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The origin of halitosis in cystinotic patients due to cysteamine treatment

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Abstract

Introduction: Cystinosis is a rare autosomal recessive disorder characterized by the intralysosomal accumulation of cystine. Cysteamine removes cystine from the lysosome and slows down the progression of the disease. One of its side effects is the induction of halitosis, which can interfere with patients' willingness to comply with cysteamine treatment.

Objective: To identify breath sulphur compounds causing halitosis induced by cysteamine therapy in patients with cystinosis.

Study design: After the ingestion of 15 mg/kg cysteamine whole blood ($n = 4$), urine ($n = 4$) and breath ($n = 8$) volatile sulphur compounds levels were measured every 60 min over a 360 min period by gas chromatography and the cysteamine plasma concentrations ($n = 4$) were measured by high-performance liquid chromatography.

Results: The expired air of cystinotic patients contained elevated concentrations of methanethiol (MT, median maximum value 0.5 (range 0–11) nmol/L) and, in particular, dimethylsulphide (DMS, median maximum value 15 (range 2–83) nmol/L). DMS concentrations higher than 0.65 nmol/L are known to cause halitosis. Maximal plasma values of cysteamine (median 46 (range 30–52) μmol/L) preceded those of MT and DMS, confirming that cysteamine is converted to MT and DMS. Less than 3% of the amount of cysteamine ingested was excreted as MT and DMS via expired air and 0.002% via urine.

Conclusion: Halitosis induced by cysteamine intake is caused by DMS and to a lesser extent by MT, excreted via the expired air. Further studies should focus on the possibilities of reducing the formation of these volatile sulphur compounds or masking their odour, which would improve the rates of compliance with cysteamine treatment.

Keywords: Cystinosis; Halitosis; Cysteamine; Dimethylsulphide; Methanethiol

Introduction

Cystinosis is a rare autosomal recessive disorder caused by mutations in the *CTNS* gene which encodes the lysosomal cystine carrier cystinosin, exporting cystine out of the lysosomes [1]. In infantile cystinosis, the most frequent form, lysosomal cystine accumulation manifests at around 3–6 months of age with generalised proximal tubular damage, the renal Fanconi syndrome and generally progressing towards end stage renal disease at around the age of 10 years. As cystine accumulates in virtually all tissues, extra-renal organs including the eyes, endocrine organs, muscles and the central nervous system are also affected by the disease [2].

Cystinosis is treated by cysteamine, which removes cystine from lysosomes via a disulphide exchange reaction with cystine, resulting in the formation of cysteïne and cysteamine-cysteine disulphide which exit the lysosome via the cysteine and lysine carriers, respectively, thus bypassing the defective cystinosin. Cysteamine slows down the progression of renal disease and protects extra-renal organs [2].

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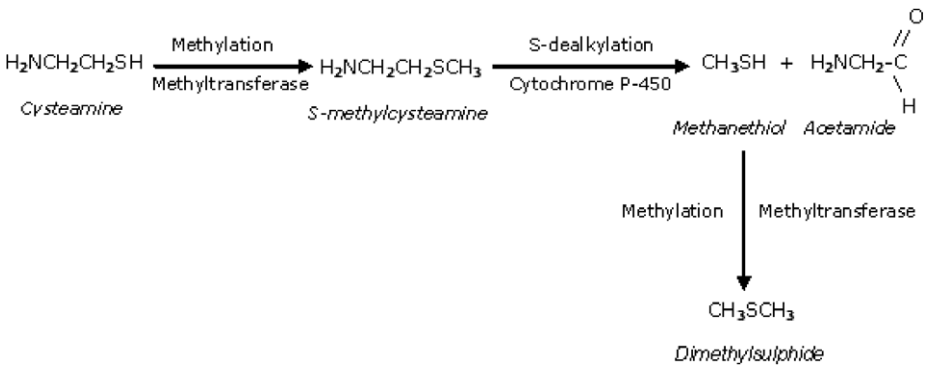

Fig. 1. Hypothesized route of cysteamine catabolism into MT and DMS. Cysteamine is methylated by a methyltransferase to *S*-methylcysteamine, followed by a cytochrome P-450 mediated *S*-dealkylation resulting in the formation of methanethiol. The latter is further methylated by a methyltransferase to form dimethylsulphide [3].

The cystine depleting effect of cysteamine is of short duration and the drug must therefore be taken every 6 h [2]. Cysteamine also has several side effects, such as bad breath and gastrointestinal discomfort, which, together with the four times daily dosage regime can interfere with patients' willingness to comply with cysteamine treatment. This problem of compliance worsens during puberty. As a consequence, patients who do not take cysteamine as prescribed risk suffering both a more rapid decline in renal function as well as a more rapid progression in the extra-renal complications of cystinosis.

In 1995 Gahl et al. proposed a mechanism by which cysteamine is metabolized to the volatile sulphur compounds methanethiol (MT) and dimethylsulphide (DMS)[1] which are both excreted via the lungs (Fig. 1) [3]. DMS had been considered to be the main cause of bad breath induced by intravenous cysteamine administration in one patient. However, to our knowledge, bad breath odour after oral cysteamine administration in cystinotic patients has not been extensively studied thus far. The aim of this study was to investigate the presence of volatile sulphur compounds in the expired air of cystinotic patients treated with oral cysteamine.

Subjects and methods

Subjects

After signing an informed consent form, eight patients were included in this study. Their clinical data are presented in Table 1. The patients were between 7 and 30 years of age and four of them were male. Cystinosis had been diagnosed between the ages of 9 months and 4 years by the measurement of elevated cystine levels in leucocytes and had been confirmed by mutation analysis of the *CTNS* gene in all patients. Five patients had a functioning renal graft, three had renal Fanconi syndrome. All patients were receiving maintenance treatment with cysteamine bitartrate (median dose 51, range 42–70 mg/kg/day). Patients with Fanconi syndrome (patients 6, 7 and 8) were additionally being treated with potassium, phosphate, citrate and carnitine supplements.

[1] *Abbreviations used:* DMS, dimethylsulphide; MT, methanethiol.

Study design

All patients took 15 mg/kg cysteamine bitartrate orally after having had a standard light breakfast. Blood and urine samples were collected from patients 1, 2, 3 and 4, breath samples were collected from all patients. Both blood and breath samples were obtained every 60 min during the 6 h after cysteamine intake, except for the first sampling of blood and breath, which was done immediately before cysteamine intake. The urine samples were collected over the same 6 h period.

Determination of volatile sulphur compounds

Samples of end-expiratory breath were collected in a balloon (Anagram International, Inc.), which was immediately closed with a 3-way valve. The balloons were tested in advance to make sure no leakage or binding of volatile sulphur compounds could occur. MT and DMS were measured according to Tangerman [4,5]. Venous heparinized blood and urine samples (both 2 ml) used in the detection of volatile sulphur compounds were injected into an evacuated 15 ml glass vial immediately after sampling. The vials were sealed with an air-tight rubber lid to prevent the vaporization of volatile sulphur compounds into the open air. All samples were processed using gas chromatography [4,5]. The air containing volatile sulphur compounds was then passed through a glass Tenax trap tube, which completely adsorbed all volatile sulphur compounds. Subsequently, the trap tube was placed in the injection port of the gas chromatograph. The sulphur compounds were thermally liberated into the gas carrier stream and transferred to the gas chromatographic column. By using this method the presence of several volatile sulphur compounds can be demonstrated, amongst which hydrogen sulphide (H_2S), methanethiol (MT), dimethylsulphide (DMS) and dimethyldisulphide (DMDS). The median area under the curve (AUC) was calculated from the measured levels of MT and DMS, respectively, using the trapezoid rule.

Determination of cysteamine

Venous blood samples were collected into ethylenediamine tetraacetic acid (EDTA) containing vacutainer tubes. The plasma was immediately separated from the leukocytes and erythrocytes by centrifugation at 2000*g* for 5 min at 4 °C. The plasma layer was collected and stored at −20 °C until analysis. Cysteamine was determined as total cysteamine, which includes free, protein and thiol bound cysteamine. After thawing the stored plasma samples, reduction and derivatization was performed as described by Fiskerstrand et al. using sodium borohydride ($NaBH_4$) for reduction and monobromobimane (mBrB) for derivatization [6]. After this step, samples were processed using high-performance liquid chromatography (HPLC) with reversed-phase separation and fluorescent

Table 1
Patient data

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Gender	Male	Female	Male	Male	Female	Female	Male	Female
Age	19 years	18 years	30 years	12 years	13 years	11 years	9 years	7 years
Age at diagnosis	4 years	3 years	2 years	11 months	1 year	2 years	9 months	1 year
CTNS mutation	57 kb del/?	57 kb del/?	57 kb del/537 del 21 bp	1261 G ins/1261 G ins	57 kb del/ 57 kb del	57 kb del/ 57 kb del	57 kb del/ 57 kb del	57 kb del/ 57 kb del
Renal status	Functioning renal graft	Functioning renal graft	Functioning renal graft	Functioning renal graft	Functioning renal graft	Native kidney, Fanconi syndrome	Native kidney, Fanconi syndrome	Native kidney, Fanconi syndrome
Glomerular filtration rate	109 ml/min/1.73 m^2	100 ml/min/1.73 m^2	72 ml/min/1.73 m^2	90 ml/min/1.73 m^2	84 ml/min/1.73 m^2	40 ml/min/ 1.73 m^2	82 ml/min/ 1.73 m^2	40 ml/min/ 1.73 m^2
Concomitant medications	Prednisone Cyclosporine A Amlodipine Enalapril Omeprazole	Prednisone Mycophenolate mofetil Amlodipine Losartan Omeprazole	Prednisolone Mycophenolate mofetil Atenolol Levothyroxine Fluvastatin Calcium carbonate	Prednisone Mycophenolate mofetil Amlodipine Enalapril	Prednisone Mycophenolate mofetil Growth hormone	Losartan Levothyroxine Alpha calcidol Growth hormone Indomethacin	Losartan Levothyroxine Alpha calcidol Growth hormone Indomethacin	Losartan Alpha calcidol Growth hormone

detection [7]. Automated reduction, derivatization and sample injection were executed using a programmable sample processor (Gilson, model 232 BIO).

Statistical analysis

Values were expressed as median and range. Spearman rank correlation and Mann–Whitney *U* test were used for statistical analysis. Values were considered statistically significant at $p < 0.05$.

Results

Blood

Blood samples were taken from patients 1 to 4. The median maximum plasma cysteamine level was 46 (range 30–52) μmol/L. Plasma cysteamine levels were below the detection limit in the control subjects [8]. Whole blood MT levels were detectable only in patients 2 and 3 in relatively small amounts (5–9 nmol/L) during the first 2–3 h after oral cysteamine intake. Whole blood DMS levels in patients 1–4 were higher than those of MT. The median maximum whole blood DMS level was 436 (range 298–617) nmol/L. MT is not detectable and DMS is found in very small amounts (<6 nmol/L) in control subjects [9].

Urine

Urine samples were collected from patients 1 to 4. Median urinary excretion of MT and DMS during the 6 h collection period was 52 (range 15–160) nmol and 233 (range 160–659) nmol, respectively. The normal value for DMS in urine is <2 nmol/L [10]. Since MT can be found in even smaller amounts than DMS, MT would not be detectable in the urine of healthy persons.

Breath

Breath samples were taken from all eight patients. No MT was detectable in the samples taken from patients 7 and 8. The median maximum MT breath level was 0.5 (range 0–11) nmol/L. DMS was detectable in all breath samples. The median maximum DMS breath level was 15 (range 2–83) nmol/L. Values measured in nose breath were identical to values measured in mouth breath (data not shown). MT breath levels are not detectable and DMS breath levels are below 0.65 nmol/L in control subjects [5]. The AUC of MT breath levels was 2.6 (range 1.2–36.9) nmol/L/6 h in patients 1–5, who had had a kidney transplant and 0 (range 0–0.3) in patients 6–8 with renal Fanconi syndrome. The mean AUC of DMS breath levels was much higher than that of MT and amounted to 57 (range 34–404) nmol/L/6 h in patients 1–5 and 16 (range 9–21) nmol/L/6 h in patients 6–8 (Fig. 2).

In patients 1–4, maximum cysteamine plasma levels preceded maximum MT and DMS breath levels. A typical example of one patient is shown in Fig. 3. At each time

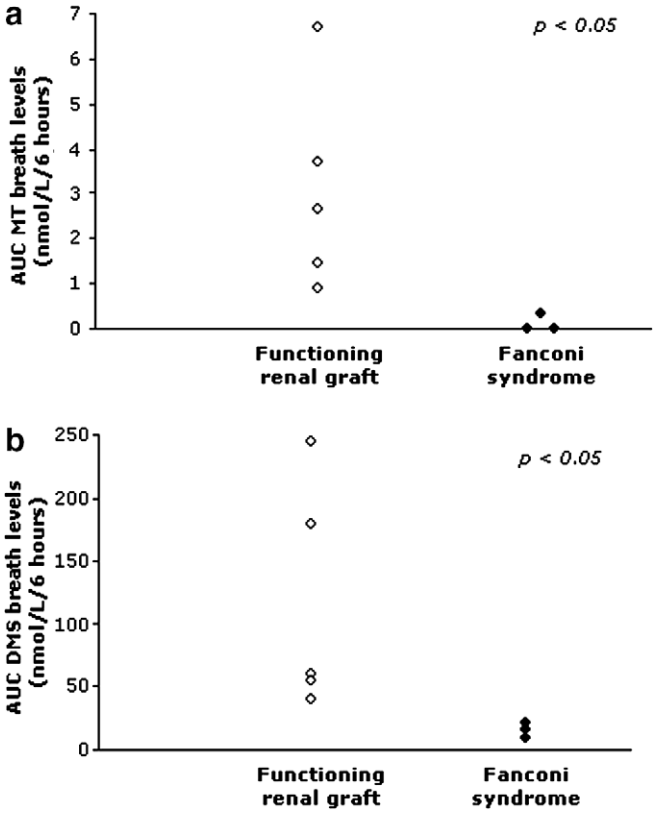

Fig. 2. Area under the curve of MT (a) and DMS (b) breath levels in nmol/L/6 h of patients 1–5, with a functioning renal graft (◇) and patients 6–8, with renal Fanconi syndrome (◆).

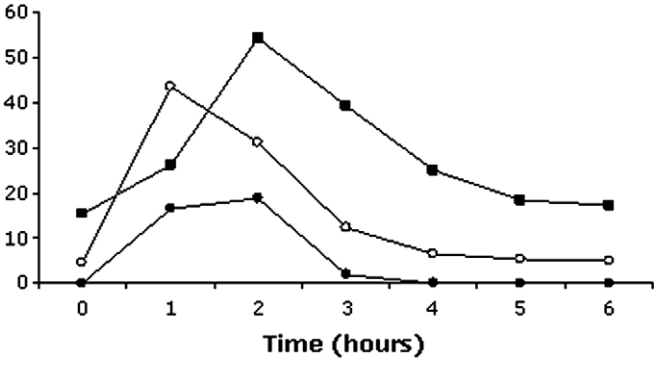

Fig. 3. Cysteamine plasma levels in μmol/L (—○—), breath MT levels × 10 in nmol/L (—■—) and DMS breath levels in nmol/L (—■—) in patient 2, before and until 6 h after cysteamine intake.

point studied, the concentration of plasma cysteamine significantly correlated with blood and breath concentrations of DMS ($r = 0.37$, $p = 0.043$ and $r = 0.46$, $p = 0.013$, respectively). Furthermore, a strong and significant correlation was observed between blood and expired air concentrations of DMS (Fig. 4). Simulated gas mixtures with the same concentration of DMS produced the same odour as that of the patients' breath.

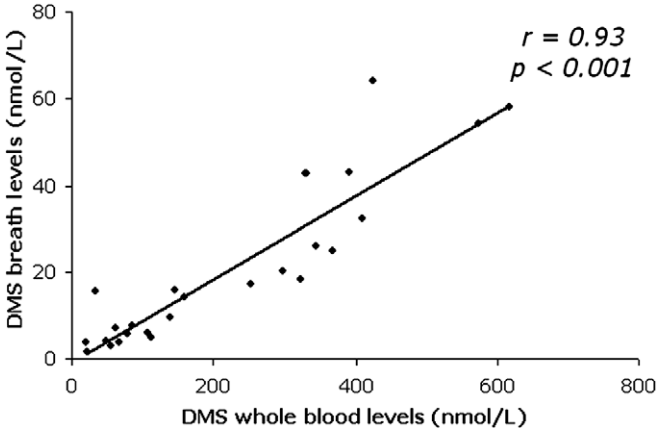

Fig. 4. Correlation between DMS levels in whole blood and breath at each time point studied in patients 1–4.

Discussion

Cysteamine has been proven to be effective in the treatment of cystinosis. It slows down the progression of renal disease and prevents extra-renal complications [2]. Although 4 h after ingestion cysteamine plasma levels decline almost to the levels before cysteamine intake, the cystine depleting effect of cysteamine lasts for 6 h. This can be explained by the fact that there is a time-lag before the drug is distributed to its intracellular point of action, the lysosomes [11]. Because of the rapid declining effect of cysteamine after 6 h, measured as a rising cystine concentration in polymorphonuclear leukocytes, it is advised that cysteamine is taken every 6 h, even at night [12]. Furthermore, the administration of cysteamine induces halitosis. In the general population, most cases of halitosis originate from the mouth and can be treated effectively by good dental hygiene, tongue cleaning and mouth washes [13]. In the case of cystinosis, when halitosis is caused by an extra-oral origin, symptomatic treatment of masking the bad breath is often the only option. Psychological support can be of additional help in some cases. The strict dosage regimen and the occurrence of halitosis after the ingestion of cysteamine hamper the compliance with the treatment and therefore worsen the prognosis of cystinotic patients.

As far as we know, this is the first extensive study of volatile sulphur compounds in the breath of cystinotic patients after oral cysteamine intake following the study of Gahl et al. in 1995 based on observations of one patient receiving intravenous cysteamine therapy. They proposed that cysteamine was metabolized to DMS via the intermediate MT [3]. MT was not detected but was assumed to be an intermediate because of the presence of small amounts of DMDS, the oxidation product of MT, in the patient's breath. At that time, breath was concentrated in Tenax trap tubes and measured by gas chromatography about 1 week after sampling. In the meantime the trap tubes were preserved on dry ice, to prevent the escape of sulphur volatiles from the tubes. In the present study, measurements

were done immediately after the trapping of breath samples in the Tenax tubes. Instead of DMDS now small amounts of MT were detected in the patients' breath, confirming the proposed mechanism. No traces of DMDS were present in the breath samples. In the study of Gahl et al. the 1 week delay in chromatographic measurements of the Tenax trap tubes after breath sampling had probably resulted in the oxidation of MT to DMDS within the trap tubes.

Patients 1–4 all showed a rise in cysteamine plasma levels after their intake of the drug. In blood samples taken from 234 children for the determination of reference values of total homocysteine by HPLC, no traces of cysteamine were found in the chromatogram [8]. By using this method, cysteamine should be detected when present. We demonstrated that maximum cysteamine plasma levels preceded maximum MT and DMS breath levels. In addition, cysteamine plasma levels correlated with DMS breath levels. The excretion of both MT and DMS via the urine is negligible compared to the amounts excreted through the lungs (0.002% versus 2.6% of the amount of cysteamine administered, respectively). The strong correlation between whole blood and breath levels of DMS and the observation that values measured in nose breath were identical to values measured in mouth breath, indicate that DMS and its precursor MT in the expired air of patients treated with cysteamine originates from the blood and not from other sources (e.g. the mouth or the stomach). All these observations confirm our assumption that cysteamine is first metabolized into MT which is then converted to DMS.

In normal subjects, DMS breath levels are below 0.65 nmol/L [5]. This limit is exceeded by far in all patients resulting in permanent halitosis. Gas mixtures containing the same concentrations of DMS as measured in the breath of cystinotic patients produced the same smell, thus confirming that the halitosis of these patients was indeed caused by DMS. DMS breath levels reached their maximum 2 h after cysteamine intake, which is in line with the patients' experience that halitosis is maximal around 2 h after ingestion of cysteamine.

In all patients, MT breath levels were increased only temporarily and to a much lesser extent compared to DMS breath levels. This same difference between DMS and MT was found in the blood samples. MT was only detected in the blood of two patients and in relatively small amounts, whereas DMS blood levels were much higher. This difference in behaviour between MT and DMS can be explained as follows. In vitro experiments have shown that the thiol CH_3SH (MT) containing a free, very reactive –SH group, immediately reacts with blood proteins and other blood compounds, resulting in irreversible binding and oxidation of MT [14]. The transportation of MT from the blood into alveolar air and into the breath is therefore hindered. Only the addition of very high concentrations of MT to whole blood leads to the detection of some unbound "free" MT. In contrast to MT, DMS is a relative neutral molecule, which is more stable in blood and can be transported from blood into alveolar air and breath.

Surprisingly, we found higher MT and DMS breath levels in patients with a functioning renal graft as compared to patients with renal Fanconi syndrome. We could speculate that immunosuppressive drugs given after renal transplantation were interacting with cysteamine catabolism. The induction of cytochrome P-450 could be promoting the metabolism of cysteamine into MT and DMS. Therefore, the use of cytochrome P-450 inhibitors might lessen halitosis in cystinotic patients under cysteamine treatment. However, the prescription of cytochrome P-450 inhibitors should be done with caution, since it will influence the pharmacokinetics of several other medications, such as immunosuppressive drugs, prescribed for these patients.

Our findings are in line with the metabolic pathway of cysteamine conversion postulated by Gahl et al. in 1995. Although only a small amount of the administered cysteamine is excreted as MT and DMS, this metabolic pathway is clinically relevant since the amount of DMS found in all patients continuously highly exceeds the level which leads to halitosis. Interference with this metabolic pathway might open therapeutic possibilities allowing the reduction of the formation of volatile sulphur compounds after cysteamine intake, which would be of paramount importance in improving patients' compliance with cysteamine treatment.

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