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Case report

Seven cases of fatal aconite poisoning: Forensic experience in China

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ABSTRACT

This paper presents seven fatal cases of *aconite* poisoning encountered in the Tongji Center for Medicolegal Expertise in Hubei (TCMEH), China, from 1999 to 2008 retrospectively. In six of the cases, deaths occurred after drinking homemade medicated liquor containing *aconite*, and in one case death was due to ingestion of traditional Chinese medication containing aconite. Forensic autopsy and pathological examinations ruled out the presence of physical trauma or life-threatening diseases. Diagnosis of *aconite* poisoning was made after postmortem toxicological analysis. Animal experiment was performed in one case demonstrating that the medicated liquor could cause death rapidly. We present the autopsy and histopathological findings, toxicological analysis, and results of animal experiment done on samples from those seven cases. As an important herbal Chinese medicine, *Aconitum* species deserve special attention, especially because it contains poisonous alkaloids.

1. Introduction

Aconitum species have been used as an important drug in Traditional Chinese Medicine (TCM) in China for over 2000 years. The tubers and roots of *Aconitum* (*Ranunculaceae*) are commonly used to treat various illnesses and poor health conditions, such as syncope, rheumatic fever, painful joints, gastroenteritis, diarrhea, oedema, bronchial asthma, various tumors, and in some endocrine disorders like irregular menstruation [1]. "Fuzi" (the daughter root of *Aconitum carmichaeli* DEBX.) is one of the most important TCM drugs derived from *aconite*. Moreover, in some areas in China, aconitum is used in 'diet therapy', such as cooking chicken with "Caowu" (the root of *Aconitum kusnezoffii* REICHB.), or soaking "Chuanwu" (the main root of *A. carmichaeli* DEBX.) and/or "Caowu" in homemade tonic liquor. *Aconitum* contains aconitine, mesaconitine, hypaconitine, and other aconitum alkaloids, which are known cardiotoxins and neurotoxins. Aconitine is the main toxic alkaloid found in the *Aconitum* species. The improper use of *Aconitum* in many countries, especially in Asian countries, still results in high rates of severe intoxications [2]. However, deaths from *aconite* poisoning are somewhat difficult to diagnose during medicolegal expertise because no specific morphologic findings are present. Here, we report 7 fatal cases due to acute *aconite* poisoning. We also discuss some difficult points which may be encountered when dealing with such cases in forensic practice.

2. Case history

2.1. Case one

A 55-year-old male was found unresponsive in his residence. Previously, he had been drinking medicated liquor for 18 days to treat his rheumatic disease until found dead. His family said that he bought the medicated liquor from a veterinarian and drank it twice a day (approximately 15 ml each time). The man had complained of numbness of lips and cramps in the legs and died an estimated 7 h after his last drink. Police investigation showed that raw herbs, such as Chuanwu and Caowu were found in the medicated liquor.

Animal experiments were carried out to determine whether the medicated liquor contained poisonous substances. Aconitine was determined in the medicated liquor through thin-layer chromatography (TLC). Toxicological analysis showed no any other toxicants or illicit drugs in the heart blood of the victim. Therefore, death was due to *aconite* poisoning.

2.2. Case two

A 51-year-old man was found unresponsive at home and he was sent to hospital rapidly. However, he was pronounced dead there. The man had a history of sciatica. His wife explained that he bought two packets of medicinal herbs which contained 18 g *Chuanwu*. The man had complained of palpitation, numbness of tongue and formication soon after taking the medicinal herbs.

A complete autopsy and microscopic examinations found no evidence of trauma and fatal disease. Aconitine was detected in both gastric content and heart blood through TLC.

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2.3. Case three

A 49-year-old male farmer with a history of rheumatism died in his residence. According to his neighbors, his friends prepared medicated liquor which they gave him to treat his disease. It is believed that the deceased had drunk eight times the required volume of medicated liquor in the morning and then presented with vomiting and weakness. Emergency physicians pronounced him dead at the scene at 4 pm. Toxicological analysis done by using TLC found aconitine in the liquor and heart blood.

2.4. Case four

A 50-year-old male worker was found unresponsive in his friend's house after drinking a kind of homemade liquor. His friend told the police investigators that the liquor was made by himself and that it contained some herbs. The deceased had complained of severe numbness of the lips, back and legs soon after he drank about 100 ml of liquor. Forensic autopsy was performed and neither trauma nor fatal disease was found. High performance liquid chromatography (HPLC) was used for toxicological analysis. Aconitine was found in heart blood and liquor. No other drugs, sedatives, etc. were detected but the concentration of blood ethanol was 490 mg/l.

2.5. Case five

A 40-year-old man was pronounced dead in hospital ER. According to his friends, the man was having dinner at their residence and he drank some medicated liquor made by the host. The liquor contained raw "*Caowu*". One hour later, the man felt very sick, vomited and complained of numbness. He was sent to hospital in less than 2 h. The medical records described coma, cyanosis and foaming at his mouth. His vital signs were as follows: respiratory rate, 20 per minute; heart rate, 110 per minute; blood pressure, 100/60 mmHg. The diameters of both pupils were 6 mm and were fixed. Resuscitation attempt failed. Forensic autopsy indicated no trauma or fatal disease. TLC technique was used to detect aconitine in the gastric content, heart blood, urine and the liquor found at the scene.

2.6. Case six

A 38-year-old male farmer was pronounced dead in hospital ER soon after his arrival. His family explained he was drinking some kind of medicated liquor which contained raw "*Caowu*" and then felt very sick and complained of numbness about an hour later. His medical records showed that his vital signs were so weak that breathing rates, blood pressure and pulse were hardly noted. The man fell unconscious and could not be resuscitated successfully. Aconitine was found in the medicated liquor, gastric content, heart blood and urine through HPLC.

2.7. Case seven

A 57-year-old man died suddenly and unexpectedly in hospital. According to his medical records, the deceased previously found a bottle of liquor outside his house and drank almost 10 ml of it that morning. Half an hour later, he felt dizzy, had abdominal pain and numbness of extremities. Emergency team was called and on arrival, they found that the man's heart rate was 204. The electrocardiogram showed ventricular tachycardia. One hour later, he suddenly collapsed and passed away. Toxicological analysis using HPLC–MS verified aconitine from the gastric content and liver.

3. Materials and methods

3.1. Data source

The records of 7 aconite poisoning deaths from the Tongji Center for Medicolegal Expertise in Hubei (TCMEH), the department of forensic medicine of Huazhong University of Science and Technology, China, from January 1999 to December 2008 were retrospectively reviewed. For each case, the possibility of mechanical asphyxia, mechanical trauma and disease have been excluded after a thorough autopsy and pathological examination. The final conclusion was reached by comprehensive analysis of the investigation reports, medical history and toxicological reports.

3.2. Toxicological analysis

Poison detection in these cases was carried out by toxicological laboratories of national/provincial/municipal public security departments, or by our toxicological laboratory (TCMEH). General drug screening for tetramine, cyanide, sedative-hypontic drugs, illicit drugs and anesthetics from the postmortem blood serum was performed in each case using gas chromatography with mass-spectrometry (GC–MS), enzyme immunoassay and/or high performance liquid chromatography (HPLC). Quantitative analysis for ethanol was performed in case four using headspace gas chromatography with flame ionization detection (GC-FID).

Samples for aconitine analysis in these seven cases included heart blood, urine, gastric contents, liver and the remains of the suspected substance. Thin-layer chromatography (TLC), HPLC or HPLC–MS was used for detecting aconitine in different toxicological laboratories.

3.2.1. TLC qualitative analysis

Briefly, 1 ml of whole blood or urine sample was added to 5 ml of aether and 0.8 g $(NH_4)_2SO_4$ powder, and 10% ammonia solution was added to keep pH at 10. The mixture was centrifuged for 5 min at 3000 rpm to separate layers. The top aether layer was removed and kept. Samples were extracted one more time with 5 ml aether and the upper layer was pooled with the previous. The extract was acidified by adding 1 ml of 0.1 mol/l HCl and back extracted by vortexing for 10 min. The layers were separated by centrifugation and the aether layer was abandoned. The residual was added 1 ml dichloromethane and 0.3 ml 10% ammonia solution and centrifuged to get the organic layer. The extractant was evaporated to dryness at 40 °C under a nitrogen stream and reconstituted in 20 μl dehydrated ethanol. Spotted amount was 10 μl. GF_{254} plate was used for TLC analysis. The outspread solvent contained cyclohexane, ethyl acetate and diethylamine (8:1:1, v/v/v). Aconitine displays orange color when combining with dragendorff reagent and the Rf value is 0.846. Limit of detection for aconitine was 1 μg/ml by this method.

3.2.2. HPLC qualitative analysis in case six

HPLC analysis was performed using a GME-714 high performance liquid chromatogram with a UV-116 detector (Gilson, France). The chromatographic conditions were as follow: ODS C_{18} column (particle size 10 μm; 4.0 mm id × 250 mm); column temperature, 35 °C; mobile phase, methanol (0.04 mol/l):ammonium carbonate:methanedioxide = 90:15:2 (v/v/v); flow rate, 1.0 ml/min; UV spectroscopic wavelength, 235 nm.

3.2.3. HPLC/MS qualitative analysis in case seven

Analysis was performed with a Agilent 1100 Series high performance liquid chromatogram (Agilent, Germany) hyphenated to a Thermo Finnigan LCQ mass spectrometric detector (MSD 5973inert, Agilent Technologies). For chromatography, a Xterra™ RP_{18} column (150 mm × 2.1 mm, i.d. 5 μm) was used, mobile phase consisted of CH_3CN (10 mmol/l) and NH_4HCO_3 (pH = 9.5) (50:50, v/v). Injection volume was 10 μl. Operation conditions were: flow rate 0.2 ml/min, column temperature 35 °C. The MS used positive ion electrospray with capillary voltage held at 42 V and source voltage at 5.0 kV. Flow rate of nitrogen stream was 0.15 l/min and that of helium stream was 0.2 l/min.

3.2.4. HPLC quantitative analysis in case four

0.5 ml sample was added to 0.5 ml ammonia solution and homogenized, then 4 ml ether was added and extracted by vortexing for 10 min. The layers were separated by centrifugation for 10 min at 3000 rpm. The supernatant was evaporated to dryness under a nitrogen stream and reconstituted in 0.4 ml aether. Injection volume was 20 μl. HPLC analysis was performed using a LC-6 high performance liquid chromatogram with a SPD-6A ultraviolet detector (Shimadzu, Japan). After prepreparation of the specimen, the separation of aconitine was performed on a ODS2 (5 μm) column (particle size 5 μm; 4.6 mm id × 200 mm; Shimadzu, Japan) at 32 °C. Analysis of aconitine was isocratic at a 1 ml/min flow rate with an acetonitrile–tetrabutylammoniumbromide (5 mmol/l) buffer solution (30:70, v/v) as the mobile phase. The absorbance of aconitine was observed at 235 nm and it was free from any interference. Calibration curve was used for quantitative analysis. The quantification range was 0.1–5.0 μg/ml (r = 0.9998) and the limit of detection for aconitine was 0.1 μg/ml. The imprecision of the assay for aconitine was 3.2% (n = 5 replicates) at 5 μg/ml.