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Detection of toluene, methanol and formic acid in the autopsy case of a solvent abuser

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SUMMARY
A fatal case of abuse of solvent containing mixture of toluene and methanol is presented. Concentrations of toluene, methanol and formic acid in a femoral venous blood sample were 20.1 mg/L, 210 mg/L and 25.2 mg/L, respectively. From the autopsy findings and toxicological examination, we concluded that the cause of death was poisoning by toluene and methanol.

Keywords: solvent – methanol – toluene – formic acid – abuse – fatal intoxication

Detekce toluenu, metanolu a kyseliny mravenčí při pitvě pacienta závislého na rozpouštědlech

SOUHRN
V tomto článku je prezentován smrtelný případ zneužití rozpouštědla. Čtyřicetiletý muž byl nalezen mrtvý ve svém domě s mnoha prázdnými vinylovými sáčky a lahvemi obsahujícími směs toluenu a metanolu v okolí. Pitva neprokázala žádné známky vnějšího poranění a vyloučila i fyzická onemocnění. Následné šetření odhalilo, že oběť dlouhodobě zneužívala toluen. V krvi ze stehenní žíly byla zjištěna koncentrace toluenu 20,1 mg/l, metanolu 210 mg/l a kyseliny mravenčí 25,2 mg/l. Nebyl detekován žádný etanol a screening léků pomocí TriageTM (Biosite Diagnostic, San Diego, CA) byl rovněž negativní. Koncentrace toluenu ve femorální krvi byla na letální úrovni a koncentrace metanolu byla v toxickém rozmezí. Těžká, relativně dlouhodobá expozice toluenu vyplývala z vysoké koncentrace kyseliny hippurové v moči (12,91 g/l). Z pitevních nálezů, výsledků toxikologického vyšetření a vyšetřování ze strany úřadů vyplývalo, že příčina smrti byla otrava toluenem a metanolem.

Klíčová slova: rozpouštědlo – methanol – toluen – kyselina mravenčí – abusus – fatální intoxikace

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Various cases of sudden death due to solvent abuse have been reported (1-3). Toluene is used in industrial and household articles, paints, paint thinners and glues (4), and is one of the most abused solvents in Japan (5). Toluene poisoning is caused not only by accidental exposure, but also by solvent abuse, and fatalities have been reported (6-10). Methanol is likewise widely used in paints, varnishes and other industrial products (11). Various types of fatal methanol poisoning have also been reported, with outbreaks occurring frequently worldwide (12-16). In addition, relatively high concentrations of methanol can be detected in cases of poisoning by methanol-containing pesticides (17). Here we report a fatal case of solvent abuse in which toluene, methanol and formic acid were detected in blood.

CASE REPORT

A 40 year-old Japanese man (height, 165 cm; weight, 65.5 kg) was found dead in his house with numerous empty vinyl bags and filled bottles nearby. The liquid in the bottles was identified as toluene (ca. 70% by volume) and methanol (ca. 30 % by volume). Subsequent investigation by the authorities showed the victim had been abusing toluene for a long period. Autopsy showed no evidence of external injury. The heart weighed 315 g and contained 70 mL of blood without coagulum. The brain weighed 1259 g without any evident injury. Pleural effusion was observed in the thoracic cavity. The left and right lungs weighed 693 g and 692 g, respectively, and appeared edematous and congested. The stomach contained approximately 10 mL of brownish liquid. Approximately 150 mL of urine was collected in the bladder. Internal examination revealed no diseases. Signs of congestion were noted in other organs. Urine drug screening using the Triage™ test panel (Biosite Diagnostic, San Diego, CA) yielded negative results. Postmortem samples of femoral venous blood, urine, bile, tissues (brain, liver and fat) and stomach contents were collected for toxicological investigations.

Determinations of toluene, ethanol and methanol concentrations were performed using headspace gas chromatography-mass spectrometry (GC/MS) and headspace gas chromatography, slightly modified from previous reports (9, 16). Determination of formic acid concentration was performed using headspace gas chromatography following methylation with sulfuric acid (18).

In brief, toluene analysis was performed by GCMS-QP2010 (Shimadzu, Kyoto, Japan). Chromatographic separation was performed with a fused-silica capillary column DB-5MS (30 m × 0.25 mm I.D., 0.25 µm film thickness; Agilent, Santa Clara, CA,

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Table 1. Concentrations of toluene, methanol and formic acid in postmortem samples.

	Toluene (mg/L or mg/kg)	Methanol (mg/L)	Formic acid (mg/L)
Femoral venous blood	20.1	210	25.2
Urine	5.4	430	113.8
Bile	161.7	310	35.9
Stomach contents	74.9	200	Below LOD
Brain (gray matter)	5.3	N.A	N.A
Brain (white matter)	26.1	N.A	N.A
Liver	19.3	N.A	N.A
Fat	628.9	N.A	N.A

LOD : limit of detection (<1μg/mL)
N.A: not analyzed

USA). Operating conditions for GC/MS were as follows: carrier gas, helium 1.78 mL/min: injector temperature 200 °C. Oven temperature was set at an initial temperature of 40 °C for 2 min, and was programmed to then rise by 20 °C/min to 220 °C , then maintained at 220 °C for 7 min. The MS system was operated in electron impact mode with an electron energy of 70 eV and an ion source temperature of 200 °C.

The GC-2014 (Shimadzu, Kyoto, Japan) with FID connected to a Turbomatrix 40 autosampler (Perkin-Elmer, Waltham, MA, USA) was used for ethanol, methanol and formic acid measurement. Chromatographic separation was performed with a Supercowax-10 (60 m × 0.53 mm I.D., 2.0 μm film thickness: Superco: Sigma-Aldrich Japan, Tokyo, Japan). Temperatures at the injector, oven and detector were set at 90 °C, 90 °C and 200 °C, respectively.

Concentration of hippuric acid was measured by high-performance liquid chromatography system (Shimadzu, Kyoto, Japan). A HALO column C_{18} (3.0 mm × 100 mm, 2.7 μm particle size; Shimadzu GLC, Tokyo, Japan) was used, with operating conditions in accordance with the previous report (19).

RESULTS AND DISCUSSION

In the present case, injury and comorbid diseases were excluded from autopsy. Toxicological analysis identified toluene in each sample. Methanol and its metabolite formic acid were identified in femoral venous blood, urine, bile and stomach contents. Table 1 shows the concentrations of these chemicals in postmortem samples. No ethanol was detected in femoral venous blood, urine, bile and stomach contents. The concentration of hippuric acid in the urine sample was 12.91 g/L (normal range in a non-abusing, non-exposed individual, <1-2 g/L (20)).

A toluene concentration of 10 mg/L or more in the blood is reported lethal (6,7,21). The concentration of toluene in femoral venous blood for the present case was 20.1 mg/L, exceeding the above-mentioned lethal concentration. Assessed toluene concentrations were approximately eight-fold higher in bile than in femoral venous blood. This suggests that toluene may accumulate in bile. Toluene concentration was higher in the white matter of the brain than in the gray matter, as its distribution depends on the lipid content of the brain. This may be due to the physico-chemical properties of toluene (low molecular weight, high lipid solubility and no protein-binding capacity) (22). Accumulation of toluene in fat tissue and the high concentration of hippuric acid in urine suggested that the victim had been exposed to high concentrations of toluene over a long period (19, 20), supporting the results of investigation by the authorities.

Toxic effects of methanol are observed at concentrations greater than 100 mg/L in the blood, with severe poisoning at concentrations greater than 200 mg/L (23), and fatalities have been reported at concentrations of 200-6300 mg/L (11-13, 15). Formic acid is a metabolite of methanol, and concentrations exceeding 500 mg/L are often fatal (14, 24, 25). In the present case, the methanol concentration of 210 mg/L was within the toxic range, and the concentration of formic acid was over the normal reference range (<12 mg/L (26)), but below the lethal level.

Both toluene and methanol depress the function of the central nervous system (27). Based on the autopsy findings, results of toxicological examinations and investigation by the authorities, we concluded that cause of death was intoxication by a mixture of toluene and methanol.

Since some commercially available solvents used by abusers contain methanol or methyl esters mixed with other solvents (such as toluene), cases of methanol poisoning have been reported among inhaled solvent abusers (26, 28-31). In the present case, the concentration of methanol in blood was approximately ten-fold higher than that of toluene, although the concentration of methanol by volume was lower than that of toluene in the containers found near the deceased. This was probably due to the higher vapor pressure of methanol (125 mmHg at 25 °C (31)) compared to that of toluene (28.4 mmHg at 25 °C (32)), resulting in the main component of the gas phase of the solvent vapor being methanol in the mixture solvent (33). From this point of view, the toxicity of methanol needs to be taken into consideration in cases of solvent abuse.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding publication of this paper.

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