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Mass spectrometric profile of exhaled breath—field study by PTR-MS

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Abstract

Recently, increased interest has focused on the diagnostic potential of volatile organic compounds (VOC) exhaled in human breath as this substance group has been conjectured in indoor air quality and disease screening. Proton transfer reaction-mass spectrometry (PTR-MS) has been established as a new tool for a rapid determination of exhaled air profile. However, no investigations have been carried out into the profile of exhaled air as determined by PTR-MS. Therefore, it was the aim of the present study to determine the profile of exhaled breath in a field survey enrolling 344 persons. Analysis was performed using PTR-MS. No significant correlations with age, blood pressure, and body mass index could be observed with any molecular mass. The present study delineates possible reference values for PTR-MS investigations into exhaled air profile. In conclusion, the present study was the first to delineate mass spectrometric characteristics of an average patient sample as possible reference values.

Keywords: Compounds, organic, volatile; Disease, screening, volatile organic compounds; Gas exchange, exhaled air profile; Mammals, human; Techniques, mass spectrometry, proton transfer reaction

1. Introduction

Over the past decade, increasing evidence has been put forward that certain patho-physiologic and metabolic processes may, in principle, be mirrored by the composition of human exhaled air. Especially over the last years, the annual number of publications dealing with diagnostic breath tests has steadily risen (Fig. 1). Among the hundreds of substances found in a human breath sample (Phillips et al., 1999b), it could be demonstrated that certain clusters of volatile organic compounds (VOC) could serve as non-invasive biomarkers of various disease states such as hyperlipidemia (Rieder et al., 2001a), lung cancer (Phillips et al., 1999a), breast cancer (Phillips et al., 2003b), and recent smoking behavior (Lindinger et al., 1998).

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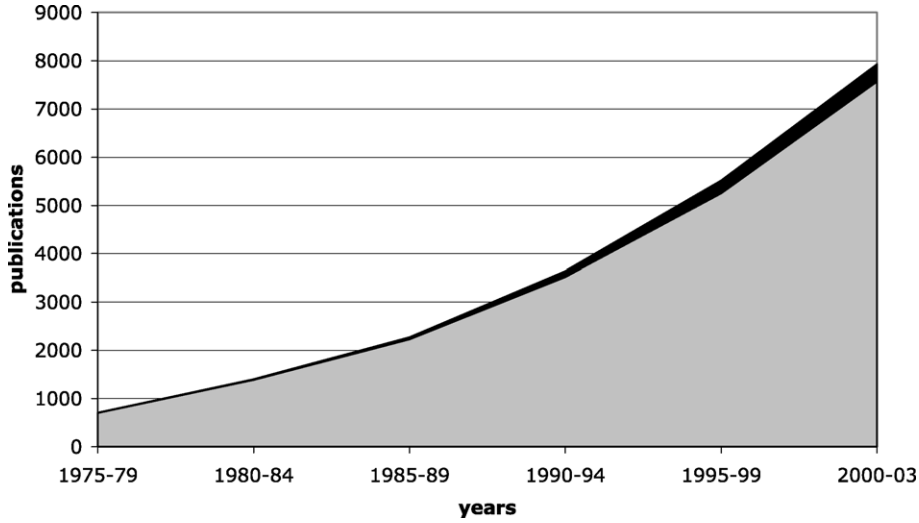

Fig. 1. Cumulative publications retrieved from a simple MEDLINE search using the keywords “volatile organic compounds” (grey) and a search for studies designated as “clinical studies” under the keyword “volatile organic compounds” (black) during 5-year intervals.

Analysis of these VOC patterns may, in principle, be achieved using combined gas chromatography–mass spectrometry (GC-MS) (Phillips, 1997) or mass spectrometry (MS) (Davies et al., 2001). Recently, Proton transfer reaction-mass spectrometry (PTR-MS) has been introduced as a new possible method for rapid screening of large collectives for risk factors, potential disease biomarkers, and ambient air (Rieder et al., 2001a; Rieder et al., 2001b). However, no studies have been conducted surveying the physiologic distribution in mass spectrometric profile as determined by PTR-MS of exhaled air in large human collectives.

Therefore, it was the aim of the present study to determine the PTR-MS profile of human in a collective of test persons attending a health fair.

2. Methods

Three hundred and forty-four test persons attending a public health fair were enrolled in the present study. Written and informed consent were obtained from each participant. Test persons were asked to exhale into a sample bag (Adtech, Gloucestershire, UK) as previously described (Rieder et al., 2001a). In brief, patients had to discard the first part of expired air and exhaled only the deep portion into the sample bag. A minimum of 5 min of rest before samples were obtained was mandatory. Parallel evaluation of room air was performed to unveil possible interactions with exogenous parameters able to spoil measurements like, for example, cleaning substances (Rieder et al., 2001b), medication, and hemodynamic variables (Lirk et al., 2003). To avoid baseline contamination of sample bags, these were flushed with pure nitrogen and subsequently voided three times.

2.1. Analysis

Analysis of samples was performed using proton transfer reaction-mass spectrometry (PTR-MS) as previously described (Hansel et al., 1995). In brief, 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 volatile organic compounds are to be analyzed (Hansel et al., 1995). 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 $1.5 \times 10^{-9}\,\mathrm{cm^3\,s^{-1}} < k < 4 \times 10^{-9}\,\mathrm{cm^3\,s^{-1}}$. 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

Table 1
Demographic data of test persons

Age (mean ± S.D., years); median [25%-percentile, 75%-percentile]	61.6; [50.9, 69.6]
Male/female (%)	63.37/36.63
Smoker/nonsmoker (%)	13.95/86.05
Fasted/non-fasted (%)	9.01/90.99

straightforward pattern, e.g. the ejection of an H_2O molecule from protonated alcohols.

Analysis was conducted with the sample bags heated to 37 °C to impede condensation of compounds on the inner bag surface. For the displayed substances, the background VOC concentration was negligible, and these VOC had a positive alveolar gradient.

2.2. Statistics

Descriptive statistics included the minimum, maximum, median, the 2.5%-, 25%-, 75%-, and 97.5%-percentile for continuous data, absolute and relative frequencies for categorical data. For analysis of dichotomy factors influencing the mass measurements the Mann–Whitney *U*-test was used. The correlations between the mass measurements and continuous factors were described with Spearman's correlation coefficient. Measurements for 179 different masses were made for 344 people, whose demographics are delineated in Table 1. Statistical significance was assumed at $P < 0.05$.

3. Results

Detailed results with minimum, maximum, median, the 2.5%-, 25%-, 75%-, and 97.5%-percentile for the measured molecular masses in the collective of 344 persons are given in Table 2.

The correlation between mass measurements and continuous factors were small. For body mass index, age, pulse, diastolic and systolic blood pressure the highest correlation coefficient achieved with any single mass was 0.139, 0.241, 0.164, 0.137 and 0.120, respectively.

Significant differences in exhaled breath composition could be found between smokers and non-smokers in 32 out of the 179 masses, with the most significant difference in mass 42. In 10 out of 179 masses a significant difference was found between male and the female test persons with $P = 0.002$ found in mass 70. Similarly, correlation between fasted and non-fasted test persons was found in three masses with the most significant difference in mass 28 ($P = 0.015$).

The mean age of test persons was 61.6 years. No correlation could be observed between any molecular mass concentration and age. The most significant correlation on mass 24 yielded a Spearman's coefficient of $r = -0.2411$.

Table 2
Results of breath sample analysis for substances detected using PTR-MS given as parts per billion (ppbv) (Lindinger et al., 1998)

Mass	Substance	Minimum	2.5%-percentile	25%-percentile	Median	75%-percentile	97.5%-percentile	Maximum
31	Formaldehyde	1,230	1,738	3,104	4,263	6,332	39,814	72,729
33	Methanol	13,367	59,736	106,227	161,179	243,185	643,614	1536,499
35	Hydrogen suplhide	0,000	0,096	0,227	0,382	0,680	2,198	4,661
42	Acetonitrile	1,425	4,534	6,857	9,091	16,151	97,065	266,250
43	Benzene	29,762	59,092	100,330	133,070	186,333	442,401	1391,078
45	Acetaldehyde	12,466	24,005	30,719	37,943	52,488	130,489	1397,457
47	Formic acid, ethanol	11,583	13,254	23,100	34,664	64,240	549,240	9779,768
59	Propanal, acetone	12,420	258,431	413,749	542,340	778,830	1965,427	6320,508
61	Acetic acid	14,557	17,564	42,529	61,620	89,883	182,490	473,334
63	Dimethyl sulphide	0,873	5,025	7,926	10,847	20,341	63,140	87,770
69	Isoprene	1,944	14,071	35,816	51,674	71,357	143,903	195,279
79	Benzene	0,537	0,765	1,100	1,575	2,589	13,064	18,976
93	Toluene	1,134	2,860	6,703	11,212	18,197	41,056	150,848
94	Phenol	1,103	1,681	4,125	6,320	9,761	25,465	32,182
107	Xylene	0,695	1,014	1,796	2,731	4,090	8,775	42,701

4. Discussion

The main result of the present study is the first determination of reference values for an exhaled air profile as determined by PTR-MS.

The screening of human exhaled breath for VOC characteristic for certain disease states has gained increasing attention in recent literature as shown in Fig. 1, correlation of original published manuscripts connected with diagnostic potential of breath test. This collective of hydrocarbons is present in body fluids and detectable in human breath in patterns depending upon nutrition (Lindinger et al., 1998), disease (Phillips et al., 1999a), and physical activity (Karl et al., 2001). Furthermore, exhalation rates of individual, blood-borne, VOCs in human breath are dependent upon Henry's constant and, therefore, molecular weight and hydrophobicity (Hansel et al., 1995). PTR-MS has been repeatedly described to allow for both single and real-time measurements of volatile organic compounds in exhaled air (Karl et al., 2001; Lirk et al., 2003; Rieder et al., 2001a; Summer et al., in press). One major advantage of the presented method is the high speed of measurements. Even when performing measurements for several times to monitor stability of the system, typical analysis times are in the range of 5–10 min. The entire patient collective enrolled in the present study could be analyzed within 3 days.

Several masses allow for a precise allocation to predominant breath VOC. Among the most important substances are isoprene and methanol.

Protonated mass 69 has been described as reflecting exhaled isoprene. This substance has recently been the focus of considerable interest since in vivo, isoprene could be shown to increase during haemodialysis (HD) in patients with end-stage renal disease (ESRD). This has been ascribed to oxidative stress elicited by bioincompatible membranes, metabolic changes, and physiological parameters in connection with haemodialysis. One recent study into VOC kinetics carried out by Capodicasa et al. found dramatic rises in the VOC isoprene of up to 270% in patients undergoing haemodialysis. The authors concluded that this rise could, in principle, be elicited by lipid peroxidation during HD (Capodicasa et al., 1999). The presence of higher isoprene levels following HD has been confirmed by Davies et al. (2001). Lirk et al. aimed to investigate in detail the kinetics of isoprene in 50 patients scheduled for elective hemodialysis using PTR-MS for sample analysis. In concordance with previous literature, a highly significant elevation of breath isoprene levels following HD could be demonstrated. The mandatory resting times introduced to assure basal blood pressure and breathing rate values lead to lower values of isoprene than previously determined (Lirk et al., 2003). In comparison to the latter study, which stipulated baseline values of 39 ppbv for isoprene at rest, our findings of a mean exhaled isoprene concentration of 52 ppbv seems slightly elevated. Mandatory resting times have been described as essential for the conduct of isoprene analysis in breath samples (Lirk et al., 2003). Therefore, the higher isoprene concentrations found in studies by Taucher et al. (1997), Mendis et al. (1994), and Davies et al. (2001) are most likely caused by the breaching of resting times necessary for a sensible evaluation of breath samples for isoprene (Lirk et al., 2003).

In analogy, methanol and ethanol have been investigated using PTR-MS (Lindinger et al., 1997; Taucher et al., 1995). Whereas ethanol had been studied previously, simultaneous analysis of methanol was, until recently, virtually impossible using conventional mass spectrometric methods, since the ensuing fragmentation superposed individual molecular rudiments (Taucher et al., 1995). Methanol has been shown to be a multipotential carcinogenic agent (Lindinger et al., 1997). Therefore, additional means of elucidating its role both in colonic physiology, neoplastic disease, and liver degeneration are of significant importance (Taucher et al., 1995). It has been suggested that breath-based analysis by PTR-MS may, in principle, be employed to test inborn variations in the metabolic pathways of alcohol breakdown (Taucher et al., 1995). Previous small-scale studies stipulated the average methanol excretion in human breath to be about 150–600 ppbv. This is in full concordance with our results, which show an average concentration of 161 ppbv for mass 33, corresponding to protonated methanol (Lindinger et al., 1998), and feature a large interpersonal variability, most probably due to ingestion of foods known to elicit rises in endogenously produced methanol, such as apples (Lindinger et al., 1997).

Furthermore, the ammonium content in exhaled air has been linked to blood ammonium levels,

thereby possibly representing a non-invasive marker of dialysis success and quality (Narasimhan et al., 2001). Most notably, patients with lung cancer and hemato-oncologic disorders have been reported to feature distinct patterns of exhaled volatile organic compounds (VOC). Phillips et al. (2003a) and Rieder et al. (2001a) conjectured *ortho*-toluidine as a possible marker substance for neoplastic disease.

Risk factor assessment has been attempted in hyperlipidemia, in which elevations of isoprene may serve as a rapid screening marker (Rieder et al., 2001a), whereas increased levels of acetonitrile in exhaled air are indicative of recent smoking behavior (Lindinger et al., 1998).

Furthermore, PTR-MS has been applied to monitor occupational exposure to noxious agents (Summer et al., in press).

Finally, some limitations of the presented study should be discussed: PTR-MS detects the concentration of specific molecules according to their individual molecular mass. Therefore, interference of molecular species other than the specific molecule in question is a potential source of error. Whereas measurements of abundant substances such as sevoflurane and isoprene in exhaled air may be performed by PTR-MS alone (Karl et al., 2001; Summer et al., in press), analysis of molecules less abundant may need to be performed using combined methods of GC and MS. Nevertheless, PTR-MS represents an interesting tool for a fast screening of patient collectives for certain diseases or occupational exposure to noxious agents.

Furthermore, the test person collective enrolled in the present study was recruited from attendants of a health fair, and mean age was 61.6 years. Even though no significant correlation was found between concentrations of any measured molecular mass and age in the present study, possible age-dependency has been suggested for isoprene in a smaller collective (Hansel et al., 1995). However, it should be noted though that the present collective of older persons may be a suitable reference collective for the age group from 50 to 70 years, which is most prone to develop diseases for which breath test have been proposed (Karl et al., 2001; Phillips et al., 1999a; Rieder et al., 2001a). The influence of the factors smoking, sex and fasting on mass measurements were small. The differences in mass measurements for alpha = 0.05 for smoking, sex and fasting were significant for only 32, 10 and 3 masses, respectively.

In conclusion, taking into account the rapidly growing number of publications into the diagnostic potential of VOC (see Fig. 1), this sub-area in medical research is certain to increase in impact upon basic and clinical research. PTR-MS may represent a valuable tool in the rapid screening of large patient collectives. The present study was the first to delineate mass spectrometric characteristics of an average patient sample as possible reference values.

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