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CASE REPORT

Aconitine involvement in an unusual homicide case

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Abstract We describe a homicide complicated by an aconitine poisoning, which was initially thought to be a strangulation case. Routine toxicological analyses demonstrated only a small amount of alcohol in the blood and the urine. The case could not be clarified until 5 years after the event. A new element in the investigation made the wife the prime suspect, and finally, after thorough interrogation, she confessed her crime. She had mixed a decoction of three plants of *Aconitum* with red wine. Additional toxicological analyses, using the liquid chromatography–tandem mass spectrometry (LC-MS-MS) technique demonstrated 810 ng/ml of aconitine in urine, 6.5 ng/g in liver and 1.3 ng/g in the kidneys. Even though aconitine poisoning is still rare in Europe, it should be taken into account in suicides and homicides, particularly in unclarified cases.

Keywords Aconitine · Intoxication · Homicide · Strangulation · LC-MS-MS

Introduction

Aconitum is a genus of plants belonging to the Ranunculacea family, consisting of about 60 species. The blue-flowered *Aconitum napellus* L., also known as monkshood (Fig. 1), is an ornamental plant in Europe, and together with other *Aconitum* species, is used in Asian herbal medicines [2, 5, 7, 8, 13, 14, 34, 35, 37, 40]. The roots of *Aconitum* can be processed by soaking or boiling in water, which leads to hydrolysis of the aconite alkaloids into less toxic derivatives [2, 39]. The substance aconitine, belonging to the group of diesterditerpene alkaloids [1, 9, 35, 37], is present in the root, stalks, flowers and leaves [2, 3, 13, 30]. One gram of the root has been fatal and the estimated lethal oral dose of aconitine for humans is 2 mg [4]. Aconitine poisoning is most frequently caused by accidental ingestion, as it can be mistaken for an edible grass and by the use of herbal medicines, mainly in Asian countries where these are more used [7, 8, 23–25, 35, 40, 41]. A few fatal accidents have been reported [5, 7, 8, 14, 17, 35, 36, 41], and as it is highly toxic, aconitine or *Aconitum* species are sometimes used in suicides and homicides [11–13, 20, 28, 36]. Even in ancient times it was known as a poison [29]. In the nineteenth century, a few cases were reported in which doctors committed a homicide by the use of aconitine [22]. However, aconitine poisoning remains rare in Europe.

An interesting review of the effects of aconitum alkaloids is presented by Ameri: the difference in action and toxicity of aconitine is compared with those of other alkaloids such as mesaconitine, lappaconitine [1]. Aconitine is a neurotoxin that binds to the neurotoxin site 2 of the sodium channel. This causes a continuous activation by increasing the inflow of sodium ions preventing complete repolarisation of the excitable membrane of neural, muscle and cardiac tissues [1, 16]. The latter results in severe, potentially life-threatening arrhythmias, such as cardiac flutter and fibrillation [1, 16]. Symptoms of acute poisoning usually appear within 2 h after oral intake, although the initial signs can occur within a few minutes [3, 7, 17, 20, 24, 33, 34]. The latter normally include neurological

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Fig. 1 *Aconitum napellus* (overview (**a**) and detail (**b**))

features such as numbness in the mouth and lips, dizziness, paraesthesia, intense pain and generalised muscle weakness due to parasympathic activation and sensory nerve ending stimulation. Afterwards, gastro-intestinal features (e.g. vomiting, nausea, diarrhea) and cardiovascular symptoms (e.g. hypotension, bradycardia, ventricular arrhythmias) appear [6–9, 11, 12, 14, 15, 24, 28, 30, 35, 40, 41]. An intoxication can range from relatively mild symptoms to a severe life-threatening situation. In fatal intoxications, death finally occurs due to respiratory paralysis or cardiovascular collapse [1, 8, 9, 41].

As a small amount of aconite can be toxic, identification can be difficult, and therefore, a sensitive method is needed. This is indeed applicable to the toxicological analysis of all herbal medicines [10]. Gas chromatography–mass spectrometry in the selected ion monitoring mode (GC-MS/SIM) is sensitive and specific [20, 37, 38]. Solid-phase extraction using mixed-mode C8 cation exchange columns followed by liquid chromatography–tandem mass spectrometry (LC-MS-MS) is also a valid method for the precise detection of aconitine in whole blood and other body fluids down to very low concentrations [3]. Due to the less drastic analytical conditions and to the higher sensitivity, LC-MS-MS became the method of choice for the analysis of aconitine alkaloids.

We present an intricate fatal case. A man died after aconitine poisoning and possible strangulation, and only after several years did his wife admit that she had mixed a decoction of the Aconitum plants in a bottle of wine. We would like to draw attention to a relatively rare manner of intoxication and to the fact that this substance may be used for homicidal purposes. In addition, this substance is not routinely tested in current forensic practice.

Case history

About 100 km from his home, a man in his fifties was found dead behind the steering wheel of his car, the front of which was lying in a deep pitch running beside a highway. The presence of a carbonised fuse hanging from the fuel tank made the scene suspicious. In addition, the postmortem examination at the scene revealed signs of asphyxia such as a pronounced cyanotic face. At autopsy, numerous ecchymoses and abrasions were found, indicating that the man had suffered frequent blunt traumatic violence on his head, face, back, arms and legs. In addition, lesions compatible with strangulation were discovered (such as a vital fracture of the upper horn of the thyroid bone), and thus, a simple frontal deceleration was excluded.

Blood, urine (150 ml), gastric contents (50 ml), liver and kidneys were sampled for toxicological analyses, and routine screening for alcohol and drugs was performed. The blood alcohol level was 0.23 g/l and urine alcohol concentration was 0.34 g/l. No other drugs or toxic agents were initially detected.

The police investigation was unable to clarify this case for 5 years. However, DNA analysis on the stamp of a letter, which was thought to have been sent by the victim, demonstrated that it belonged to his wife and she confessed the story. She had prepared a decoction of *Aconitum napellus* made by boiling three plants, using the leaves and stalks. She mixed this decoction with a few tablets of Halcion® (triazolam) in a bottle of red wine. Her husband drank this bottle at supper, and according to her statements, he did not show any symptoms. She went to bed and about 3.5 h later, she found her husband lifeless in his chair. She could not feel a pulse or heart beat and—in her opinion—thought he had passed away, and so, she decided to move the body. Using ropes and a piece of carpet (see Fig. 2), she pulled the body up the staircase (Fig. 2a). Afterwards, she changed her mind and pulled him down the stairs (Fig. 2b,c) and placed it on the driver's seat of the car (Fig. 2d). Sitting on the lap of the dead body (Fig. 2e), she drove a distance of 100 km. After she had pushed the car into a pitch and attempted to burn the car, she left the scene and called a taxi to get back home.

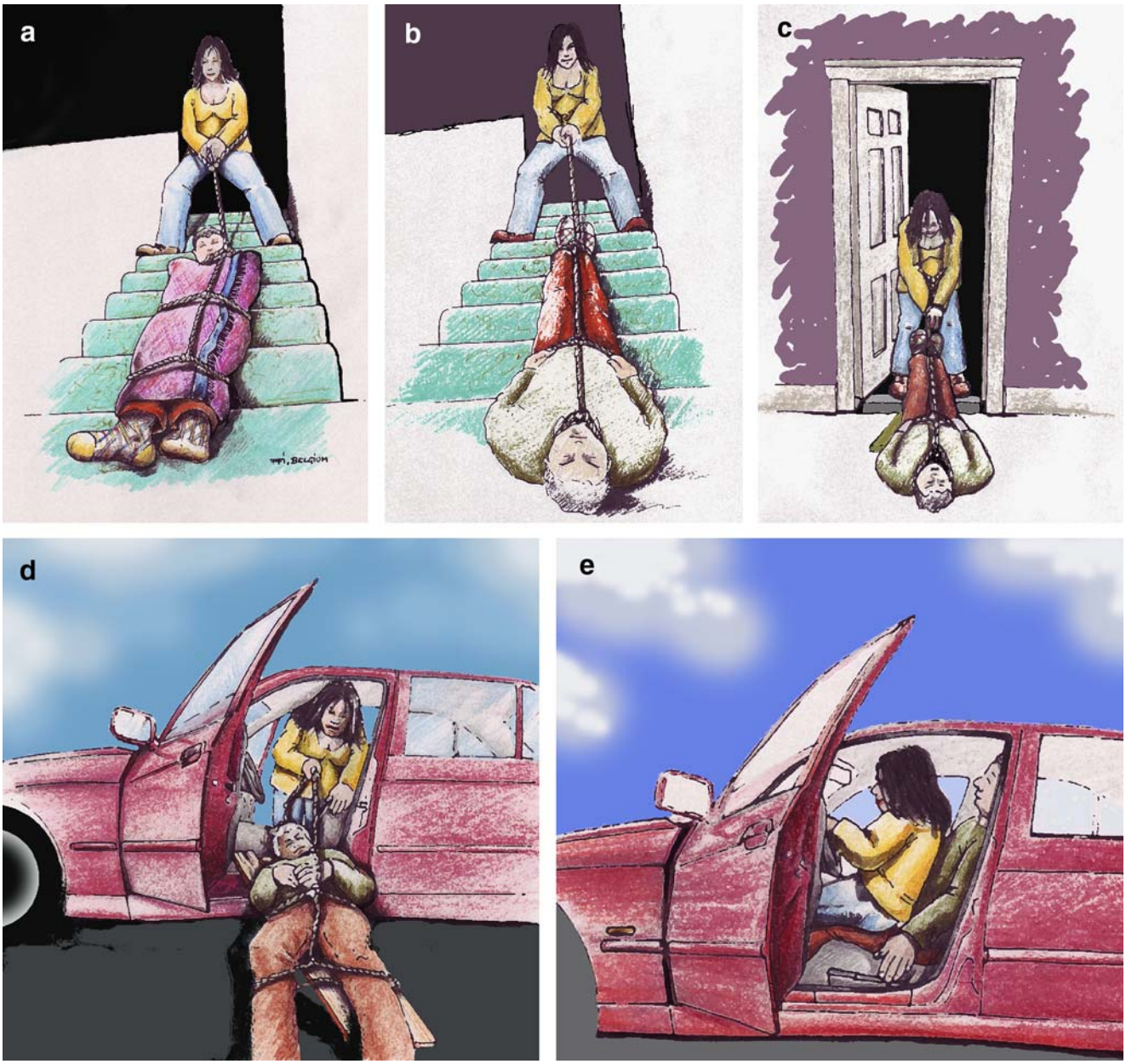

Fig. 2 Representation of the various manipulations of the body by the accused

Experimental section

Instrumentation

The experiments were carried out on a Quattro LCZ mass spectrometer (Micromass, Manchester, UK). Nitrogen was used as nebulizer and desolvation gas, while argon served as collision gas. The high performance liquid chromatography (HPLC) consisted of a Waters Alliance 2690 configuration (Milford, MA).

Chemicals and reagents

Aconitine was from Sigma (Bornem, Belgium) while mesaconitine was provided by Kishida Chemical Company (Osaka, Japan). Methanol and acetonitrile (LC-MS grade) were purchased from BDH (Poole-Dorset, England) and formic acid was from VWR (Leuven, Belgium). Double-deionized water (Milli-Q, Millipore) of 18.2 MΩ cm^{-1} resistance was used throughout.

Preparation of stock solutions and dilutions

Stock solutions of 1 mg/ml of both aconitine and mesaconitine were prepared in methanol. Working solutions were prepared with a dilution solvent (acetonitrile: water, 50:50, by vol., containing 0.1% formic acid) to concentrations of 10 and 1 ng/μl, respectively.

For the preparation of urine sample calibrators (for the screening), the 1-ng/μl working dilution was added to blank urine, resulting in 14 calibrator levels ranging between 2 and 100 ng/ml. For the confirmation procedure, calibrators at 100, 250, 500, 750, and 1,000 ng/ml were prepared.

Sample preparation

Sample preparation was kept to an absolute minimum and consisted of a tenfold dilution of the urine with the aforementioned dilution solvent. After centrifugation, a 100-μl aliquot was injected into the HPLC system and was analysed in triplicate (screening). For the confirmation analysis, the urine was diluted 50-fold and a 25-μl aliquot was injected.

Liquid chromatography

Screening procedure

For screening, the samples were injected into a Waters Oasis® HLB column (1.0×50 mm) and a gradient elution with (A) acetonitrile and (B) water, both containing 0.3% formic acid, was applied. From time 0 to 2.0 min, 100% B was used; between 2.0 and 2.5 min, the solvent was changed to 100% A, and this composition was held for 1 min. Between 3.6 and 6.0 min, the column was equilibrated under the starting conditions. The flow rate was 4 ml/min of which 400 μl/min was split to the mass spectrometer while the rest was diverted to the waste.

Final analytical procedure

The final chromatographic separation was achieved on a Waters XTerra column (150×2.1 mm ID) protected with a Waters Symmetry guard column (10×2.1 mm ID) (Waters). The same solvents were used as for the screening procedure; however, the gradient profile was different. From time 0 to 5.0 min, 25% A was used. Between 5.0 and 6.5 min, the percentage of A (organic phase) was increased to 60%, and this was held to 10.0 min. Between 10.0 and 11.0 min, the solvent composition was changed back to the starting conditions, and the system was equilibrated for 4 min under these conditions. The flow rate used was 300 μl/min.

Mass spectrometry

Optimisation of the mass spectrometric signal was performed by continuous infusion of the standards at the level of 1 ng/μl in the dilution solvent. The highest intensities were obtained by electrospray (ESI) in the positive mode. The following settings were applied: nebulizer gas (80 l/h), desolvation gas (560 l/h) and collision gas (2.3×10^{-3} mbar).

An overview of the MS/MS transitions, collision energy and cone voltages used during the screening (aconitine and mesaconitine) and the final quantitation (only aconitine) is given in Table 1. The ratio between the different transitions was also taken into account as a criterion for identification.

Toxicological data

During the initial screening procedure, two transitions were used: one for aconitine and one for mesaconitine. With this procedure, it became clear that aconitine was present in the sample while mesaconitine was not. In addition, it was shown that the aconitine level in the urine sample was far above the highest calibrator (100 ng/ml) used for screening.

For the final measurement of aconitine, we used three transitions (646→105, 646→368 and 646→586) at a different collision energy. As quantifier transition, 646→105 was selected because of its higher intensity.

A 20-μl aliquot of the centrifuged urine was diluted to 1.00 ml with the dilution solvent, and 25 μl of this dilution was injected in triplicate. The three transitions were monitored, and the ratio between the transitions was similar to the one obtained for the standard. This, together with the similar retention time of a pure standard, confirmed the identity of the peak as aconitine. The level of aconitine in the urine sample was 810 ng/ml. Other method parameters are summarised in Table 2.

Discussion and conclusion

We report a homicide case which was clarified only 5 years later. After the DNA analysis of a stamp and the confession

Table 1 MS-MS parameters

Compound	MRM transitions (m/z)	CE eV	CV V
Screening			
Aconitine	646→105	55	55
Mesaconitine	632→105	55	60
Final quantitation			
Aconitine	646→105,368,586	55,40,40	60,60,60

CE Collision energy (electron voltage), *CV* cone voltage

Table 2 Measurement characteristics

Compound	Range (ng/ml)	Equation	r^2
Aconitine	100–1,000	y=933.36x−24.70	0.9966
Transition ratios			
	646→586/ 646→105		646→368/ 646→105
Standard	0.9101		0.3058
SD	0.0351		0.0171
n	15		15
Urine	0.8863		0.3150
	0.8835		0.3105
	0.8853		0.2972

of the partner, it was confirmed that the man had been poisoned by means of aconitine.

The cause of death in aconitine poisoning is mainly attributed to ventricular arrhythmias [4, 8, 24]. As the cardiopulmonary system steadily fails, death occurs due to asphyxia, and therefore, possible postmortem signs point to the latter, even though these are nonspecific [21]. In the fatal case reported by Kämpf, generalised congestion of the organs (spleen, kidneys, gastro-intestinal tract and liver) and edema of the brain and lungs were noticed; these findings were confirmed by microscopical examination [21]. Maresch and Udermann reported another lethal case: the body showed cyanosis of the lips, petechial haemorrhages on the inside of the eyelids, pulmonary congestion and congestion of the spleen and kidneys, which was microscopically confirmed [26]. These literature data are in full accordance with our autopsy findings. In two other fatalities, the autopsy findings were not detailed but described as unremarkable [15, 17].

At first, the signs of asphyxia in our case were completely attributed to strangulation. The wife dragged her husband up the staircase using ropes around his neck and chest, in the conviction that he had passed away (see Fig. 2). Moreover, the presence of the numerous ecchymoses and abrasions on the body indicated that the man was still alive, although probably in a comatous or agonal state during these actions. On the other hand, the huge congestion of the tissues could have provoked postmortem bruises and even ‘vital-like’ strangulation signs [32]. Therefore, we can assume that the combination of aconitine poisoning and possibly ligature strangulation has contributed to the victim’s death.

Elliott reported a fatal suicide case with *Aconitum napellus* extract. The concentrations of aconitine in blood and urine were measured by HPLC-DAD (high performance liquid chromatography with diode array detection) and were 10.8 and 264 ng/ml, respectively [13]. At high concentrations, aconitine may be detectable during routine screening by HPLC-DAD [13]; however, in our case, this was not possible. HPLC has drawbacks for detection in poisoned victims, such as incomplete resolution and imprecise determination and is often not sensitive enough to determine *Aconitum* alkaloids in body fluids [19, 27]. In a more elaborate toxicological analysis, 5 years after the event, aconitine was detected in the urine (810 ng/ml), in the liver (6.5 ng/g) and in the kidneys (1.3 ng/g) by means of LC-MS-MS. The value of LC-MS-MS as an important tool to diagnose intoxications with plant material has recently been proven, e.g., in a case of oleander poisoning [31] and to demonstrate taxine B [18]. According to the available literature data, aconitine alkaloids have a relatively short half-life [28, 41]. In the case of Mizugaki et al., aconitine was detectable in the serum only within 24 h after intake [28]. On the other hand, the concentration was higher in urine than in serum, and aconitine was still detectable in the urine even 6 days after the intoxication [28]. These findings indicate a substantial urinary excretion of the substance, and therefore, urine is a useful matrix for identifying the toxicants in aconite poisoning.

Ito et al. reported a suicide by means of aconite ingestion in which GC-MS/SIM was used to quantify the aconitum alkaloids [20]. Although jesaconitine was the main alkaloid found in this case, aconitine itself was also detected. The concentrations of aconite in blood, urine, right and left lobes of the liver and kidneys were low (1.1 and 3.3 ng/ml and 4.3, 4.2 and 2.8 ng/g, respectively) reflecting the higher content of jesaconitine in *Aconitum* species in Hokkaido where the suicide took place [20]. *Aconitum* alkaloids were found in much higher concentrations in the liver and in the kidney than in the serum [20].

In our case, the victim survived less than 24 h after ingestion of the *Aconitum* extract. However, we can assume that aconitine would still have been detectable in blood, but regret that we were unable to report a blood level because the whole blood sample was used up during the first routine toxicological analyses. Although in our case, the qualified levels in urine, kidneys and liver are an appropriate proof. The toxicological analyses could not demonstrate the presence of triazolam in any of the samples. However, it cannot be excluded that the woman did not tell the whole truth, e.g., about adding triazolam tablets to the bottle of wine.

In summary, we present a peculiar homicide in which aconitine poisoning was involved. The intricate case was elucidated after a delay of about 5 years. It should be emphasised that the police inquiry in connection with criminalistics on the one hand and the forensic pathologists and toxicologists on the other hand should be in close collaboration when drawing a conclusion. Finally, it should be kept in mind that exceptional herbal intoxication should not be overlooked, and indeed, can take an interesting and unexpected turn in the investigation. In this case, initial

strangulation and kicking turned out to be an unusual poisoning and perimortem dragging.

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