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Short communication

Quantitative analysis by gas chromatography of volatile carbonyl compounds in expired air from mice and human

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Abstract

Formaldehyde, acetaldehyde and acetone expired from tumor-bearing transgenic mice and formaldehyde exhaled from breast cancer patients were analyzed using gas chromatography. The tumor-bearing mice expired significantly more formaldehyde per unit metabolic size (1.43–2.98 μmol) than did control mice (0.77–1.01 μmol). There was no detectable difference in the levels of expired acetaldehyde and acetone between the two groups of mice. The exhaled formaldehyde levels from three women with breast cancer and from three healthy women were satisfactorily determined using the method developed in this study. The results suggest that these carbonyl compounds may be used as a biomarker. © 1997 Elsevier Science B.V.

Keywords: Formaldehyde; Acetaldehyde; Acetone

1. Introduction

Many compounds have been identified in the expired air of humans and animals. Compounds in the breath reflect both the physiological state of the individual as well as exposure to various drugs and chemicals. For example, increased breath acetone levels are highly correlated with diabetes and have been used successfully to diagnose and monitor diabetic patients [1]. Expiration of the alkanes, ethane and pentane, has previously been shown to be a direct measure of the extent of in vivo lipid oxidation [2,3]. However, it is difficult to separate pentane and isoprene in breath samples by gas chromatography [4]. Aldehydes, including formaldehyde, acetaldehyde and acetone, are also produced as secondary products of lipid oxidation. These aldehydes, via their reactions with cellular nucleophiles, may be more directly responsible than the alkanes for the damaging biological effects of lipid oxidation [5,6]. The high reactivity and volatility of these aldehydes requires special precautions to ensure their accurate analysis [7].

Breath analysis is a non-invasive and relatively rapid procedure, making it ideal in situations where repeated sampling is necessary, as in monitoring patients. In addition, matrix effects are minimal and sample preparation prior to analysis is often simpler when compared to the preparation and analysis of biological specimens, such as blood and urine. Previously, we described an analytical protocol for measuring carbonyl compounds in the expired air of

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laboratory mice [7]. In the present study, levels of expired formaldehyde, acetaldehyde and acetone in expired air from tumor-bearing and non-tumor-bearing transgenic mice were quantified and investigated for their potential as biomarkers. The feasibility of this approach was also demonstrated in three healthy women and in three women with breast cancer.

2. Experimental

2.1. Collection of expired air from mice

The method of collecting expired air from individual mice and for trapping and analyzing exhaled volatile carbonyls has been described previously [7]. Briefly, mice were housed for 1 h in a cylindrical, glass metabolism chamber (5 cm diameter; 19 cm long; tapered to 1/4″ for connecting it to PTFE tubing) that was continuously flushed with 90 ml of purified air/min. Air exiting the chamber was passed through two or three impinger traps in series. Each trap contained 25 ml of 30 m*M* aqueous cysteamine solution at pH 8.5. Volatile, saturated carbonyls reacted with the cysteamine to form substituted thiazolidine derivatives.

2.2. Collection of exhaled air from women

Three healthy adult women (ages 21, 31 and 35; body weights 56.7, 58.1 and 63.5 kg) and three women with breast carcinoma (ages 79, 63 and 48) volunteered for the study. The study was approved by the University of California Davis, Human Subjects Review Committee. Prior to enrolment, informed consent was obtained from each patient after thoroughly explaining the procedures.

The exhaled air from these female volunteers was collected in a 150–200 l Douglas bag. Volunteers inhaled air (Medical Air, 99.95%, Puritan-Bennett, San Ramon, CA, USA) through a Rudolph mask equipped with two-way inlet and outlet valves and their exhaled air was collected for 10–12 min, following a 10-min equilibration period. The respiratory quotient (RQ) of these women was also determined on a separate 5–6 min collection of exhaled air, to confirm that the patients had fasted overnight prior to sample collection.

Following sample collection, a 5.0±0.1 l aliquot of the exhaled air was transferred from the Douglas bag into a Tedlar air-sampling bag (SKC-West, Fullerton, CA, USA). Before the transfer, the Tedlar bag was flushed three times with the exhaled air. The exhaled air from the Tedlar bag was then drawn through two impinger traps in series. Each impinger trap contained 35 ml of a 0.06-*M* cysteamine solution at pH 8.5. A slight vacuum was applied at the exit of the second trap in order to maintain air flow through the system at approximately 14–17 ml/min.

2.3. Analysis of expired and exhaled carbonyl compounds

At the end of the collection period, the contents of each trap were adjusted to pH 8.5 with 1 *M* NaOH. A 5-ml volume of chloroform was added to each trap to extract the carbonyl compounds as their thiazolidine derivatives. The chloroform layer was quantitatively transferred to a vial and evaporated to a volume of 1.0 ml under a N_2 stream. A 15-ml aliquot of each of two internal standards, 2-isobutylthiazole (1 mg/ml) and 2,4,5-trimethylthiazole (1 mg/ml) was added. Samples were stored at 5°C for 24–48 h prior to analysis. A 2-μl volume of each sample was injected onto a gas chromatograph (GC) equipped with a flame photometric detector (FPD) and a 30 m×0.25 mm I.D. (*df*=0.25 μm) DB-1 fused-silica capillary column (J&W Scientific, Folsom, CA, USA). The FPD was used because of its increased sensitivity and selectivity for sulfur-containing heterocyclic derivatives. The detector and injector were operated at 220 and 230°C, respectively. Helium was used as the carrier gas at a linear velocity of 38 cm/s, with a split ratio of 1:14. The oven temperature was programmed from 80 to 115°C at a rate of 4.5°C/min and then from 115 to 190°C at 15°C/min, with a 10 min hold at 190°C.

Integrated peak areas were determined and the ratio of analyte (thiazolidine) to internal standard (2,4,5-trimethylthiazole) was calculated. Peak area ratios were converted to concentrations (mg/ml) using a thiazolidine calibration curve. The thiazolidine calibration curve was used for quantitation of formaldehyde, acetaldehyde and acetone as the thiazolidine, 2-methylthiazolidine and 2,2-dimethylthiazolidine derivatives, respectively. Analyte concentrations were converted to molar values and these were expressed per unit metabolic body size

(body weight in $kg^{0.75}$) of the mice. Formaldehyde concentrations in exhaled air from human were expressed in ppm, based upon the 5 l expired air sample.

2.4. Transgenic mice

Transgenic mice carrying the human T-lymphotrophic virus Type 1 (HTLV-1) transactivator (tax_1) gene in their germ line, under control of its own long terminal repeat (LTR), the transcriptional regulatory region of the virus, were recently described [8]. These mice are predisposed to developing nerve sheath tumors that are similar to those that occur in human neurofibromatosis. Such tumors have a well characterized time of onset (90–130 days), incidence (three–ten tumors per mouse) and tissue involvement (snout, ear, foot and tail) [9]. Mice used in the present experiments were derived from these original founder lines, which are maintained as breeding colonies at the University of California at Davis (Davis, CA, USA). Genotyping was performed as follows. Tail biopsies (approximately 0.5 cm) were taken at ten days of age and digested with proteinase K. DNA was extracted, digested with BglII restriction enzymes, electrophoresed in 0.8% agarose, transferred to nylon membranes [10] and hybridized with ^{32}P-labeled HTLV-1 tax DNA [8], labeled via the random primer procedure [11]. Membranes were washed and exposed to X-ray film for 24 h. The presence or absence of a band corresponding to HTLV-1 tax DNA on the X-ray film was used to classify the mice as transgenic or as non-transgenic controls.

Male and female mice were housed individually in wire bottom cages in a controlled environment (23°C; lights on at 0700 h, lights off at 1900 h; 95% relative humidity). The mice were fed a cereal-based, closed-formula diet (Mouse Chow, Ralston Purina, St. Louis, MO, USA) and were weighed prior to being placed in the metabolism chamber to collect the expired air. Transgenic tumor-bearing mice were compared with non-transgenic controls of similar age (94–164 days old), weight range and sex distribution. At the end of the collection periods, all mice were killed and tumors were excised and weighed. Tumor burden was expressed as tumor mass/body weight×100.

2.5. Breast cancer patients

Patient number 1 was a 79-year-old Hispanic female (body weight, 63 kg) who presented to the UC Davis Cancer Center with stage IV (T4N1M1) breast carcinoma. Based upon the examination results, total tumor mass was estimated to be approximately 600 g, corresponding to a tumor burden of 1% of the total body weight. Her breath sample was obtained after initiating Tamoxifen treatment but prior to chemotherapy or surgery.

Patient number 2 was a 63-year-old African–American female (body weight, 118.4 kg) with stage IV (T3N1M1) breast carcinoma, who presented with episodic bleeding from the right nipple over the previous year and a 4-kg body weight loss. Based upon examination results, total tumor mass was estimated to be approximately 672 g, corresponding to a tumor burden of 0.5% of the total body weight. A breath sample was obtained prior to initiating therapy.

Patient number 3 was a 48-year-old Caucasian female (body weight, 62.6 kg) with a 4.5-cm mass in the right breast, which was found on a routine mammogram. Her tumor was determined to be consistent with stage I (T1N0M0) infiltrating ductal carcinoma. Total tumor mass was estimated to be 0.52 g, corresponding to 0.001% of the total body weight.

2.6. Statistical analysis

Mean differences in the composition of the expired air between the control and tumor mice were evaluated by a two-tailed Student's t-test using the StatView software package (Abacus Concepts, Berkeley, CA, USA) on a Macintosh IIci computer. A linear regression equation was developed to describe the relationship between expired formaldehyde levels (y-variable) and tumor burden (x-variable).

3. Results and discussion

Figs. 1 and 2 show the amounts of formaldehyde, acetaldehyde and acetone found in the expired air from control and tumor-bearing mice, respectively. The tumor-bearing mice expired significantly more ($p<0.001$) formaldehyde per unit metabolic size

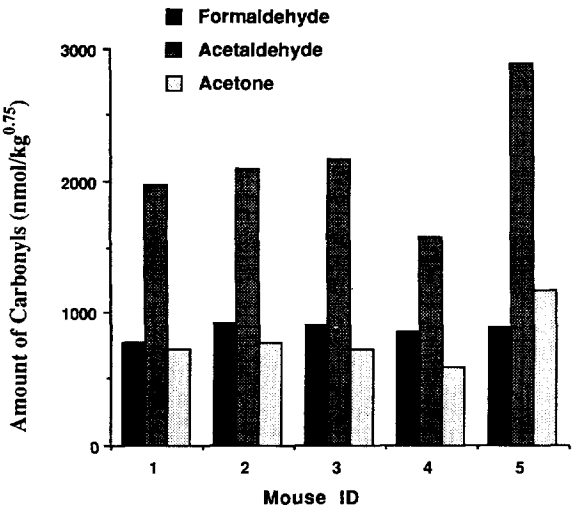

Fig. 1. Expired levels of formaldehyde, acetaldehyde and acetone from five control mice.

(body weight in $kg^{0.75}$) than did control mice. Tumor burdens greater than ~1%, when expressed as a percentage of body weight (tumor mass/body weight×100), were positively correlated with increased levels of expired formaldehyde (Fig. 3). The correlation was highly significant ($n=5$), with a linear $R^2=0.902$. Non-transgenic controls were not included in the correlation because they do not develop tumors. These results indicate that expired formaldehyde may have a relationship to the extent

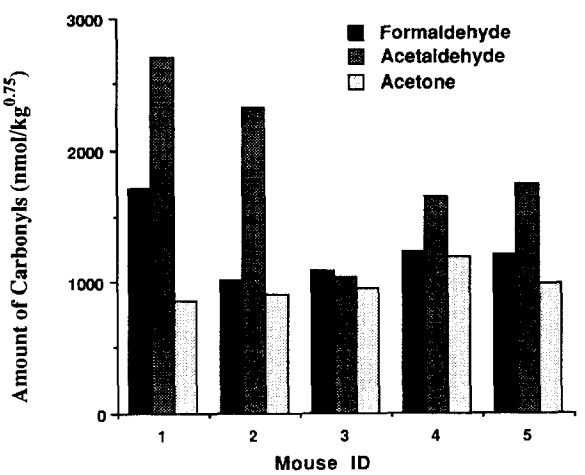

Fig. 2. Expired levels of formaldehyde, acetaldehyde and acetone from five tumor-bearing transgenic mice.

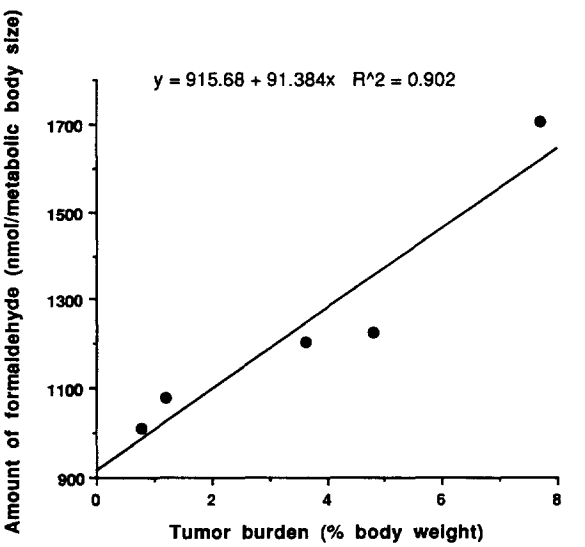

Fig. 3. Relationship between tumor burden (expressed as a percentage of total body weight) and expired formaldehyde levels for tumor-bearing transgenic mice.

of tumor progression. It did not appear to be related to diet intake at the stage at which it was studied in these mice.

Whether the observed increase in formaldehyde expiration is due to tumor formation or due to other conditions, such as inflammation, and/or necrosis and/or anoxia, is not clear at this time. No significant differences were observed in formaldehyde production between non-transgenic mice (controls, 869±52 nmol/$kg^{0.75}$) and transgenic mice prior to having visible tumors (958±134 nmol/$kg^{0.75}$). Age, weight range and sex distribution of the two groups were similar. This suggests that formaldehyde production did not precede the appearance of a visible tumor. A possible explanation is that the production of one of the known cancer-associated proteins, such as the tumor necrosis factor, may induce lipid oxidation and formaldehyde production. However, the relatively low levels of formaldehyde expired by these mice make it difficult to accurately evaluate small differences in formaldehyde levels that may have been present before tumor growth. Formaldehyde production by control mice in the follow-up experiment was identical to that of controls in the first experiment. This suggests that formaldehyde production is not related to age.

Table 1
Concentration of formaldehyde (FA) in exhaled breath from women with breast cancer

Age (years)	Weight (kg)	Cancer stage	Tumor size (g)	Tumor burden (% body wt.)	FA concentration (ppm)
79	63	IV (T4N1M1)	~600	1	1.2
63	118.4	IV (T3N1M1)	~672	0.5	0.42
48	62.6	I (T1N0M0)	~0.52	0.001	0.45

There was no detectable difference ($p>0.2$) in the levels of expired acetaldehyde and acetone between the two groups of mice (Figs. 1 and 2). Both acetone and acetaldehyde are known to be formed by in vitro lipid oxidation [12] and, presumably, they are also formed in vivo. Acetone is one of the major carbohydrate metabolites [13], and the amounts in the breath originating from lipid oxidation would be very small relative to the total acetone produced in vivo [14]. The fact that acetone levels were not significantly different between the tumor-bearing and non-tumor-bearing mice demonstrates that carbohydrate metabolism was not significantly altered as a result of carcinogenesis. The absence of a significant elevation of acetone levels in the tumor-bearing mice, along with the absence of major weight loss in the mice, suggests that the effect on formaldehyde expiration was associated with the presence of the tumor and unrelated to cachexia.

Table 1 shows the results of formaldehyde analysis in breath from human subjects. The exhaled formaldehyde level from the three women with breast cancer was 0.45–1.20 ppm, whereas the three healthy women exhaled 0.30–0.60 ppm formaldehyde. Cancer patient number 1, who had widely metastatic inflammatory breast carcinoma (tumor burden ~1% of body weight), expired the highest levels of formaldehyde, while the formaldehyde levels expired by the remaining two patients with clinically less advanced cancer were not markedly different from those of the healthy women. The results demonstrate that formaldehyde is detectable in humans using the procedures developed. However, a controlled (age-matched, diet-matched, etc.) population must be tested before conclusive results can be obtained regarding the relationship between expired formaldehyde and tumor progression.

The results of this study show that the method developed is useful for monitoring the in vivo formation of formaldehyde, acetaldehyde and acetone. Further studies evaluating the correlation of expired formaldehyde, tumor progression, and other conditions known to be associated with oxidative stress, in both animals and humans, are warranted.

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