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CASE REPORT

Suicidal poisoning with mercaptodimethur–morphological findings and toxicological analysis

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Abstract In the western countries, the number of fatal intoxications with plant protecting agents has decreased to some extent due to laws restricting the use of highly toxic pesticides like halogenated hydrocarbons. Nevertheless, in consideration of the easy availability of most plant protectants, the small fraction of such fatalities among suicides and intoxications is astonishing. An 80-year-old woman died of an intoxication with methiocarb (mercaptodimethur), a carbamate type pesticide and as such a reversible inhibitor of the acetylcholinesterase. The case is presented because it is the first explicit report on a fatal poisoning of a human with methiocarb. The methiocarb concentrations detected were 6,100 μg/g in stomach content, 4.0 μg/ml in heart blood, 11 μg/g in kidney, 1.9 μg/ml in urine, 25 μg/g in liver, 2 μg/g in bile and 2.5 μg/g in brain tissue.

Keywords Mercaptodimethur · Methiocarb · LC-MS/MS · Suicide

Introduction

Intoxications with plant-protecting agents have been reported for decades [7], and in recent years, reports on therapeutic measures outweigh forensic issues. Lethal poisonings by pharmaceutical drugs [9] and illicit drugs [4] seem to have increased. However, fatal poisonings with plant toxins are relatively rare [5, 11], and intoxications with plant protecting agents have even decreased [13].

In Germany, doctors are obliged to report on poisonings with pesticides as well as suspected cases. For 2005, the total number reported was 121 [2]. Unfortunately, these data lack a complete specification in the various agents. According to the Federal Statistical Office, among 10,733 suicides in Germany in 2005, 51 were due to pesticides (http://www.destatis.de). In consideration of the easy availability of plant protecting agents, the small proportion of fatalities is astonishing. According to the WHO, the situation in other countries is different: the percentage of intentional self-poisoning by pesticides relating to all deaths is about 27 times higher in Portugal (0.1686 %) than in Germany (0.0062 %; http://www.data.euro.who.int/dmdb). Akgür et al. [1] reported that in Turkey, organophosphate insecticides are the most common agents used for suicidal poisoning. In some agricultural districts of Sri Lanka, pesticide poisoning is the most frequent cause of death in hospitals; the easy availability and the widespread use are made responsible [8].

In the following, we want to report on a suicide case with methiocarb (mercaptodimethur). This is the first explicit report on a fatal poisoning of a human, whereas animal poisoning has been reported repeatedly [3, 6].

Case report

An 80-year-old woman was found dead in her bed 21 h after last being seen alive. At the scene, an indication of the intake of red-coloured liquid was provided, whose nature could not be revealed. Due to rheumatism accompanied by chronic pain, the woman was known to be suicidal, and there was no

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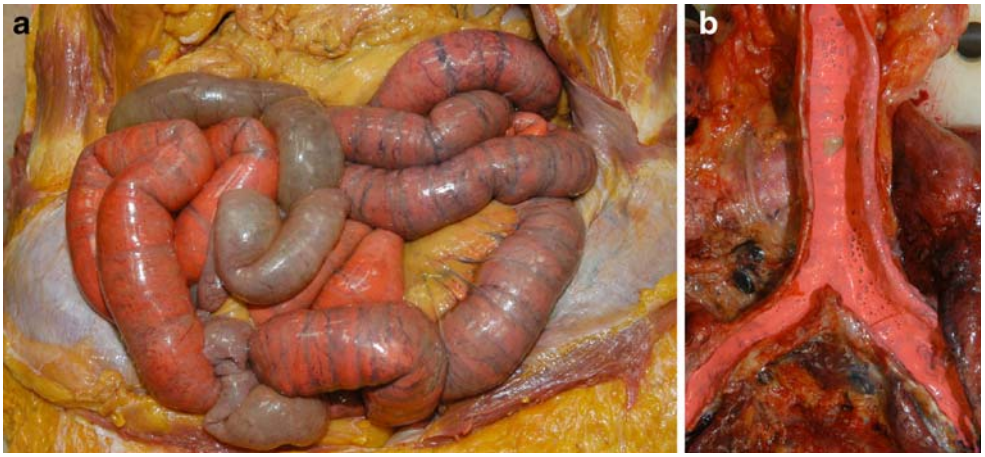

Fig. 1 **a** Coloured liquid in the gastrointestinal tract. **b** pink liquid in the respiratory tract

evidence of third-party influence. A medico-legal autopsy was performed 25 h after discovery of the body.

Morphological findings

The clothing showed extensive staining on the front with a pink liquid; the body featured similar staining on the face and both hands. The internal investigation of the corpse (166 cm/53 kg) yielded the coloured liquid in the gastrointestinal tract (Fig. 1a) from the mouth to the lower parts of the ileum as well as in the upper and peripheral parts of the respiratory tract including the trachea (Fig. 1b) and the paranasal sinuses. The lungs showed a chronic emphysema, an acute augmented volume and an oedema. The internal organs presented congestion. Further pathological findings were severe arteriosclerosis with an aneurysm of the thoracic and abdominal aorta and coronary sclerosis. Myocardial scars were found in the posterior wall of the left ventricle. The defective positioning of joints was consistent with rheumatism. Microscopic examinations confirmed the macroscopic diagnoses.

An acute intoxication with subsequent aspiration of gastric contents was assumed to be the cause of death.

Toxicology

Apart from the detection of ibuprofen and its metabolite in urine by gas chromatography–mass spectrometry (GC/MS) [10], toxicological examination of postmortem blood, urine and stomach contents provided negative results for the common legal and illegal drugs using immunochemical screening, followed by high performance liquid chromatography (HPLC)-screening (blood) and GC/MS screening (urine and gastric content). Mercaptodimethur (Fig. 2a) and its main metabolite were detected by HPLC-UV-diode-array detector (DAD) in blood and by GC/MS in gastric contents. Further quantitation of mercaptodimethur in liquids and tissues was performed by HPLC (Table 1); the identification of the metabolite was supported by LC-UV-tandem mass spectrometry (MS/MS) as described below.

Experimental

Instrumentation

The LC-DAD-MS/MS system consisted of an Agilent 1100 system (LC-System 1100 with a binary pump (G1312A), a degasser (G1312A), an autosampler (G1313A) and a 1,100 DAD (Agilent, Waldbronn, Germany)) and a column oven (Knauer GmbH, Berlin, Germany) coupled to a QTrap 2000 hybride tandem mass spectrometer with nebuliser-assisted electrospray ionisation (ESI; turboionspray source, Applied Biosystems, Darmstadt, Germany). The DAD was controlled by the Chemstation software from Agilent (Waldbronn, Germany) and the rest of the analytical system by the software Analyst 1.4 (AB). The analytical column was a synergy polar RP (150×2 mm, 4 µm; Phenomenex,

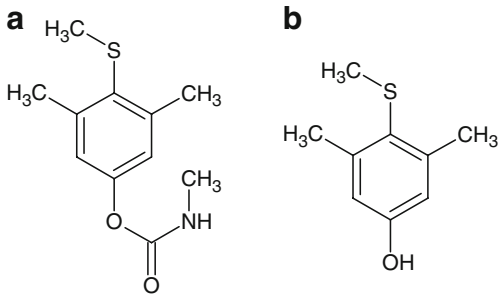

Fig. 2 **a** Structure of mercaptodimethur (3,5-dimethyl-4-(methylthio) phenyl methylcarbamate); **b** structure of descarbamoylmercaptodimethur (3,5-dimethyl-4-(methylthio)phenol)

Table 1 Results of quantitative HPLC/UV analyses

Matrix	Concentration of mercaptodimethur (mg/L)	Semiquantitative detection of mercaptodimethur-metabolite	Concentration ratio mercaptodimethur/ metabolite
Stomach	6100 μg/mL	65 μg/mL	94:1
Liver	25 μg/g	10 μg/g	2.5:1
Kidney	11 μg/g	Not detected	–
Heart blood	4.0 μg/mL	3.6 μg/g	1.1:1
Femoral vein blood	Not detected	12 μg/mL	–
Brain	2.5 μg/g	Not detected	–
Bile	2.0 μg/g	Not detected	–
Urine	1.9 μg/mL	1.5 μg/mL	1.3:1

Semiquantitative analysis was performed by the approximation of similar extinction coefficients of mercaptodimethur and its metabolite descarbamoylmercaptodimethur at wavelength 200 nm.
– Not determined

Aschaffenburg, Germany), and the solvents A and B were used with gradient mode and a flow-rate of 400 μL/min.

The above-mentioned solvents used for LC-MS were: A: water 0.1 % formic acid and 1 mM ammonium formate; B: ACN 0.1 % formic acid and 1 mM ammonium formate, and a mixture of them (A:B, 90:10, *v*/*v*) was used to redissolve the extracted samples.

The LC-UV system consisted of an isocratic pump LC10AD (flow rate: 0.6 mL/min), an autosampler SIL10A, a communication Bus module CBM-10A, a diode-array detector SPD-M10A (Shimadzu, Duisburg, Germany) and a commercialised UV-spectra library (F. Pragst, Berlin, Germany). The analytical column was a synergy polar RP (250×3 mm, 4 μm; Phenomenex, Aschaffenburg, Germany).

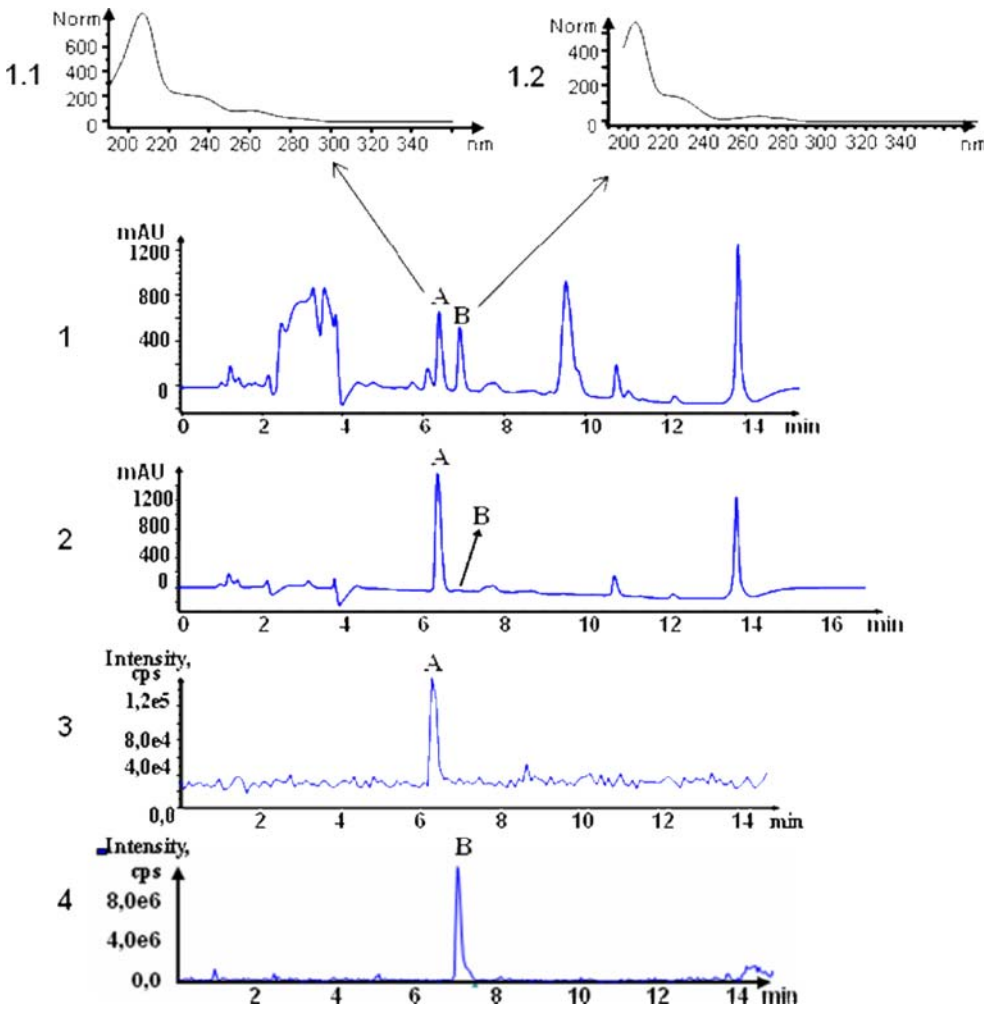

Fig. 3 HPLC-chromatogram of mercaptodimethur and its metabolite using LC-UV-ESI-MS/MS. *A* Descarbamoylmercaptodimethur; *B* mercaptodimethur. *1* LC-UV/DAD chromatogram of heart blood extract, *A* 6.39 min; *B* 6.90 min. *1.1* UV-spectrum of mercaptodimethur-metabolite. *1.2* UV-spectrum of mercaptodimethur matching the spectrum from the UV-spectra database [12]. *2* LC-UV/DAD chromatogram of mercaptodimethur after acidic hydrolysis, *A* 6.39 min; *B* 6.89 min. *3* LC-MS/MS: Total ion chromatogram (TIC) of enhanced product ion scan of mercaptodimethur after acidic hydrolysis using negative-mode ESI, precursor ion $[M-H]^-$, *m*/*z* 167.1; *A* 6.44 min (spectrum see Fig. 4b). *4* LC-MS/MS: TIC of enhanced product ion scan of mercaptodimethur using positive-mode ESI; precursor ion $[M+H]^+$, *m*/*z* 226.1; *B* 7.00 min (spectrum see Fig. 4c)

Isocratic eluent was acetonitrile:phosphate buffer pH 2.3, 36:64 (*v*/*v*) [12].

For quantitation of mercaptodimethur and its metabolite, tissue samples were homogenised and diluted with phosphate buffer, liquid samples were directly diluted with water (heart blood 1:50 *v*/*v*; urine 1:2 *v*/*v*; stomach content 1:200 *v*:*v*) and 1 ml aliquots of the diluted samples were extracted after addition of 0.5 ml borate buffer (adjusted to pH 9 with sodium hydroxide solution) with 1 ml chlorobutane. After centrifugation and phase separation, the organic phase was evaporated to dryness at 45°C under a stream of nitrogen, and the residue was redissolved in 100 µl HPLC solvent. HPLC-UV-DAD analysis was performed with isocratic elution. Quantitation was performed with an internal standard (methaqualone, 1 µg/ml) at 200 nm which was linear in the range of 0.5–50 µg/ml. Semiquantitative analysis was performed by the approximation of similar extinction coefficients of mercaptodimethur and its metabolite descarbamoylmercaptodimethur (Fig. 2b) at wavelength 200 nm.

The sample preparation for the identification of descarbamoylmercaptodimethur by means of LC-DAD-MS/MS was the same as described for HPLC, except that the dried residue was redissolved in 100 µL LC-MS solvent.

To confirm the identity of the chromatographic peak, which was assigned to descarbamoylmercaptodimethur, an acidic hydrolysis was performed prior to HPLC analysis. Therefore, 100 µL of a mercaptodimethur solution (1 mg/mL) was diluted with 1.9 mL water and 600 µL concentrated HCl (12 M). After boiling for 45 min with a reflux condenser, 800 µL NaOH (10 M) and 2 mL 30 % $(NH_4)_2SO_4$ were added. Finally, the product of the hydrolysis was extracted using chlorobutane and injected into the HPLC after reconstitution with LC-MS-solvents A:B (90:10, *v*/*v*).

Results and discussion

Generally, the autopsy findings of intoxications are affected by a lack of substance-related specificity and cannot contribute to the verification of the diagnosis. Only toxicological examinations led to the result that mercaptodimethur had been ingested, and in synopsis, with the pink colour, the agent could be determined as "Mesurol® flüssig", which is used as an insecticide as well as an agent against damage caused by game animals. Mercaptodimethur is a carbamate-type pesticide and as such, a reversible inhibitor of acetylcholinesterase with a parasympathomimetic effect. In the safety datasheet of Mesurol®, the following symptoms and potentially life-threatening conditions are listed: bradycardia, hypotension, vomitus, fasciculations, spasm, respiratory disorders up to respiratory insufficiency and paralysis, seizures, somnolence and coma. The acute oral toxicity in rats is specified as >25 to <50 mg/kg.

The identification of the metabolite and hydrolysis product descarbamoylmercaptodimethur was performed by on-line coupling of HPLC to the UV/diodearray-detector and to electrospray-ionisation tandem-mass spectrometry (LC-UV-ESI-MS/MS, Fig. 3). To confirm the structure of the metabolite, an acidic hydrolysis of methiocarb was carried out. The retention time and UV spectra of the metabolite in the sample and of the hydrolysis product were the same, which confirms the identity of the unknown compound (see Fig. 3). For the mass spectrometric identification of mercaptodimethur, positive-mode ESI was used; for descarbamoylmercaptodimethur, negative-mode ESI was more sensitive (Fig. 4).

Acute poisoning with methiocarb was identified as the cause of death. Absorption of the pesticide from the gastrointestinal tract was not completed. Only low concen-

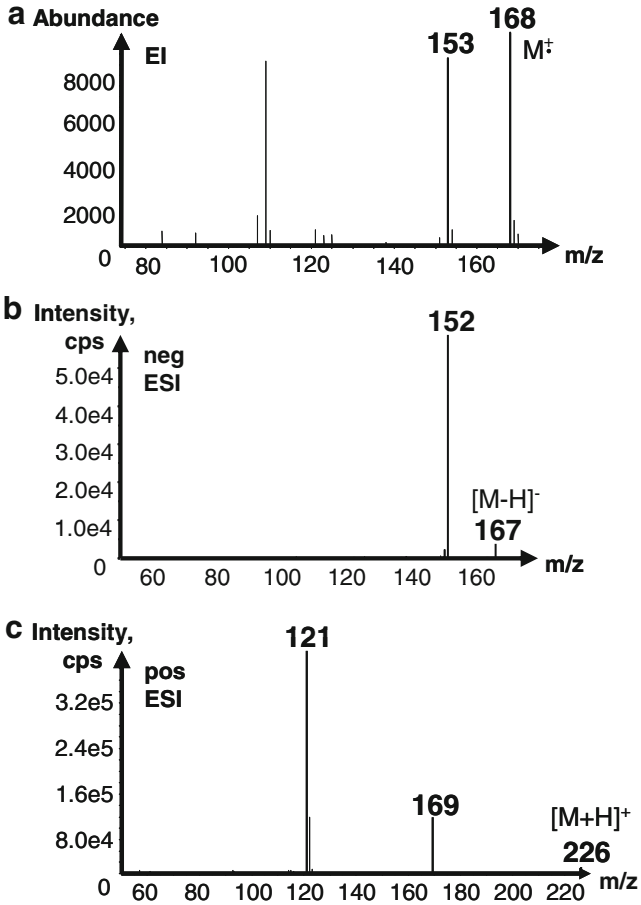

Fig. 4 **a** GC-EI/MS-spectrum of descarbamoylmercaptodimethur [10]. **b** Enhanced product ion (EPI) spectrum of mercaptodimethur after acidic hydrolysis, using LC-(negative-mode) ESI-MS/MS, precursor ion $[M-H]^-$, *m*/*z* 167.1. **c** EPI spectrum of mercaptodimethur, using LC-(positive-mode)-ESI-MS/MS, precursor ion $[M+H]^+$, *m*/*z* 226.1

trations were found in urine, whereas higher concentrations were detected in liver, brain and other tissue samples. Low concentrations in urine can be explained by the short duration of the agonal phase.

Elevated concentrations in heart blood might not only result from ante-mortem absorption but also from post-mortem redistribution from the gastrointestinal tract. As expected in the gastric content, only relatively small amounts of the hydrolysed compound were found. Only low volume of femoral vein blood sample was available. According to the regulations, femoral vein blood samples, which are used for alcohol determination, have to be stored at +4°C; therefore, this sample was not deep-frozen for several months prior to final quantitative LC analysis—in contrast to other liquids and tissue samples, which had been stored at −20°C prior to analysis. Since methiocarb was detected in frozen tissue and blood samples, and since carbamates are unstable in terms of hydrolysis, it has to be assumed that methiocarb in femoral blood was completely hydrolysed to descarbamoylmercaptodimethur prior to analysis due to its instability during storage at +4°C.

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