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Short communication

Quantitative analysis of acetaldehyde in whole blood from human and various animals by gas chromatography

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Abstract

Acetaldehyde present in the blood of bull, chicken, hamster, horse, human, monkey, pig, rabbit, rat and sheep, was quantitatively analyzed by a newly developed gas chromatographic method. Acetaldehyde in a blood sample was reacted with cysteamine to give 2-methylthiazolidine, which was extracted with dichloromethane and subsequently analyzed by gas chromatography with a fused-silica capillary column and a nitrogen–phosphorus detector. The quantities of acetaldehyde found in blood ranged from 2.04 μmol/ml (hamster) to 14.8 μmol/ml (pig). The quantity of acetaldehyde recovered from human blood was 6.17 μmol/ml.

Keywords: Acetaldehyde

1. Introduction

Low-molecular-mass aldehydes such as formaldehyde and acetaldehyde bind readily with a nucleophile site of proteins, peptide glutathione, individual amino acids, and DNA due to their highly polar carbonyl moiety [1–3]. Because of these characteristics, they are implicated in a variety of diseases, such as atherosclerosis [4,5]. For example, acetaldehyde reacts with low-density lipoprotein (LDL) to produce a modified form that directly contributes to atherosclerosis [6]. Most importantly, acetaldehyde has reportedly been associated with the risks related to ethanol intake [7]. Studies using experimental animals suggest that acetaldehyde participates in the pathogenesis of alcoholism [8]. It has also been suggested that acetaldehyde is the injurious agent in acute alcoholic liver disease [9]. Therefore, it is important to develop an accurate quantitative analytical method for trace acetaldehyde in blood in order to investigate the role of acetaldehyde in blood-related diseases.

Analysis of acetaldehyde in whole blood is, however, extremely difficult because its concentration in blood is extremely low. Moreover, whole blood has a high concentration of proteins which bind acetaldehyde readily and consequently inhibit its recovery. Many investigations which attempted to release bound acetaldehyde have been seriously hampered by artefactual acetaldehyde formations during different hemolysation, hydrolysation, heating and/or other analytical procedures [10]. Therefore, there has been no really satisfactory method for the quantitative analysis of trace acetaldehyde in whole blood. The most commonly used method for trace aldehyde analysis – use of 2,4-dinitrophenylhydrazine deriva-

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tive – has been used in the analysis of acetaldehyde in rat serum [11] but not in whole blood.

In the present study, a simple and specific method for trace analysis of acetaldehyde in whole blood has been developed. This method involves derivatization of acetaldehyde with cysteamine to form 2-methylthiazolidine in whole blood; the resulting 2-methylthiazolidine is analyzed by gas chromatography (GC) equipped with a fused-silica capillary column and a nitrogen–phosphorus detection (NPD) system.

2. Experimental

2.1. Materials

Cysteamine hydrochloride and 2,4,5-trimethylthiazole were purchased from Aldrich (Milwaukee, WI, USA). Authentic 2-methylthiazolidine was synthesized according to a method previously reported [12,13]. Whole blood samples (5 ml) obtained from a human (healthy male, 39-year-old), golden Syrian hamster (female, 5-month-old), rat (female, 3-month-old), quarter horse (male, 20-year-old), angus bull (1-year-old), Yorkshire pig (female, 2-month-old), sheep (male, 1-year-old), New Zealand white rabbit (male, 2-year-old), macaque monkey (female, 6-year-old), and white leghorn chicken (8-month-old) were placed in sterile, 10-ml tubes containing 0.01 ml of 15% K3 ethylenediaminetetraacetic acid (EDTA).

2.2. Analysis of acetaldehyde in the whole blood of a human and various animals

Each whole blood sample (50 μl) was diluted to 50 ml with deionized water and cysteamine hydrochloride (0.75 g) was added. Each sample was adjusted to pH 1.0 with 3 *M* HCl, and 40 ml of 90% methanol was added. In order to better release acetaldehyde from acetaldehyde-bound proteins, the solution was stirred for one day at room temperature. After the reaction mixture was adjusted to pH 7.4 with 6 *M* NaOH, the solution was extracted with 50 ml of dichloromethane using a liquid–liquid continuous extractor for 6 h. The extract was dried over anhydrous sodium sulfate for 10 h. After removal of sodium sulfate, the volume of the extract was adjusted to exactly 50 ml with dichloromethane. 2,4,5-Trimethylthiazole (100 μl) standard solution (10 mg/ml) was added as a GC internal standard prior to analysis. The entire system was covered with aluminum foil to avoid any influence of light. The quantitative analysis of acetaldehyde in each whole blood sample was replicated three times. A sample without blood was prepared by exactly the same method as above for each experiment and used as a blank control.

2.3. Recovery of acetaldehyde from whole blood

The 50 μl whole blood samples of the horse and the human were diluted to 50 ml with deionized water and spiked with 500 μl of an acetaldehyde solution (60 mg acetaldehyde/50 ml water). The samples were prepared and analyzed by the method described above. The experiment was replicated three times.

2.4. Instrumental analysis

A Hewlett-Packard Model 5890A gas chromatograph equipped with a NPD system and a 30 m× 0.25 mm I.D. fused-silica capillary column coated with DB-1 was used for quantitative analysis of 2-methylthiazolidine derived from acetaldehyde with cysteamine. The oven temperature was programmed from 60°C to 180°C at 4°C/min and held for 10 min. Peak areas were integrated with a Spectra Physics SP 4290 integrator. The injector and detector temperatures were each 250°C. The linear velocity of helium carrier gas was 30 cm/s with a split ratio of 21:1.

3. Results and discussion

As mentioned above, the high concentration of proteins in whole blood inhibits recovery of free acetaldehyde because acetaldehyde readily binds covalently to proteins. Therefore, it is necessary to release acetaldehyde from proteins. This problem was solved by diluting whole blood with deionized water and treating it with acid for a prolonged time. Therefore, even though acetaldehyde readily reacts with cysteamine under mild conditions at room

temperature and neutral pH, acidic condition was required to release acetaldehyde from proteins. The lowest detection level of 2-methylthiazolidine by NPD was 16.7 pg, which was equivalent to 7.1 pg of acetaldehyde. The recovery of acetaldehyde using aqueous solutions of the horse and the human blood (272 μmol/ml of blood) was 80.5±4.3 and 77.5±3.1%, respectively. These values are mean±standard deviation (n=3). Fig. 1 shows a typical NPD gas chromatogram of the dichloromethane extract from a blood sample. Baseline separation of 2-methylthiazolidine was obtained.

Table 1 shows the results of acetaldehyde analysis of the whole blood of the various animals and of the human. The values are the mean±standard deviation (n=3), and a correction was made by subtraction of the blank value (13.6 μmol/ml of aqueous solution). The blank value was somewhat high. Therefore, several clean-up methods were tried to remove trace acetaldehyde which may be present in the solvent, such as washing with sodium bisulfite, and treatment with active charcoal or Porapak Q porous polymer, but both were without success. Trace levels of acetaldehyde in the solvent may be impossible to remove. Moreover, additional treatment may damage the purity of the solvent. However, quantitative analysis can be conducted satisfactorily as long as reliable blank values are obtained.

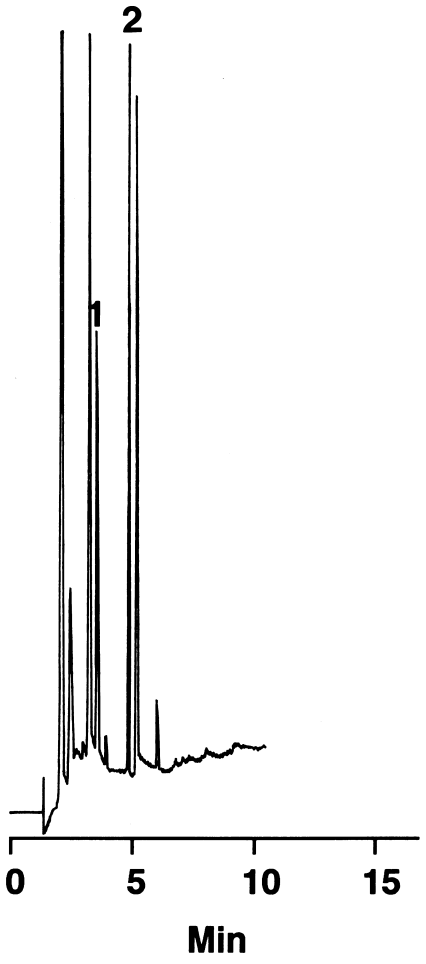

Fig. 1. A typical gas chromatogram of the extract from a horse blood sample. Peaks: 1=2-methylthiazolidin (acetaldehyde derivative), 2=internal standard (2,4,5-trimethylthiazole).

Table 1
Results of acetaldehyde analysis in a human and in various animals

Subjects	Concentration of acetaldehyde (μmol/ml of blood)[a]
Bull	8.64±3.80
Chicken	10.1±1.55
Hamster	2.04±1.14
Horse	3.02±2.57
Human	6.17±3.72
Monkey	3.54±2.35
Pig	14.8±2.39
Rabbit	7.47±3.52
Rat	13.8±0.18
Sheep	5.02±2.54

[a] Values are mean±standard deviation (n=3) and are corrected for a control value which was obtained from subtraction of the value for the acetaldehyde recovered from a blank without blood.

The average amount of acetaldehyde detected in whole blood samples was 7.46±2.38 μmol/ml of blood. Although the results showed rather high deviation values, quantitative measurement was effectively performed by using the ratio of peak areas of derivative against an internal standard, 2,4,5-trimethylthiazole [14]. Among the kinds of animal blood examined, the pig blood contained the greatest amount of acetaldehyde (14.8 μmol/ml) and the hamster blood contained the least amount of acetaldehyde (2.04 μmol/ml). In the case of the healthy human, the amount of acetaldehyde found was 6.17 μmol/ml, which was below the average found in the other animals. It was postulated that there may be some relationship between acetaldehyde formation

and lipid content in blood because acetaldehyde is considered to be produced by lipid peroxidation [15]. However, there was no appreciable relationship between the amount of acetaldehyde found in the blood samples in the present study and the values of lipid contents reported in the literature [16–20], suggesting that acetaldehyde in blood derives not only from lipid peroxidation but also from other sources. It is necessary to know the exact content of unsaturated fatty acids in each blood sample in order to know the relationship between acetaldehyde formation and the lipid content in whole blood. However, investigation of the role of lipid peroxidation in acetaldehyde formation in blood is not within the scope of this study.

The results of this study show that the method developed is useful to quantitate trace amounts of acetaldehyde in various kinds of blood. There is no report on quantitative analysis of trace acetaldehyde in whole blood prior to this study. Quantitative analysis in biological samples, such as whole blood, may be a valuable approach to risk assessment of toxic aldehydes.

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