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Case report

Determination of breath isoprene and acetone concentration with a needle-type extraction device in gas chromatography–mass spectrometry

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ABSTRACT

Background: Isoprene in human breath is said to be related to cholesterol metabolism, and the possibility of the correlations with some clinical parameters has been studied. However, at this stage, no clear benefit of breath isoprene has been reported for clinical diagnosis. In this work, isoprene and acetone concentrations were measured in the breath of healthy and obese subjects using a needle-type extraction device for subsequent analysis in gas chromatography–mass spectrometry (GC-MS) to investigate the possibility of these compounds as an indicator of possible diseases.
Methods: After measuring intraday and interday variations of isoprene and acetone concentrations in breath samples of healthy subjects, their concentrations were also determined in 80 healthy and 17 obese subjects. In addition, correlation between these breath concentrations and the blood tests result was studied for these healthy and obese subjects.
Results: The results indicated successful determination of breath isoprene and acetone in this work, however, no clear correlation was observed between these measured values and the blood test results.
Conclusions: Breath isoprene concentration may not be a useful indicator for obesity or hypercholesterolemia.

1. Introduction

Previous studies have reported that thousands of volatile organic compounds (VOCs) could be found in human breath [1,2], and much attention has been paid to their clinical purposes because a particular compound may be an indicator for certain diseases, such as breath acetone for diabetes [3–5]. Isoprene in human breath is said to be related to cholesterol metabolism [6], and the possibility of the correlations with some clinical parameters has been studied, such as age [6,7] or blood cholesterol level [8]. Intraday variation of isoprene concentration in the breath of smokers has been reported [9]. However, at this stage, no clear benefit of breath isoprene has been reported for clinical diagnosis.

Off-line analysis by gas chromatography–mass spectrometry (GC-MS) is one of the most promising techniques for the analysis of breath VOCs owing to its high selectivity and sensitivity. However, the concentration of VOCs in the breath is typically low; thus, a kind of preparation process for samples is required in most cases. For the subsequent GC analysis, extraction of VOCs from gaseous samples using a needle-type extraction device is one of the most promising techniques because of some advantageous features over conventional extraction methods [10–12]. These needle-type devices consist of sorbent particles in a stainless-steel needle, and the analytes are extracted as the gaseous sample when they passed through a needle packed with sorbent. The extracted analytes can then be thermally desorbed in the conventional GC injection port, typically without a desorption solvent [13–16]. Therefore, the use of a needle-type device for extraction of VOCs can be regarded as a simple, rapid, and useful sample preparation method.

Needle-type extraction devices have also been applied to breath analysis [17–19]. Previously, our research group developed a new measurement technique for the analysis of breath acetone using a needle-type extraction device packed with a copolymer of methacrylic acid (MA) and ethylene glycol dimethacrylate (EGDMA) [18], in which a clear correlation between the developed method and conventional method was confirmed along with successful extraction and desorption of breath acetone. More recently, another type of the extraction needle was developed for the extraction of very VOCs (VVOCs) from gaseous and human breath samples [20]. The newly developed extraction needle was packed with Carbopack X and carbon molecular sieve

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(CMS) particles as sorbents. With this needle, the extraction and desorption were successfully performed for several VVOCs from gaseous samples, including acetaldehyde, isoprene, pentane, acetone, and ethanol. The determination of VVOCs using the developed extraction needle was reported, where no significant interference by the sample humidity or CO_2 in the extraction/desorption process was observed. In the study, a future possibility of using the newly developed extraction needle for breath analysis in a clinical scene was also demonstrated.

2. Method and patients

2.1. Chemicals

Acetone (>99.8%) was purchased from Wako Pure Chemicals. Isoprene (>99%) was purchased from Tokyo Kasei Kogyo. The nitrogen gas and air (>99.99% purity) used for the desorption of the extracted analytes and that for the preparation of the standard gas sample were purified using a gas filter packed with molecular sieves 5 Å to remove organic contaminations.

2.2. Extraction of gaseous samples

As the extraction device, an extraction needle packed with Carbopack X (Supelco, 60/80 mesh) and a CMS absorbent (Shinwa Chemical Industries, 70/80 mesh) was used. The lengths of the packed section of these sorbents were 15 mm each.

For sampling of gaseous samples, the extraction needle was attached to a commercially available vacuum sampling device (Komyo Rikagaku Kogyo). Typical sampling volume of gaseous samples was 50 mL, and the extraction time for 50 mL of gaseous sample using extraction needle was approximately 5 min. All the standard samples and breath samples were collected in 1 L gas sampling bags (Smart Bag PA, GL Sciences). After the extraction process, the extraction needle was attached to an injection syringe and then N_2 gas was collected via the extraction needle as the desorption gas. The extraction needle was then inserted into the heated GC injection port and thermally desorbed analytes were extruded into a column by desorption gas.

2.3. GC-MS measurements

A Shimadzu QP5050A GC-MS system was used for all the GC measurements. As a carrier gas, He (>99.999% purity) was used. The head pressure and split ratio were 100 kPa and 15:1, respectively. The separation was performed with a capillary column of HP-INNOWAX, 30 m × 0.2 mm I.D., 0.4 μm d_f (J&W Scientific). The column temperature was initially maintained at 40 °C for 2 min and then programmed to increase to 80 °C at a rate of 20 °C/min. The GC-MS interface temperature and the ionization voltage were set to 250 °C and 70 eV, respectively. The mass spectrometer was operated in the selected ion monitoring mode (*m/z*: 44, 46, 58 and 67).

2.4. Subjects

All the breath samples were collected with the official approval of the Ethical Committees of Ena Hospital, Ishiguro Clinic, and Toyohashi University of Technology, and written consent was obtained from all of the sample donors before the sampling. All the subjects were non-smokers and did not have any significant diseases, and at least 12 h had passed after drinking an alcohol beverage or coffee. The breath samples were collected from 80 healthy volunteers in Ena Hospital and 17 obese subjects in Ishiguro Clinic.

3. Results

Breath isoprene and acetone concentrations of 10 healthy subjects (mean age: 35.9 y) were measured, and their observed concentrations ranged from 80 to 227 ppb and from 64 to 856 ppb, respectively. Intra-day variations of breath isoprene and acetone concentrations in healthy subjects were in the range of approximately 150 ppb and 300 ppb, respectively. No clear trend was observed for the concentration of breath isoprene; however, most subjects showed a higher breath acetone concentration in the morning, which subsequently decreased gradually. Interday variations of breath isoprene and acetone concentration in the healthy subjects were measured for 2 weeks. It was found that these concentrations showed variations in the range of approximately 200 and 300 ppb, respectively. The results could be valuable

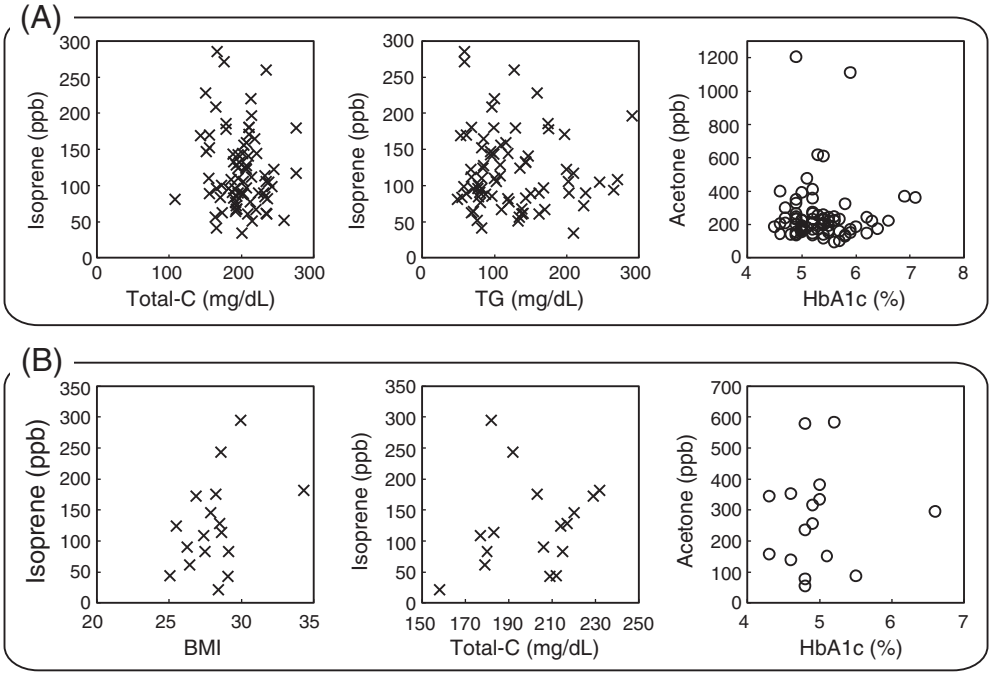

Fig. 1. Correlation between breath isoprene or acetone concentration and blood test results for (A) 80 elderly and (B) 17 obese subjects.