correlations were observed between isoprene quotients and variations in blood pressure during HD, calorie intake, ingestion of lipid-lowering drugs or serum lipid levels.

Conclusions. Isoprene concentration was higher in the exhaled air of patients after HD as compared with values before HD. Large interindividual variability existed in isoprene kinetics. Oxidative stress appears to be an unlikely cause for this rise. An alternative hypothesis is an influence of respiratory variables on isoprene exhalation based upon Henry's law constant. We therefore propose to perform online monitoring of isoprene exhalation by PTR–MS during the HD session to investigate the possible influence of respiratory variables.

Keywords: breath test; haemodialysis; isoprene; proton transfer reaction–mass spectrometry; volatile organic compounds

Introduction

One characteristic feature of patients presenting with end-stage renal disease (ESRD) is unusual breath odour. For decades, definitions of this 'uraemic breath' have been included in nephrology and medicine textbooks [1].

Substances principally involved in the generation of odours are volatile organic compounds (VOCs). This collective of hydrocarbons is present in body fluids and detectable in human breath in patterns depending upon nutrition [2], disease [3] and physical activity [4]. Furthermore, exhalation rates of individual, blood-borne VOCs in human breath are dependent upon Henry's constant and, therefore, molecular weight and hydrophobicity [5].

For a long time, uraemic odour was attributed to oral cavity ulceration and bacterial overgrowth. As early as 1963, Simenhoff *et al.* [6] detected relevant amines in body fluids of ESRD patients. Yet, it was not until the 1970s that the same author characterized di- and trimethylamine as the substances possibly responsible for this odour by combined gas chromatography/mass spectrometry also in human breath [1] and speculated on the intestinal origin of these substances [7]. Although it is therefore clear that certain VOC species related to uraemia appear to be exhaled by ESRD patients, few investigations into respective patterns have since been carried out.

A recent study into VOC kinetics by Capodicasa *et al.* [8] found dramatic rises in the VOC isoprene, by a factor of up to 2.7, in patients on intermittent haemodialysis (HD) treatment. Isoprene has been considered as a marker of oxidative stress but this issue remains controversial [8]. The presence of elevated isoprene levels following HD has been confirmed by Davies *et al.* [9]. One major cause of

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oxidative stress during HD is dialyser membranes exhibiting different levels of biocompatibility [10]. Bioincompatible membranes, as for example haemophan, are more likely to cause activation of neutrophils and subsequently oxidative stress than biocompatible membranes, which are increasingly in use [11]. Proton transfer reaction–mass spectrometry (PTR–MS) has been established as a new and valuable tool in screening human breath samples [4,12]. Moreover, new insights into the metabolism of isoprene have been reported [13].

Therefore, the focus of the present study was to screen the breath of 50 patients with ESRD before and after HD using two different membranes. We attempted to re-examine HD-related kinetics in breath isoprene and to track possible correlations with metabolic, drug-induced and haemodynamic parameters. The working null hypothesis was that isoprene would not increase during HD using either membrane.

Subjects and methods

Patient population

Fifty patients on intermittent HD treatment at the local Clinical Division of Nephrology were enrolled in the present study. After approval by the local Ethics Committee, written and informed consent was obtained. Patients were dialysed using either a HG600 haemodialyser (Gambro Hospital, Wiener Neustadt, Austria; $n=23$) or an F Series haemodialyser (Fresenius Medical Care, Vienna, Austria; $n=27$). Mean duration of HD was 246 (± 44) min. Patients were randomly recruited from three different HD sessions (06.00–11.00, 12.00–17.00 and 18.00–23.00 h). Lipid-lowering medication and routinely acquired serum levels of total cholesterol and low density lipoprotein (LDL) cholesterol were recorded (Table 1). Blood pressure and heart rate were recorded each time breath samples were attained and after 2 and 3 h into HD (Figure 1). Calorie intake, antihypertensive medication and heparin/lovenox administration of patients were recorded.

Table 1. Patient characteristics

	Haemophan ($n=23$)	Polysulphone ($n=27$)
Age (years)	61.4 \pm 16.4	50.3 \pm 14.6
Body weight before HD (kg)	75.6 \pm 14.1	76.8 \pm 12.2
Body weight after HD (kg)	72.1 \pm 13.2	74.3 \pm 11.9
Smokers	$n=1$	$n=0$
Statin therapy	$n=1$	$n=4$
Heparin therapy	$n=20$	$n=25$
Lovenox therapy	$n=2$	$n=2$
Duration of HD (min)	233.5 \pm 57.9	258.5 \pm 33.3
On HD for (months)	35.1 \pm 34.7	46.3 \pm 49.5
Calorie intake during HD (kcal)	476.2 \pm 208.4	395 \pm 198.6
Mean cholesterol levels (mg/dl)	175.9 \pm 31.2	171.5 \pm 49.1
Mean LDL levels (mg/dl)	93.9 \pm 30	95.7 \pm 41.8
Mean HDL levels (mg/dl)	43.7 \pm 14.1	39.6 \pm 13.7
Mean TG levels (mg/dl)	197.7 \pm 142.5	200 \pm 168.2

Data are given as means \pm SD. HDL, high density lipoprotein; TG, triglycerides.

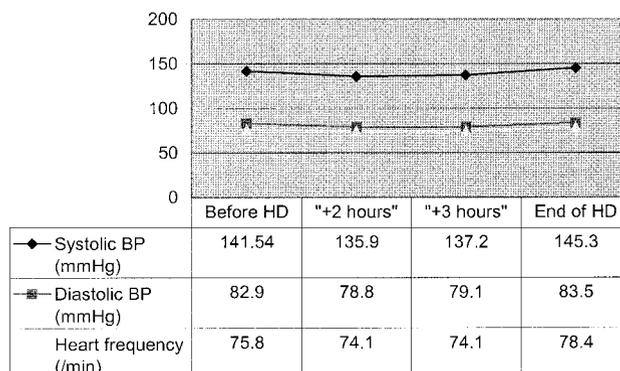


Fig. 1. Haemodynamic variables immediately before, after and 2 and 3 h into HD. Data are given as the mean. BP, blood pressure.

Breath sample collection

Patients were asked to exhale into a sample bag (Adtech, Gloucestershire, UK) as previously described [4]. Samples were collected before HD was initiated and immediately after the patients had been disconnected from the dialysis machine. Patients had to rest for a minimum of 5 min before pre-dialysis samples were obtained. Post-dialysis samples were collected with the patients still at rest to minimize the previously reported influence of blood pressure upon isoprene concentration [4]. Kinetics were expressed as a quotient calculated as concentration of isoprene after HD divided by concentration of isoprene before HD (ppbv). Parallel evaluation of room air was performed as previously described [14] before and after dialysis to unveil possible interactions with exogenous parameters able to spoil measurements, like, for example, cleaning and disinfecting substances [14].

Breath analysis

PTR–MS allows online monitoring of VOCs with volume mixing ratios as low as a few pptv [5]. Chemical ionization is applied based on proton transfer reactions, with H_3O^+ as the primary reactant ion, which is most suitable when air samples

containing a wide variety of trace VOCs are to be analysed [5]. Almost all VOCs have proton affinities larger than H_2O and therefore proton transfer occurs on every collision with rate constants k that are well known, having typical values of $1.5 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1} < k < 4 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$. A decisive advantage of using primary H_3O^+ ions is that many of their proton transfer processes are non-dissociative, so that only one product ion species occurs for each neutral reactant. In cases where dissociations do occur, they frequently follow a straightforward pattern, e.g. the ejection of a H_2O molecule from protonated alcohols. In PTR-MS measurements, protonated mass 69 predominantly represents exhaled isoprene [4,15].

Statistical analysis

Results are given as means \pm SD. Patient characteristics in haemophan and polysulphone study groups were compared using analysis of variance (ANOVA) or Fisher's exact test as applicable. For statistical comparisons of isoprene concentrations before and after HD, a paired t -test was used after Kolmogorov-Smirnov testing assured normal distribution of values. For correlation of isoprene quotient with physiological parameters, Pearson's correlation coefficient was employed. Blood pressure during HD was statistically evaluated using ANOVA for repeated measurements. For *post hoc* multiple comparisons, the Bonferroni type I error correction procedure was applied. Statistical significance was assumed at $P < 0.05$ and results were considered highly significant at $P < 0.001$.

Results

Significant changes in exhaled mass 69 (protonated isoprene) were found comparing patients before (39.1 ± 14.9 ppbv) and after (63.5 ± 27.5 ppbv) dialysis ($P < 0.001$) (Figure 2). The quotient of values before and after dialysis was 1.84 ± 1.41 .

No differences in isoprene kinetics were observed between haemophan and polysulphone membranes (Figure 3).

No significant differences in the rise in exhaled isoprene concentration were found in patients under statin therapy as opposed to patients without statin therapy. There was no significant correlation between isoprene quotients and serum levels of total cholesterol or LDL cholesterol and no correlation between calorie intake and isoprene rise.

Using ANOVA, blood pressure values were not significantly different at any time point between membrane groups. For the whole patient population, diastolic blood pressure significantly decreased 2 h after initiation of HD as compared with values before HD. However, this effect was not significant after Bonferroni correction (Figure 1).

Mean levels of isoprene concentration in ambient air were $1.55 (\pm 0.58)$ ppbv.

No significant differences in patient characteristics were encountered, as listed in Table 1, with the exception of age, which was significantly ($P < 0.05$)

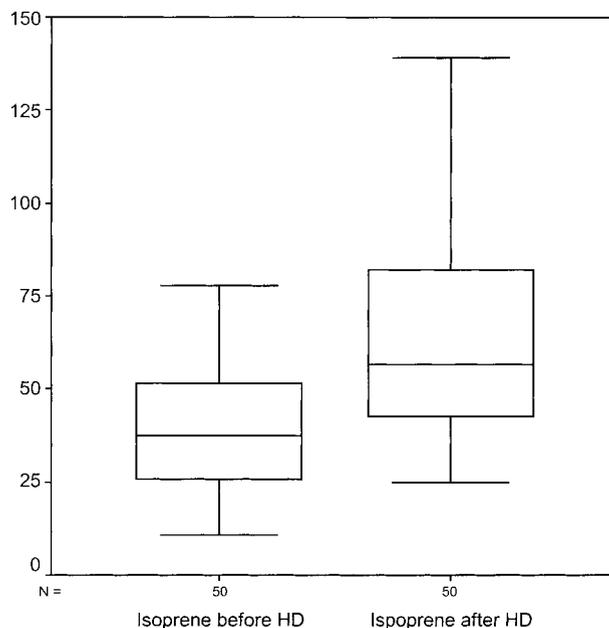


Fig. 2. Isoprene concentrations of patients ($n = 50$) before and after HD are given as ppbv on the y -axis. Boxes represent mean \pm SD; bars represent 5% and 95% percentiles.

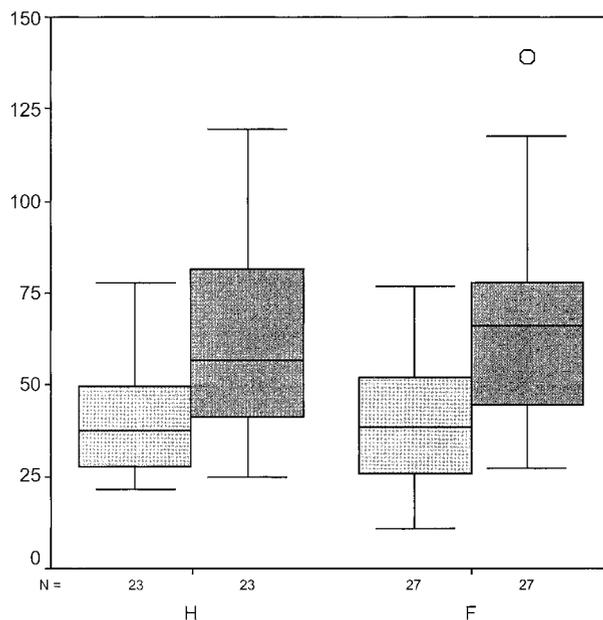


Fig. 3. Mean exhaled isoprene values for patients dialysed using either haemophan (H, $n = 23$) or polysulphone (F, $n = 27$) membranes, before (light grey) and after HD (dark grey), are given as ppbv on the y -axis. Boxes represent mean \pm SD; bars represent 5% and 95% percentiles.

higher in the haemophan group. However, analysis of covariance with age as a possible confounder did not show a significant effect on the relationship between membrane type and isoprene quotient.

Discussion

The present investigation aimed to investigate in detail the kinetics of isoprene in 50 chronic HD patients. In concordance with previous literature, a highly significant increase of breath isoprene levels following HD could be demonstrated. The mean post-/pre-dialysis quotient was 1.84 ± 1.41 . Large interpersonal variations in isoprene kinetics were, however, observed. The parallel evaluation of room air confirmed the endogenous origin of breath isoprene.

Compared with values reported previously [15], the air isoprene content found by PTR-MS was lower considering mean values, but high interpersonal variability was confirmed, as acknowledged previously [15]. This is reflected by the range of isoprene values before and after HD (Figure 2). Reported values for isoprene vary greatly according to the method used [9, 15]. Furthermore, a profound influence of haemodynamic parameters upon breath isoprene concentration has recently been reported [4, 15]. Therefore, a strict set-up of sample collection after resting times should decrease measured isoprene concentrations. The method that is most comparable with the PTR-MS system used in the present study to investigate breath isoprene during HD is the selected ion flow tube-MS used by Davies *et al.* [15]. In their study, the experimental set-up included no resting times and patients had to move to a laboratory adjacent to the HD facility for sample collection even though transient increases in blood pressure may have significantly influenced isoprene exhalation rates [15]. 'Mandatory' breaching of necessary resting times in the study conducted by Davies *et al.* may therefore, in principle, explain the higher isoprene concentration in baseline (138 ± 63 ppbv) and post-dialysis (184 ± 95 ppbv) samples [9]. Therefore, special interest focused on parallel evaluation of blood pressure, as this has been demonstrated to influence levels of exhaled isoprene [4]. It is conceivable to assume that during mild hypotension, which may occur during dialysis, isoprene accumulates in body fluids. Cessation of dialysis could then hypothetically increase blood pressure and trigger increased exhalation of isoprene. However, no significant alterations of blood pressure were observed in our patient groups after *post hoc* correction of ANOVA results (Figure 1).

Oxidative stress has been repeatedly conjectured as a conceivable cause of isoprene rise [8,9]. Contact of human leucocytes with bioincompatible dialyser membranes elicits an oxidative burst by neutrophils [16]. The involvement of specific hydrocarbons (e.g. ethane and pentane) in oxidative stress has long been acknowledged [17]. However, consistent evidence concerning the generation of isoprene via oxidative stress is not available. A recent trial in cystic fibrosis patients during acute exacerbations could not establish correlations between breath isoprene levels and blood-borne markers of oxidative stress [18]. Furthermore, the two dialysis membranes used in the present study differed with respect to biocompatibility. Whereas

polysulphone can be considered as biocompatible, haemophan is more likely to cause complement activation and neutrophil activation via two distinct pathways [11,19]. Therefore, if isoprene were a marker of oxidative stress, one should expect differences in isoprene rise after the dialysis session. However, this was not the case (Figure 2). Furthermore, in contrast to findings in HD patients, no isoprene overproduction was observed during continuous ambulatory peritoneal dialysis (CAPD) by Capodicasa *et al.* [20]. This study, together with recent evidence showing no significant differences in oxidative stress status between HD and CAPD based on circulating biomarkers [21], would argue against an oxidative genesis of increased breath isoprene.

A possible influence of the circadian rhythm of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase upon cholesterol synthesis, and therefore isoprene production, has also been proposed [13]. However, in the patients of the present study who were selected randomly from three different daytime schedules of HD, the increase in exhaled isoprene concentration after HD was the same in patients independent of daytime. Therefore, it appears to occur independently of circadian rhythm which would, at best, explain a nocturnal rise in isoprene.

Patient demographics showed no significant influence upon isoprene exhalation in the present patient population.

Based upon the Henry's constant of isoprene (0.029 M/atm) [15], an alternative hypothesis is conceivable. The low constant indicates that diffusion across the pulmonary barrier readily occurs. Therefore, changes in respiratory frequency and/or tidal volume readily occurring during HD [22] may considerably influence isoprene exhalation. This possibility is underpinned by the rapid reactions of exhaled breath isoprene to changes in blood pressure [4,15]. Individual variations in ventilatory drive would therefore offer the most plausible explanation for the large interindividual differences in isoprene kinetics. According to this hypothesis, a decrease in respiratory drive would lead to endogenous accumulation of isoprene, whereas the increase of ventilation back to baseline after HD would then release the accumulated isoprene over the consecutive hours. This would be in concordance with a recent trial demonstrating a time-dependent decay of elevated isoprene levels following HD [23].

Finally, some limitations of the present study should be discussed. The device chosen for isoprene monitoring (PTR-MS) does not allow a precise differentiation of substances, i.e. all substances with a common molecular mass are determined together. Thus, interfering molecular species with the same molecular mass as isoprene (68 unprotonated atomic mass units) constitute a potential error source, although a significant impact of possible substances in exhaled isoprene measurements by PTR-MS has recently been dismissed [24].

In conclusion, taking into account the variables, such as ambient air, lipid-lowering drugs, heart rate,

blood pressure and membranes of different biocompatibility, the previously reported increase in breath isoprene content following HD could be confirmed. Oxidative stress seems an unlikely cause for this rise since patients exposed to two membranes with differing biocompatibility exhibited no significant difference in isoprene increase. An alternative scenario might be the direct influence of respiratory variables (respiratory frequency, tidal volume) on isoprene exhalation based upon Henry's law constant. We therefore propose a future study with online monitoring of isoprene exhalation during HD by PTR-MS to investigate respiratory variables and their possible direct influence on isoprene exhalation.

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Conflict of interest statement. None declared.

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