Fourier Transform Infrared (FTIR) Analysis of Volatile Compounds in Expired Gas for the Monitoring of Poisonings 1.Ethanol

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INTRODUCTION

Many chemical compounds in the environment can cause poisonings. To determine and evaluate medical treatments, and to prevent poisonings, qualitative and quantitative analyses of chemical compounds are necessary. In practical poisoning cases, analyses are usually performed using urine and/or blood of patients. GC and HPLC are the most popular methods for this purpose.

In patients poisoned with volatile compounds, some of the compounds are usually eliminated in the expired gas. Therefore, expired gas can be a useful sample, but is rarely used clinically. As the method of obtaining expired gas is the least invasive, it could be the most practical material in such cases if an appropriate analysis technique is developed.

Infrared (IR) spectroscopy is a useful instrument for the qualitative and quantitative analysis of gases, liquids and solid samples. However, it was previously very difficult to apply this method to biological samples due to interference by the strong absorption of water. Recently, the introduction of the Fourier transform infrared system (FTIR) overcame this problem, and now FTIR is applicable to biological samples.

However, clinical use of FTIR is rare. Setiawan *et al.* (1) measured the concentration of boron compounds in human blood using FTIR. The practical application of FTIR to monitor inhalational compounds is mainly limited to industrial hygienic approaches. Some studies (2–5) dealt with the application of FTIR for monitoring volatile organic compounds in the workspace. Among them, Franzblau *et al.* (2) examined human exposures to methanol vapor using a 47 m^3 stainless steel chamber. They measured the concentrations of methanol in the chamber, expired gas and blood using transportable FTIR and HS-GC.

We examined the availability of FTIR to the clinical analysis of expired gas for monitoring volatile compounds poisonings. In the present study, we used ethanol as a volatile compound. For the human study using volunteers, ethanol is the most suitable volatile compound. With respect to other organic compounds, many ethical problems would occur to administer.

For ethanol, many sensitive alcohol checkers are working worldwide (6,7), because it is the most common organic compound and its poisoning is sometimes a serious problem. We did not use alcohol checkers but used a common FTIR spectrometer. This instrument was laboratory based, not so expensive, and can detect most organic compounds while alcohol checkers can detect only ethanol. The method described here with ethanol could be easily applied to other organic compounds.

Using human volunteers and rabbits, ethanol concentrations in expired gas after oral administration were measured by FTIR. The obtained data were compared with the reported values, and the applicability of this method was discussed. To evaluate the correlation between concentrations in expired gas and blood, the concentration in blood was also measured with GC.

MATERIALS AND METHODS

Human Volunteers

This study was approved by the ethical committee of the Kagawa Medical University, and informed consent was obtained from all volunteers. After 12 hours fasting, 20 healthy volunteers (male: n = 15, age: 24.9 ± 3.4 y, weight: 67.5 ± 6.3 kg; female: n = 5, age: 22.4 ± 0.9 y, weight: 48.6 ± 2.9 kg) ingested 0.4 g/kg of ethanol in alcoholic beverages (whisky (ethanol 40%, n = 7), beer (4%, 6), Japanese sake (14%, 7)) within 10 minutes. The expired gas and blood were collected just before and 15, 30, 60, 120 and 180 minutes after drinking.

Animal Experiments

This study was approved by the Kagawa Medical University Animal Investigation Committee. After fasting 6 male Japanese white rabbits (2.4–3.7 kg) for 12 hours, anesthesia was induced by administration of pentobarbital (50 mg/kg). A tracheotomy was performed during spontaneous respiration, and an end tracheal tube was inserted. After the administration of gallamine (100 mg/kg), mechanical ventilation was initiated with a tidal volume of 25 mL and respiratory rate of 20/min. Ethanol was administered as a 20% aqueous solution through a gastric tube (2.0 g of ethanol/kg). The expired gas and the blood were collected just before and 10, 20, 30, 40, 50, 60, 90, 120, 150 and 180 minutes after administration.

Sample Preparation

The expired gas was collected in a gas sampling bag (Tedlar fluorine contained resin bag made of a mixture of $-(CF_2 - CF_2)_n-$ and $-(CFCF_3 - CF_2)_n-$, 50 μm thick, 155 × 250 mm, ca. 1 L, equipped with 2 Teflon sleeves with a valve). Human volunteers rinsed their mouth 3 times with water, took a deep breath and held it for 20 seconds. Then the last 500 mL of the expired breath was collected in the bag by blowing into the sleeve. The expired gas of rabbits was collected in the bag through the expiratory route of the ventila-

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tor. As the dead volume of the expiratory route was very small (0.3 mL) compared to the tidal volume (25 mL), dilution by the dead volume was considered to be negligible. Blood samples were collected from the basilic vein (human volunteers) and from the femoral vein (rabbits). Each blood sample (0.1 mL) was transferred into a glass sample bottle (ca. 22 mL) with 0.1 mL of an internal standard (0.02% t-BuOH aqueous solution). The bottle was immediately sealed with an air tight cap for the measurement of HS-GC.

Quantitation Procedure of Ethanol in Expired Gas by FTIR

FTIR spectra were measured with a Perkin Elmer FTIR spectrometer 1650 equipped with a long gas cell (Infrared Analysis Inc. (1424 N. Central Park Ave. Anaheim, CA 92802) Long Path Mini Cell, ca. 450 mL, path length 1.8–7.2 m, window: ZnSe). The cell was preliminary vacuumed with an electric vacuum pump. The valves of the cell and the gas sampling bag were connected with a short silicone tube, and then by opening the valves, the sample gas filled the cell. The gas sampling bags were made of very flexible material so the inner pressure was always 1 atm during the procedure.

The calibration gases were prepared in the gas sampling bags using a Hamilton micro syringe (10 μL) and a 200 mL glass syringe. The reagent grade of ethanol was used. For the calibration, the path length was adjusted at 7.2, 5.4 and 2.4 m for the concentration range 0.0158–0.158 mg/L, 0.0631–1.58 mg/L and 1.58–7.89 mg/L, respectively. With respect to all human and rabbit samples, the path length was fixed at 5.4 m.

Each spectrum was measured from 400 to 4,400 cm^{-1} with the resolution of 4 cm^{-1} and 16 scans were co-added to improve the signal/noise ratio. Each measurement took only 1 minute.

Quantitation Procedures of Ethanol in Blood by HS-GC

An HS-GC system (Perkin Elmer Augosystem Gas chromatograph equipped with HS-40 Headspace sampler) was used. The incubation was performed at 60°C for 15 minutes. The samples were analyzed on a 60 m long × 0.53 mm I.D. capillary column (CPP1 Supercowax™) at 90°C. The concentration of ethanol was measured from the peak area ratio to that of the internal standard.

RESULTS

FTIR spectra of the expired gas and ethanol in ambient air are shown in Fig. 1. Only H_2O and CO_2 showed strong absorption in the expired gas and/or in the ambient air. Absorption peaks of ethanol around 2,800–3,000 cm^{-1} did not overlap with the peaks of H_2O) or CO_2. Using a C-H stretching band of ethanol at 2,988 cm^{-1}, the concentration was determined. The absorbance was closely correlated to the concentration within the range from 0.016 to 8 mg/L (Fig. 2).

The peak area of gas chromatograms was also closely correlated to the concentration within the range from 0.15 to 3.0 mg/mL.

At each time point, the ratio of the concentrations in blood (mg/L) to that in expired gas (mg/L) was calculated as the B/G ratio.

Fig. 3 and Fig. 4 show the time course changes of the concentrations of human and rabbit samples, respectively.

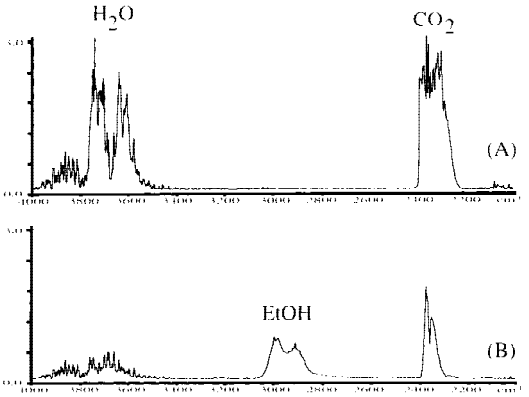

Fig. 1. FTIR spectra of expired gas and ethanol. The spectra were measured from 16 scans with resolutions of 4 cm^{-1}. Path length was 5.4 m. (A): Collected expired gas was filled into the pre-vacuumed cell. Only H_2O and CO_2 show absorption peaks. (B): 2 μL of ethanol was evaporated in 1,000 mL of ambient air (1.58 mg/L). Then the prepared gas was transferred to the cell. Absorbance at 2,988 cm^{-1} was used for the calibration. Absorption peaks of H_2O and CO_2 are weak compared with those in the expired gas.

The time course changes of the B/G ratio are also shown in Figs. 3 and 4.

DISCUSSION

The toxic and fatal blood concentration of ethanol is over 0.84–4.5 mg/mL, and 2.3–6.0 mg/mL, respectively (8). A blood concentration over 0.5 mg/mL is illegal for drivers in Japan. In this experiment, the quantitative range of ethanol in expired gas was 0.016 to 8 mg/L, which approximately corresponds to the concentration range of 0.04 to 20 mg/mL of blood. It appeared that FTIR can monitor the concentration of ethanol in poisoning cases.

The human B/G ratio was small just after the administration, and then approached a constant value: ca. 2,500 which was similar to the previously reported value (6,7). The ethanol concentration in the stomach should be very high at the beginning of the experiments. As the expired gas was col-

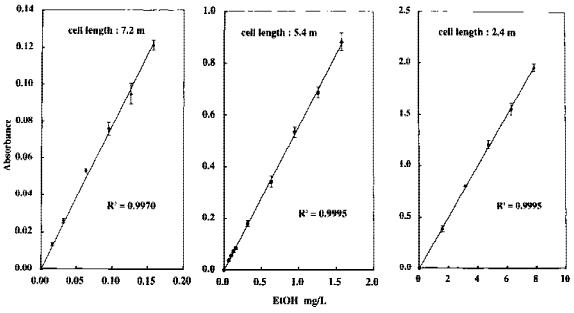

Fig. 2. The relationship between the absorbance and the concentration of ethanol 5,000 mL of air was filled into a pre-vacuumed polymer film bag (10 L) followed by 1 or 50 μL of ethanol ([EtOH] = 0.158 or 7.89 mg/L). The film bag was flexible enough that the dead volume was negligible, and inner pressure was always 1 atm. Using a 200 mL glass syringe, an appropriate amount of the standard gas was transferred into another bag, and diluted with air. Absorbance at 2,988 cm^{-1} was measured by the same method as shown in Fig. 1. At each concentration, more than 5 preparations were independently performed and measured. The average and standard deviations are shown with error bars.

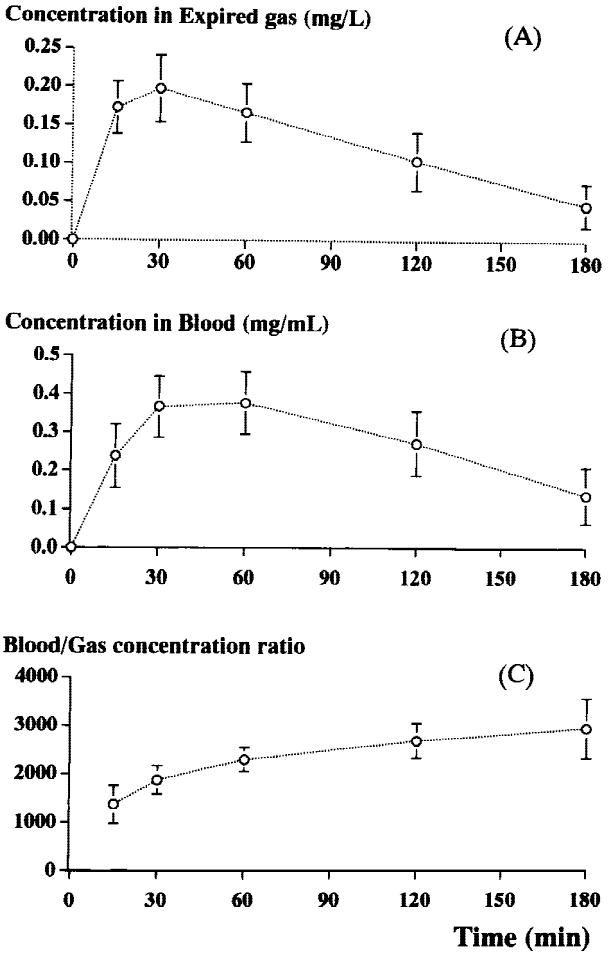

Fig. 3. Time course change of ethanol concentrations in the human samples. After the oral administration of ethanol (0.4 g/kg), the concentrations of ethanol in the expired gas and blood were measured by FTIR and HS-GC, respectively. Measurements were performed using 20 volunteers and averaged. Standard deviations are shown as error bars. (A): Concentration in the expired gas. (B): Concentration in the blood. (C): The ratio of the concentrations in the blood to that in the expired gas.

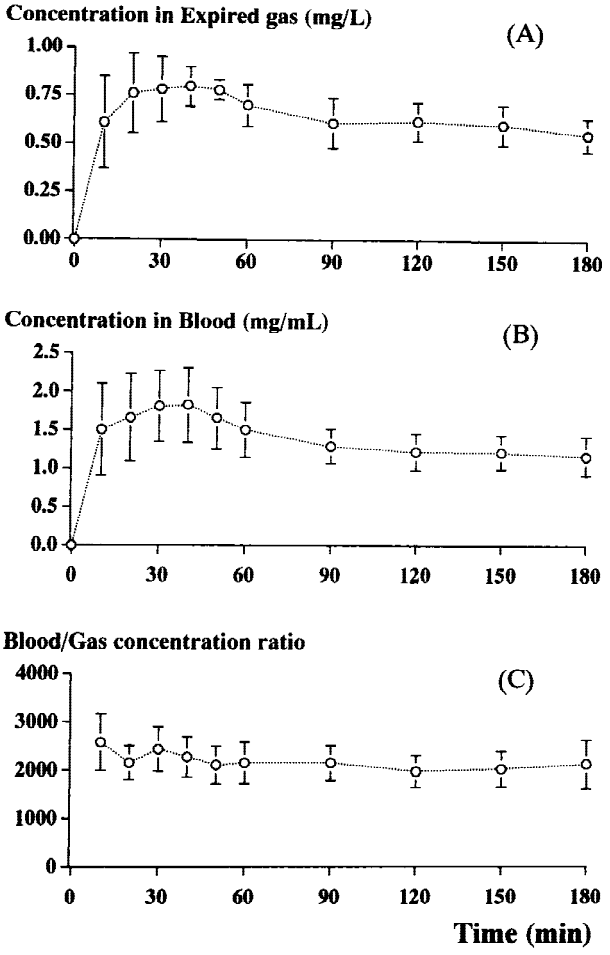

Fig. 4. Time course change of ethanol concentrations in the rabbit samples. After the oral administration of ethanol (2.0 g/kg), the concentrations of ethanol in the expired gas and blood were measured by FTIR and HS-GC, respectively. Measurements were performed using 6 rabbits and averaged. Standard deviations are shown as error bars. (A): Concentration in the expired gas. (B): Concentration in the blood. (C): The ratio of concentrations in the blood to that in the expired gas.

lected from the oral cavity, the effect of ethanol diffusion from the mucosa of digestive tracts should be considered.

To confirm the effect of the diffusion, the ethanol concentration in the gas intra-tracheally collected was measured using rabbits. In contrast to the human findings, the B/G ratio of rabbits was almost constant during the experiments (Fig. 4). These findings indicated that the diffused ethanol from the mucosa of the digestive tracts affected the concentration in the expired gas just after administration.

The final B/G ratios of human and rabbits were similar. The transfer of ethanol from blood to expired gas should be performed in a similar manner with respect to humans and rabbits. The transfer is mainly controlled by physicochemical parameters such as transmittability through biomembranes. These parameters should not be markedly different between species. From this point of view, we can estimate the human B/G ratio of other volatile compounds from the animal experiments. Analyses of other volatile compounds with this method will be reported in the near future.

Although most organic compounds have absorption around 3,000 cm^{-1}, the quantities included in the normal expired gas are extremely small, and their absorptions do not interfere with the analyses of the poison compounds.

In industrial hygienic studies (2–5), ambient gases were analyzed by FTIR with significantly longer scan times, higher resolution and a longer path length than the present method. However, the sensitivity of the present method was adequate to monitor the poisonings. It required no incubation, no internal standard, and only 1 minute for the measurement. Additionally, since the expired gas was the least invasive sample, this method is very suitable for clinical purposes.

REFERENCES

1. Y. Setiawan, T. Rise, and D. E. Moore. Fourier transform infrared (FTIR) spectrometry for the assay of polyhedral boron compounds in plasma and pharmaceutical formulations. *Pharm. Res.* **11**:723–727 (1994).
2. A. Franzblau, S. P. Levine, L. A. Burgess, Q. S. Qu, R. M. Schreck, and J. B. D'Arcy. The use of a transportable Fourier transform infrared (FTIR) spectrometer for the direct measure-

ment of solvents in breath and ambient air I: Methanol. *Am. Ind. Hyg. Assoc. J.* **53**:221–227 (1992).
3. Y. Li-shi, S. P. Levine, C. R. Strang, and W. F. Herget. Fourier transform infrared (FTIR) spectroscopy for monitoring airborne gases and vapors of industrial hygiene concern. *Am. Ind. Hyg. Assoc. J.* **50**:354–359 (1989).
4. Y. Li-shi and S. P. Levine. Evaluation of the applicability of Fourier transform infrared (FTIR) spectroscopy for quantitation of the components of airborne solvent vapors in air. *Am. Ind. Hyg. Assoc. J.* **50**:360–365 (1989).
5. Y. Li-shi and S. P. Levine. Fourier transform infrared least-squares methods for the quantitative analysis of multicomponent mixtures of airborne vapors of industrial hygiene concern. *Anal. Chem.* **61**:677–683 (1989).
6. J. Olsson and R. G. Hahn. Analysis of ethanol in expired air during low-flow isoflurane anaesthesia. *Br. J. Anaesth.* **76**:85–89 (1996).
7. A. W. Jones, Å. Norberg, and R. G. Hahn. Concentration-time profiles of ethanol in arterial and venous blood and end-expired breath during and after intravenous infusion. *J. Forensic Sci.* **42**: 1088–1094 (1997).
8. A. H. Stead and A. C. Moffat. A collection of therapeutic, toxic and fetal blood drug concentrations in man. *Human Toxicol.* **3**: 437–464 (1983).