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CASE REPORT

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The usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning: three cases

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Abstract We examined the usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning by analysing sulfide and thiosulfate in three cases. In the first (non-fatal) case sulfide and thiosulfate were not detected in the blood samples from any of the four workers involved in the accident. In the urine samples, only thiosulfate was detected in three out of the four workers at a concentration of 0.12–0.43 μmol/ml, which was 4–14 times higher than the level in a healthy person. In the second (fatal) case sulfide and thiosulfate were detected in the blood sample at concentrations of 0.007 μmol/ml for sulfide, and 0.025 μmol/ml for thiosulfate. The thiosulfate concentration was at least 8 times higher than the level in a healthy person. In the third (fatal) case sulfide and thiosulfate were detected in the blood sample at concentrations of 0.95 μmol/ml for sulfide, and 0.12 μmol/ml for thiosulfate. Based on the above results, we concluded that thiosulfate in urine is the only indicator to prove hydrogen sulfide poisoning in non-fatal cases, while the analysis of sulfide in fatal cases should be accompanied by the measurement of thiosulfate in blood.

Key words Toxicology · Hydrogen sulfide · Thiosulfate · Metabolite · Analysis

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Introduction

Hydrogen sulfide poisoning is frequently encountered in the practice of forensic science. Although identification of sulfide can be made in cases of fatal poisoning [1–6], the detection of sulfide in non-fatal cases is often difficult because this compound is unstable and is rapidly metabolized within the human body [7–9]. There are also problems such as the postmortem production of sulfide due to putrefaction of blood and tissues.

Thiosulfate is one of the major metabolites of sulfide and is formed by the oxidation of sulfide [8, 9]. Vivian et al. [10] reported that thiosulfate remained stable in urine for at least 3 days at room temperature, and for up to 18 months when the urine was stored at –20° C. We carried out animal experiments using rabbits to examine if thiosulfate is a useful indicator of hydrogen sulfide poisoning [11] and ascertained that the measurement of thiosulfate rather than sulfide was useful for the identification of hydrogen sulfide poisoning.

Since we have experienced three hydrogen sulfide poisoning cases, we examined whether or not the above results obtained through animal experiments could be applied to human cases.

Case reports

Case 1 (non-fatal case)

Four workers lost consciousness in an underground tank (length 2.9 m, width 3.3 m, height 2.0 m) in a factory producing regenerated paper. The tank contained a liquid mixture of used paper and sodium sulfite to a depth of 50 cm. Two workers (A: 54-year-old man, B: 36-year-old man) entered the tank to remove plastic rope and plastic film which had twisted around the blades of the mixer set at the side of the tank. As A lost consciousness, B rushed out to call for help. He and other workers (C: 61-year-old man, D: 54-year-old man) entered the tank to rescue A, however, all of them lost consciousness inside the tank. The four men were sent to 2 hospitals and all survived. The concentrations of hydrogen sulfide and oxygen within the tank, measured on an XP-302II model gas analyzer (Shincosmos Electric Japan), were 114 ppm and 20.7% respectively 3 h after the accident.

Case 2 (fatal case)

One worker died in an underground tank (length 2.6 m, width 4.5 m, height 2.2 m) for waste water in a hospital. There was waste water and sludge in the tank to a depth of 1.94 m. After the water had been removed, one worker (48-year-old man) entered the tank to remove the sludge. He suddenly collapsed and died soon after. The cadaver was kept at room temperature and a forensic autopsy was carried out 22 h after death. At the time of inspection of the scene, 1 month after the accident, the concentrations of hydrogen sulfide and oxygen within the tank were in excess of 150 ppm and 21.0%, respectively.

Case 3 (fatal case)

One worker died in an underground drainage pump room (length 4.0 m, width 5.0 m, height 2.5 m) in a fish market. Two workers (A: 37-year-old man, B: 23-year-old man) entered the pump room to repair the pump. When A loosened a check valve, sewage within the pipe gushed out. Because B smelled the odor of hydrogen sulfide, he rushed out to fetch a ventilator. When he returned, A was lying beneath a pool of sewage and died soon after. The cadaver was kept at 0° C and a forensic autopsy was carried out 24 h after the accident. The concentrations of hydrogen sulfide and oxygen in the pump room were 123 ppm and 21.0%, respectively 4 h after the accident.

Materials and methods

Analytical procedure for thiosulfate

Blood and urine samples were used directly for analysis by a previously published method [12]. Thiosulfate was detected as bis(pentafluorobenzyl)disulfide ($C_6F_5CH_2SSCH_2C_6F_5$) as follows: 0.2 ml of the sample was added to the mixture of 0.5 ml of 20 mM pentafluorobenzyl bromide (PFBBr) solution in acetone, 0.05 ml of 200 mM L-ascorbic acid solution and 0.05 ml of 5% sodium chloride. The preparation was vortexed for 1 min and 2 ml of 25 mM iodine solution in ethyl acetate and 0.5 ml of internal standard solution (40 μM 1,3,5-tribromobenzene (TBB) in ethyl acetate) were added to the mixture. The preparation was again vortexed for 30 s and the mixture was centrifuged at 2,500 rpm for 15 min and allowed to stand for 1 h. An aliquot of the organic phase was injected into a GC-ECD apparatus.

Analytical procedure for sulfide

Blood and urine samples were used directly for analysis by a previously published method [13]. Sulfide was detected as bis(pentafluorobenzyl)sulfide ($C_6F_5CH_2SCH_2C_6F_5$) as follows: 0.2 ml of the sample was added to the mixture of 0.5 ml of 20 mM PFBBr solution in toluene, 2.0 ml of internal standard solution (10 μM TBB in ethyl acetate) and 0.8 ml of 5 mM tetradecyldimethylbenzylammonium chloride solution in oxygen-free water saturated with sodium tetraborate. The preparation was vortexed for 1 min and 0.1 g of potassium dihydrogenphosphate was added to the mixture as a buffer to stop excessive alkylation by tissue protein. The preparation was again vortexed for 10 s and centrifuged at 2,500 rpm for 10 min. An aliquot of the organic phase was injected into a GC-ECD apparatus.

GC conditions

The apparatus used was a Shimadzu Model GC-14AE gas chromatograph (Kyoto, Japan), equipped with a ^{63}Ni electron-capture detector and connected to a computerized recorder, Shimadzu Model C-R5A Chromatopac. The column comprised a glass tube of 2.1 m × 3 mm I.D. packed with 5% Apiezon grease L on Uniport HP, 60–80 mesh. The temperatures of the column, the injection port and the detector were 220° C, 270° C and 270° C, respectively. Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min.

Results and discussion

Sulfide and thiosulfate concentrations in the blood and urine samples in each case are shown in Table 1. Figure 1 shows the typical gas chromatograms of the derivatized extract from blood samples.

In Case 1 (non-fatal case) sulfide was not detected in the blood or urine samples from any of the four workers. This result was in agreement with the results of our animal experiments on non-fatal exposure to hydrogen sulfide gas [11]. Therefore, it was considered that the sulfide had rapidly been metabolized in the bodies of the surviv-

Table 1 Sulfide and thiosulfate concentrations in blood and urine in three hydrogen sulfide poisoning cases

Case	Sample	Concentrations (μmol/ml)	
		Sulfide	Thiosulfate
1	blood (A–D)[a, b]	N.D.	N.D.
non-fatal	urine (A)[a]	N.D.	0.43
	urine (B)[b]	N.D.	0.39
	urine (C)[a]	N.D.	0.12
	urine (D)[b]	N.D.	N.D.
2			
fatal	blood[c]	0.007	0.025
3			
fatal	blood[d]	0.03	0.12
	blood[c]	0.95	0.12

[a] Collected 6 h after the accident
[b] Collected 15 h after the accident
[c] Collected at the time of autopsy
[d] Collected 4 h after death in a hospital
N.D. = Not detected, (lower detection limits of sulfide and thisulfate were 0.3 nmol/ml and 3 nmol/ml, respectively)

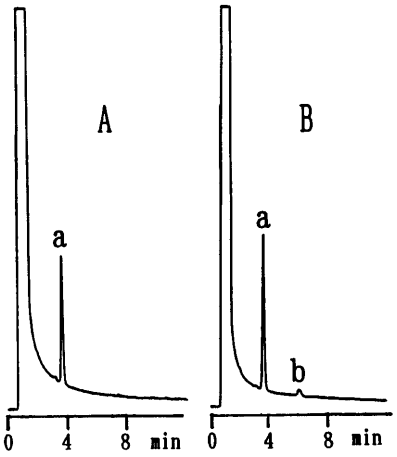

Fig. 1 The typical gas chromatograms of the derivatized extract from blood samples; **A** control blood, **B** blood containing 0.05 μmol/ml of thiosulfate. *a* TBB (I.S.), *b* the derivative of thiosulfate

ing workers. Thiosulfate was not detected in the any of the blood samples collected 6 or 15 h after the accident. Since the blood level of thiosulfate decreased rapidly from 0.061 μmol/ml to trace levels within 2 h during our animal experiments, thiosulfate probably no longer existed in the blood at the time of collection. Thiosulfate was clearly detected in the urine samples from workers A, B and C at concentrations of 0.43, 0.39 and 0.12 μmol/ml, respectively. These values were 4–14 times higher than 0.03 μmol/ml in healthy persons [14–16]. These results are in agreement with the results of our animal experiments. Thiosulfate was not detected in the urine sample from worker D, probably because the hydrogen sulfide gas was only inhaled for a short time and because the sample collection was delayed (15 h after the accident).

From the above data, we concluded that thiosulfate in urine was the only indicator to prove hydrogen sulfide poisoning in non-fatal cases.

In Case 2 (fatal case) sulfide and thiosulfate were both detected in the blood sample at concentrations of 0.007 μmol/ml and 0.025 μmol/ml, respectively. The sulfide concentration was higher than that in healthy persons which was below the lower detection limit of 0.3 nmol/ml. The value of thiosulfate was at least 8 times higher than that of healthy persons which was less than 0.003 μmol/ml [16]. In the fatal cases of our animal experiment [11], where exposure to 500–1,000 ppm of hydrogen sulfide gas continued until death, the thiosulfate concentration in the blood was 0.08 μmol/ml, which was 7 times higher than the level of sulfide itself. Therefore the data obtained from this case resembled the data obtained from our animal experiments.

In Case 3 (fatal case), sulfide and thiosulfate were detected in the blood samples of the victim at the concentrations shown in Table 1. The concentration of sulfide in the blood at the time of autopsy was 0.95 μmol/ml, which was extremely high compared to the levels obtained following the exposure to hydrogen sulfide gas [1–5, 17]. In our animal experiments comprising oral administration of sodium sulfide [6], the concentration of sulfide (0.32 μmol/ml) was much higher than that of thiosulfate (0.04 μmol/ml). We therefore thought that the victim had collapsed due to an exposure to hydrogen sulfide gas and ingested the sewage containing sulfide [6]. The sulfide concentration in the blood collected in the hospital was very low, 0.03 μmol/ml. Since this sample was kept at room temperature for several hours and then sent to our laboratory for analysis, the sulfide had presumably decomposed during this storage period. On the other hand, the thiosulfate concentration in the blood obtained at the time of autopsy was 0.12 μmol/ml, which was the same as the value for the blood sample collected in the hospital. This value was at least 40 times higher than that in healthy persons. Therefore, it was concluded that thiosulfate is stable and that the concentration of thiosulfate is not affected by the conditions of storage.

From the results obtained in Cases 2 and 3 we concluded that the analysis of sulfide in fatal cases should be accompanied by the measurement of thiosulfate in the blood.

In conclusion, from the analysis of sulfide and thiosulfate in human blood and urine samples of three hydrogen sulfide poisoning cases, we were able to conclude that thiosulfate in urine is the only indicator to prove hydrogen sulfide poisoning in non-fatal cases, while the measurement of thiosulfate in the blood is recommended together with the analysis of sulfide in fatal cases.

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