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Case report

Fatal cytisine intoxication and analysis of biological samples with LC–MS/MS

F. Musshoff *, B. Madea

Institute of Forensic Medicine, Stiftsplatz 12, 53111 Bonn, Germany

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ABSTRACT

We report about a fatal cytisine intoxication in a 20-year-old man who, according to his mother, had drunken tea prepared from plant material of *Laburnum anagyroides* with the toxic pyridine-like alkaloid as ingredient, which exhibits pharmacological effects similar to nicotine. Using a liquid chromatographic–mass spectrometric (LC–MS) procedure cytisine was quantified in post-mortem specimens. By exclusion of other causes of death an intoxication was determined as the cause of death with respiratory failure as the pathophysiological mechanism.

1. Introduction

Cytisine is a toxic pyridine-like alkaloid. Due to a structural similarity of the two molecules, it exhibits pharmacological effects similar to nicotine and is partial agonist of nicotinic acetylcholine receptors (nAChRs) with high affinity for α4β2-nAChRs [1–3]. Since the 1960s it has been used as a smoking cessation drug in East and Central European countries, where it is marketed as Tabex, registered for this purpose in 20 countries [4].

Plants containing the alkaloid cytisine in various concentrations are found in several genera of the *Faboideae* subfamily, including *Laburnum anagyroides* (also known as golden rain/chain or *Cytisus laburnum*), which is a smallish, decorative tree, often planted in parks and gardens. In late spring it is covered in longish, bright yellow inflorescences. Seeds develop in pea-like pods in summer and often remain on the tree all winter. All parts of the tree, especially the bark and seeds, contain the toxin cytisine and there are numerous reports of people getting poisoned with seeds of *C. laburnum*. It belongs to the toxic plants most often asked for in poisoning centers [5]. In spite of many fright news, serious cases of poisoning are an exception. Fortunately, ingestion of laburnum usually causes gastrointestinal upset only. Severe intoxication is rare but may result in neurological symptoms. Effects may appear within 1 h and include a burning sensation in the oropharynx, nausea, vomiting, abdominal pain and diarrhoea. Headache, dizziness, confusion, dilated pupils, clammy skin, tachycardia, pyrexia, dyspnoea and drowsiness are possible successive symptoms. Recovery is usually complete within 12–24 h. Although large doses of cytisine could cause hallucinations, convulsions, respiratory failure, coma and even death, fatal cases of poisoning are extremely rare due to the compound's innate emetic effect. One fatal case was reported in a psychiatric patient who also used the antipsychotic drug chlorpromazine. This patient absorbed 23 pods of *C. laburnum*, corresponding to approximately 50 mg of cytisine [6]. In a second case a 50-year-old man died after ingestion of approximately 25 pods, because his medical doctor had applied a central suppressant with antiemetic properties [7]. The lethal dose in human is still unknown. Poisoning or collective poisoning in children who eat laburnum seeds is frequent [8–11]. It was reported, that in an average summer over 3000 children are admitted to hospitals in England and Wales because of laburnum poisoning, but that it is not as dangerous as been thought and many of these admissions were unnecessary [12]. One report described two non-lethal suicide attempts by the same patient, a pharmacist who swallowed 40–50 Tabex tablets (containing 60–75 mg cytisine) on her fist attempt and 90 tablets (135 mg cytisine) on her second suicide attempt [13].

2. Case report

We report a fatal cytisine intoxication in a 20-year-old man who, according to his mother, had drunken a tea prepared from

* Corresponding author. Tel.: +49 228 738316; fax: +49 228 738368.
E-mail address: f.musshoff@uni-bonn.de (F. Musshoff).

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plant material. The young man was found dead in bed in the morning by his mother. On a table two glasses with a stone, charcoal and plant fibres (golden rain) were found, furthermore a bottle with a brownish liquid and beside a bottle a strainer with leaves. According to the mother only a few days ago her son has bought a book on poisonous plants. The autopsy was performed 2 days later. Main autopsy findings were: body of a 20-year-old man, weight 58.7 kg, height 181 cm. No signs of external violence. Severe brain oedema (1630 g). In the urinary bladder 220 mL urine. In the stomach 200 mL brownish liquid with small greenish leaves. Very small greenish leaves also in the duodenum and the oesophagus.

Histology: slight pulmonary congestion and moderate alveolar oedema (organ weight right lung 490 g, left lung 470 g). Hepatic congestion with dilatation of sinusoids. Brain oedema. No evidence of pre-existing diseases, no concurrent cause of death.

3. Toxicological analyses

Various body fluids and tissues were collected at the autopsy and stored at −20 °C until further analysis. Specimens were assayed for ethanol and drugs of abuse (acidic, basic and neutral organic drugs) using routine methods including immunochemical tests and liquid–liquid as well as solid-phase extraction procedures, with subsequent analysis by gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography with diode array detection (HPLC/DAD). For analysis of alkaloids in biological specimens we used a LC–ESI-MS/MS procedure similar to a procedure recently published by Beyer et al. [14] using d_3-benzoylecgonine as internal standard. Cytisine and d_3-benzoylecgonine were purchased from Promochem (Wesel, Germany).

For extraction 1 g or mL of post-mortem sample was homogenized in 3 mL borate buffer (pH 11) and extracted twice with 5 mL dichloromethane after the addition of 10 ng IS. Calibration samples (plasma) were prepared in the same way. The combined organic phases were evaporated to dryness and redissolved in 100 μL of HPLC mobile phase A, a 10 μL aliquot was injected into the chromatographic system.

A LC-20AD HPLC system (Shimadzu) coupled with an API 4000 Q-Trap with an Aqua C18 column (3 μm, 150 mm, Phenomenex (Aschaffenburg, Germany) and gradient elution using 5 mM ammonium formate in H_2O:acetonitrile (pH 3.5) was used. The ESI-MS/MS with a turbo ion spray source was used in positive ion mode at 425 °C and ion spray voltage of 5000 V. From the molecular ions ([M+H+]) generated in this way in multiple reaction monitoring (MRM) following transitions were monitored: cytisine m/z 191.19 → 147.9/76.9; d_3-benzoylecgonine m/z 293.16 → 170.9/76.9. For quantification, peak-area ratios of the analyte to the internal standard were calculated as a function of the concentration of the substances.

4. Results

The LC–ESI-MS/MS procedure described above was useful for the analysis of cytisine. In Figs. 1 and 2 the product ion scan of cytisine and the calibration curve are demonstrated. Compared to an APCI procedure in the ESI mode the sensitivity was markedly increased. In order to evaluate method selectivity, five blank plasma samples and five liver blank samples were prepared as described and analysed to check for interferences. Using the mean values of the duplicate analyses, calibration curves were checked for variance homogeneity (F-Test) and for linearity (Mandel-Test). The linear regression is described by the following equation: $y = 0.0299x + 0.0185$ ($r = 0.9981$). The limit of detection (LOD), characterized by a signal-to-noise ratio of 3, was determined to be 0.65 ng/mL, the limit of quantification (LOQ), defined as three times the LOD, was calculated to be 2 ng/mL. In the present case cytisine was found in various samples in concentrations demonstrated in Table 1. It has to be considered, that these concentrations

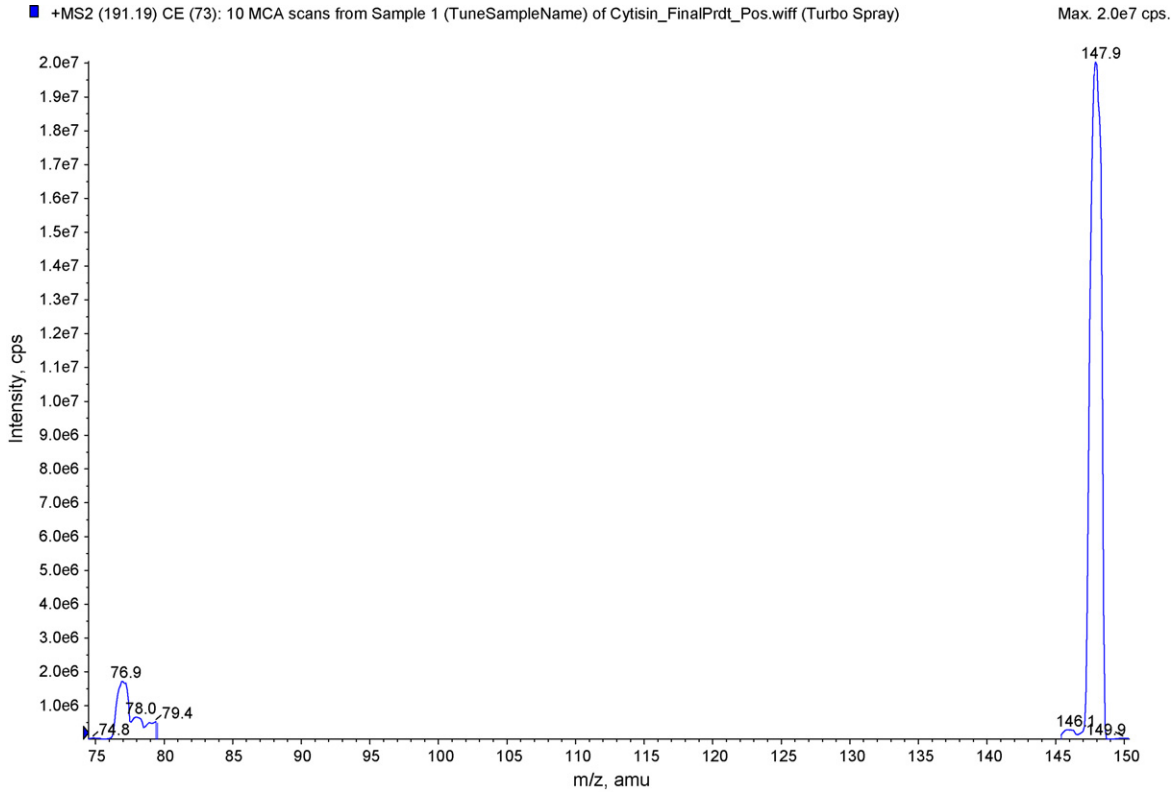

Fig. 1. Final product ion scan of cytisine (CE 73).