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Serum Levels of Volatile Organic Compounds in Patients with Sick Building Syndrome

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Volatile organic compounds (VOCs) pose possible health risks that could result from exposure to indoor airborne VOCs as suggested by the causal associations with symptoms of sick building syndrome (SBS) (Hodgson 2002). People can be exposed to VOCs through various products and processes, including building materials, paints, cleaning agents, pesticides, tobacco smoke, and personal care products in addition to the traditional sources of exposure such as occupation and ambient air pollution (Ashley et al. 1996). There is no universally accepted clinical definition of SBS and no adequate theory for its occurrence (Redlich et al. 1997). The characteristics of SBS are non-specific symptoms which include dryness and irritation of skin, eyes and air-ways and general symptoms. In Japan, SBS symptoms associated with indoor air VOCs in new or newly remodeled houses have been increasingly highlighted, and they are called 'sick house syndrome'.

Although past researchers have tried to understand the causes of SBS, they did not generally succeed in showing direct evidence that elucidates the relationship between SBS and VOCs (Hodgson 2002). For example, information concerning serum levels of VOCs in patients with SBS symptoms is not available because measuring sub ng levels of VOCs in serum with the exclusion of experimental contamination is very difficult. To address this issue, we examined (1) whether headspace gas chromatography/mass spectrometry (HS-GC/MS) was applicable to the measurement of serum VOC concentrations in SBS patients and volunteer controls, and (2) whether the elevation of serum VOC levels correlated with SBS symptoms.

MATERIALS AND METHODS

For contamination control, blood-collecting equipment was treated as described below. Glass syringe barrels were sterilized in an autoclave and dried in a drying oven. Test tubes and screw caps were washed with detergent, water and methanol, and then heated at 180 °C for 5 hours. They were fitted with screw caps and then stored. A saturated saline solution was prepared by mixing sodium chloride and distilled water, which was subsequently subjected to aeration using helium gas and to degassing in an ultrasonic bath under reduced pressure. The prepared solution

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Table 1. Retention times, quantification and confirmation ions.

Analyte	Retention time (min)	Quantification ion	Confirmation ion	Quantification ion (internal standard)
Benzene	12.7	78	51	84
Toluene	15.3	91	92	98
Ethylbenzene	17.3	91	106	98
m,p-Xylene	17.4	91	106	98
o-Xylene	18.2	91	106	98
Styrene	18.3	104	78	112
p-Dichlorobenzene	21.2	111	146	115
Naphthalene	25.1	128	127	136

was immediately placed in headspace vials previously treated as for test tubes. Internal air space in the vial was replaced with helium gas. The headspace vials were sealed with Teflon-backed septa and crimp caps.

Fortified sample recovery was carried out using pig serum. Although human blood is easily available in hospitals, it is difficult to obtain it in quantity without contamination problems when baseline levels are measured. We therefore procured pig serum using the contamination control described above.

For the measurement of VOCs in serum, 1 mL of serum was diluted with 14 mL of saturated saline solution and analyzed by HS-GC/MS. VOCs were quantified using deuterated compounds as internal standards. Target VOCs (benzene, toluene, *o*-, *m*-, *p*-xylene, ethylbenzene, styrene, *p*-dichlorobenzene and naphthalene) were selected for analysis because they are frequently found in indoor air in Japan (Saijo et al. 2004). HS-GC/MS analysis was carried out under the following conditions:
Headspace sampler: Tekmar 7000 (Tekmar, USA), vial size: 22 mL, sample temperature: 60 °C, sample equilibrium time: 20 min, mixer: on (power 5, 3 min), sample loop size: 1 mL, sample loop temperature: 150 °C, transfer line temperature: 160 °C. GC/MS: AUTO MASS SYSTEM II (Jeol, Japan), Column: Vocol (60 m x 0.25 mm i.d., 0.1 μm film thickness, Supelco, USA), oven temperature: initial temperature 40 °C with 4 min hold, then 10 °C /min to 230 °C and post run at 230 °C for 5 min, ion source temperature: 210 °C, EI voltage: 70 eV, scan range: m/z 46-260. Table 1 shows the retention times and quantification masses used for each of the analytes and internal standards.

The study participants were 18 patients with SBS and 32 volunteer controls. Seven patients live in Sapporo, and 11 live in Aichi Prefecture, Japan. The patients were diagnosed according to the following criteria: 1) a typical setting for symptoms is a new or newly remodeled house; 2) symptoms generally improve when the patient is away from the house. The possible factors of SBS onset were moving into a new or remodeled house (56%, 10/18), use of chemicals such as insecticides, mothballs and bleach (33%, 6/18), exposure to organophosphorus pesticides (6%, 1/18) and unknown (6%, 1/18). The volunteer controls were recruited from the staff of Aichi Prefectural Institute of Public Health, Nagoya, Japan. All volunteer controls live in Aichi Prefecture, Japan. Five volunteer controls were excluded because they are smokers. Elevated serum VOC levels due to smoking have been reported

previously (Mannino et al. 1995), and smoking is the largest confounder in discerning the influence of other environmental exposure (Ashley et al. 1996). In fact, the detection rates and levels of toluene, styrene and benzene in the smokers' serum were higher than in nonsmoker volunteer controls (data not shown).

Of the patients, 89% (16/18) were women and 11% (2/18) were men. Previous studies have shown the same pattern (Stenberg et al. 1995). Among patients, 22% (4/18) were under 29 years of age, 44% (8/18) were 30-49 years of age, and 33% (6/18) were over 50 years of age. Representative symptoms were classified into 10 categories as follows: eye (irritation, dry eyes, eye congestion); nose (stuffy or runny nose); throat (sore throat, itchy throat); respiratory system (cough, shortness of breath); skin (itching and dry skin); general symptoms (fatigue, headache, dizziness); psychological symptoms (difficulty concentrating, insomnia, depression); musculoskeletal system (joint pain, numbness in the hands or feet); gastrointestinal system (nausea, stomachache, diarrhea); genitourinary system (increased urinary frequency, menstrual pain, menorrhagia). The prevalence of patients' symptoms was significantly greater than for controls as follows: (patients vs controls) eye, 67% vs 16%; nose, 83% 16%; throat, 56% vs 3%; respiratory system, 50% vs 3%; skin, 44% vs 9%; general symptoms, 72% vs 9%; psychological symptoms, 56% vs 3%; musculoskeletal system, 44% vs 0%; gastrointestinal system, 44% vs 0%; genitourinary system, 50% vs 0%. The data analyzed in this paper were collected in 2001 and 2002 in Japan. This study was conducted according to the Declaration of Helsinki and signed informed consent was obtained from all subjects.

For statistical evaluation of the prevalence of symptoms between patients and controls, the proportions of positive symptoms were compared using the Chi-square test or Fisher's exact test for the resulting 2x2 contingency table. To compare VOC concentrations between patients and controls, Mann-Whitney's U-test was used. Differences in the mean number of symptoms within the patient group and between the two groups dichotomized at the limit of quantification of serum VOCs were evaluated using Mann-Whitney's U-test. In all statistical analysis, a 5% level of significance was applied.

RESULTS AND DISCUSSION

Reproducible calibration curves for all target VOCs were obtained with correlation coefficients greater than 0.998 (known concentration vs analyte/internal standard ratio) by HS-GC/MS analysis of standard VOCs. The method was sensitive with limits of detection between 0.1 and 0.5 ng in 1 mL of serum for all analytes. The method does not require complicated sample preparation procedures and takes only 1 hour to complete all procedures starting from sample dilution to quantification by HS-GC/MS analysis.

In order to examine the applicability of this method to real sample analysis, VOC recoveries were determined. The recoveries from blank pig serum spiked with target VOCs and their internal standards are summarized in Table 2. Spiked VOCs

Table 2. Recovery of VOCs from serum.[a]

VOCs	Scan mode (with IS)[b]			SIM mode (with IS)[b]			Scan mode (No IS)[c]
	LOQ[d] (ng/mL)	Recovery (%)	CV[e] (%)	LOQ[d] (ng/mL)	Recovery (%)	CV[e] (%)	Recovery (%)
Benzene	0.5	106	2.8	0.1	104	4.7	85.1
Toluene	0.5	103	1.4	0.1	101	6.0	65.6
Ethylbenzene	0.5	101	2.2	0.1	96.6	4.4	44.3
m,p-Xylene	0.5	97.3	1.7	0.1	100	4.5	42.1
o-Xylene	0.5	100	2.7	0.1	89.8	4.3	44.8
Styrene	0.5	101	1.9	0.1	105	4.9	48.6
p-Dichloro-benzene	2.0	98	6.4	0.5	114	6.3	35.2
Naphthalene	2.0	101	0.4	0.5	105	3.2	24.8

[a]A mixture of VOCs was added at 15 and 1.5 ng/mL for scan and SIM mode analysis, respectively.

[b]Results are the means of five replicate determinations. With IS; with internal standard correction

[c]Results were obtained from a single set of measurement. No IS: without internal standard correction

[d]LOQ, limit of quantification (S/N>5)

[e]CV, coefficients of variation

were satisfactorily recovered with the internal standard correction (scan mode; 97.3-106.3%, selected ion monitoring mode; 89.8-114%), whereas they were poorly recovered without the correction (24.8-85.1%). The coefficients of variation values were acceptable with the internal standard correction (scan mode; 0.4-6.4%, selected ion monitoring mode; 3.2-6.3%). The selected ion monitoring mode had greater recovery and coefficients of variation values than in scan mode, which might be derived from lower concentrations in the selected ion monitoring mode. These results indicate that internal standard correction is indispensable for the headspace analysis of VOCs in biological samples because headspace analysis tends to show less reproducibility than direct measurement procedures.

Measuring low levels of VOCs in human biological samples is very difficult because highly sophisticated techniques and contamination control are required (Ashley et al. 1996). VOCs are ubiquitous components in many consumer products and the laboratory environment. Thus, for contamination control, we carefully washed the blood-collecting equipment and removed VOCs from saturated saline solution as described in MATERIALS AND METHODS. These treatments reduced the VOC background to sub ng/mL level (data not shown).

Table 3 shows a summary of the serum VOC levels of 18 patients with SBS symptoms and 27 controls. Three of the most often detected VOCs among these 45 subjects were *p*-dichlorobenzene, toluene, and xylene which were found in 61%, 44%, and 44% of the patients, and 85%, 67%, and 15% of the controls, respectively.

Table 3. Serum VOC levels in patients (n=18) and controls (n=27).

	No. of participants with indicated levels of VOCs (ng/mL)					No. of positives[b]	Mean conc. in positives (ng/mL)	Maximum conc. (ng/mL)
	<0.1	0.1-0.4	0.5-1.0	>1.0				
Toluene								
Patients	10	6	2	0		8 (44)[c]	0.3	0.8
Controls	11	13	3	0		18 (67)	0.4	1.3
Xylene[a]								
Patients	10	7	1	0		8 (44)	0.3	0.8
Controls	23	4	0	0		4 (15)	0.3	0.4
Benzene								
Patients	16	1	1	0		2 (11)	0.4	0.7
Controls	26	1	0	0		1 (4)	-	0.1
Ethylbenzene								
Patients	16	2	0	0		2 (11)	0.1	0.1
Controls	27	0	0	0		0 (0)	-	-
Styrene								
Patients	17	1	0	0		1 (6)	-	0.1
Controls	25	2	0	0		2 (7)	0.2	0.2
	No. of participants with indicated levels of VOCs (ng/mL)					No. of positives[b]	Mean conc. in positives (ng/mL)	Maximum conc. (ng/mL)
	<0.5	0.5-0.9	1.0-4.9	5.0-10	>10			
p-Dichlorobenzene								
Patients	7	4	5	0	2	11 (61)	5.1	25.4
Controls	4	3	9	4	7	23 (85)	16.8	171
Naphthalene								
Patients	18	0	0	0		0 (0)	-	-
Controls	27	0	0	0		0 (0)	-	-

[a]Results are expressed as the sum of *o*-, *m*-, *p*-xylenes.

[b]*p*-Dichlorobenzene and naphthalene; >0.5 ng/mL, toluene, xylene, benzene,

[c]Number in parentheses indicate percentages. There is a significant difference in the ratio of positive samples of xylene (P <0.05).

Among the most often detected VOCs, only xylene was significantly more prevalent in the patients. The positive rates of *p*-dichlorobenzene and toluene in the patients showed lower frequency than in the controls although there were no significant differences. The mean concentrations of *p*-dichlorobenzene, toluene and xylene in positive cases were 5.1, 0.3 and 0.3 ng/mL for the patients and 16.8, 0.4 and 0.3 ng/mL for the controls, respectively. The differences in the concentrations of *p*-dichlorobenzene and toluene were not statistically significant between the patients and controls. The low positive rates of other VOCs did not allow us to perform statistical analyses.

Table 4. Differences in the mean number of symptoms within the patient group and between the two groups dichotomized at the limit of quantification of serum VOCs.

Compound	Mean No. of symptoms		*p* Value
	Serum VOC positive	Serum VOC negative	
p-Dichlorobenzene	5.5	7.0	0.3187
Toluene	7.5	5.0	0.0701
Xylene	6.0	6.2	0.8906

Table 4 shows a summary of the mean number of symptoms within the patient group. The differences in the number of symptoms are not statistically significant between the two groups dichotomized at the limit of quantification of serum VOCs.

These results indicate that it is difficult to determine the serum VOC levels responsible for inducing symptoms.

Several reports have suggested that SBS symptoms are related to VOCs in the indoor air environment (Norbäck et al. 2000; Kamijima et al. 2002; Saijo et al. 2004); however, there has been no report on the relationship between serum VOC levels and SBS symptoms to our certain knowledge. In this study we measured serum levels of VOCs in patients with SBS symptoms and volunteer controls. Measuring chemicals in blood is advantageous because we can calculate the body burden precisely. However, for nonoccupationally exposed people, it is generally difficult to clarify the relationship between adverse effects and VOC exposure or serum VOC levels because of the relatively low levels of VOCs found in serum (Ashley et al. 1994). The serum VOC levels of all subjects in this study were relatively low except for *p*-dichlorobenzene, which was consistent with previous reports (Ashley et al. 1996). The number of target VOCs was limited although there are more than one hundred VOCs in indoor air. The small subject population restricted our approach to include limited statistical analysis. Detailed information on the patients' history of exposure is lacking. Further investigation to overcome these problems is required.

In this study we intended to examine (1) whether HS-GC/MS was applicable to the measurement of serum VOC concentrations in SBS patients and volunteer controls, and (2) whether the elevation of serum VOC levels correlated with SBS symptoms. Despite the successful application of our HS-GC/MS method, we found no statistical differences in the concentrations of studied VOCs between the patients and controls. We also found no relationship between serum VOC levels and SBS symptoms in the patients studied. Therefore, we must consider that it is difficult to identify the responsible VOCs and their serum levels inducing SBS symptoms from the results of this study.

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