Forensic Science International 227 (2013) 118–126

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Eight cases of fatal and non-fatal poisoning with *Taxus baccata*☆

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ARTICLE INFO

Article history:
Available online 21 December 2012

Keywords:
Fatal poisoning
European yew (*Taxus baccata*)
Taxoids
Taxine B, 3,5-Dimethoxyphenol
LC–MS/MS

ABSTRACT

This paper describes two fatalities, three non-fatal intentional and three accidental oral ingestions of yew (*Taxus baccata*) leaves. In all cases the post-mortem external examinations showed no signs of violence. Internal examinations revealed small green, needle-like particles on the tongue, in the esophagus and in the stomach. Yew leaves were also identified in the stomach contents, whereas *Taxus* leaves were cut into small pieces and then ingested in one case.

The analytical method used was based on a liquid–liquid-extraction under alkaline conditions followed by LC–MS/MS analysis (QTRAP 5500). Chromatographic separation was achieved by HPLC on a Kinetex C18 2.6u (100 × 3) mm. The analytical method allows the simultaneous identification and quantification of the commercially available yew alkaloids taxoids (*m/z*): paclitaxel (854.2 → 105.0/286.1), 10-deacetyltaxol (10-DAT: 812.2 → 105.0/286.1), baccatin III (BAC III: 604.0 → 105.0/327.0), 10-deacetylbaccatin III (10-DAB III: 562.1 → 105.0/327.0), cephalomannine [taxol B] (562.1 → 105.0/327.0) and of 3,5-dimethoxyphenol (3,5-DMP: 155.0 → 111.9/122.9) also encompassing the qualitative analysis of the alkaloidal diterpenoids (*Q1* → 194.0/107.0); reference mass spectra obtained from a yew leaves extract: monoacetyltaxine (MAT: *568.4*), taxine B (*584.2*), monohydroxydiacetyltaxine (MHDAT: *626.4*), triacetyltaxine (TAT: *652.4*), monohydroxytriacetyltaxine (MHTAT: *668.4*).

In both fatalities, paclitaxel, 10-DAT and cephalomannine were not identified in urine, cardiac and femoral blood but all taxoids and 3,5-DMP were present in stomach content and excreted into the bile. In urine, highest 3,5-DMP concentration was 7500 μg/L and 23,000 μg/L after enzymatic hydrolysis, respectively.

In intentional and accidental poisonings, when electrocardiogram (ECG) examinations revealed ventricular tachycardia and/or prolonged QRS intervals, taxines were identified in plasma/serum, even after the ingestion of a few number of yew leaves, when 3,5-dimethoxyphenol was not even found. According to the data from one near-fatal intentional poisoning, elimination half-life of MAT, TAXIN B, MHDAT and MHTAT in serum was calculated with 11–13 h and taxines were detected up to t = +122 h post-ingestion of approximately two handfuls of yew leaves.

1. Introduction

The European Yew (*Taxus baccata*, *family*: *Taxaceae*) is an evergreen poisonous conifer and the cardiotoxic effects of the yew plant has been known for more than 2000 years [1]. *T. baccata* contains a complex mixture of compounds, including phenolic constituents (e.g. 3,5-dimethoxyphenol), non-alkaloidal diterpenoids (e.g. 10-deactetylbaccatin III), alkaloidal diterpenoids (e.g. paclitaxel, taxine B) or flavonoids (e.g. myricetin) and biflavonoids (e.g. bilobetin) etc. [2–4].

The taxoid concentrations in the plant vary with the season [5]. In general, paclitaxel (taxol A), 10-deacetylpaclitaxel, cephalomannine and baccatin III were found in low concentrations (<0.48% dry wt. in leaves) [6] but the major compounds, approximately 30% of the total alkaloid fraction from *T. baccata* consist of a mixture of compounds alkaloids, e.g. taxine B – called ''taxines'' – which are responsible for the toxicity of the yew plant (approx. 1.2% dry wt. in leaves) [2,7]. The lethal dose for an adult is reported to be 50 g of yew

☆ This paper is part of the special issue entitled "The 50th Annual Meeting of the International Association of Forensic Toxicologists (TIAFT)". June 3–8, 2012, Hamamatsu, Japan. Guest edited by Adjunct Professor Einosuke Tanaka and Associate Professor Masaru Terada.

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http://dx.doi.org/10.1016/j.forsciint.2012.11.014

needles equal to 250 mg taxine alkaloids or approx. 3 mg taxine per kilogram body weight [2].

The taxoid paclitaxel is metabolized by liver enzymes and excreted into the bile [8–10]. Only a small portion is excreted in urine [11]. The general mechanism of action is the disruption of microtubule function [12,13]. Paclitaxel is used in cancer therapy; e.g. lung [14] and ovarian neoplasia [15] and advanced forms of Kaposi's sarcoma [16]. The presence of an oxetane ring and a side chain at position C-13 is believed to be essential for the cytotoxic and anticancer acitivity [17].

In the case of an overdose with yew plant material, the taxoids e.g. taxine B block calcium channels in cardiac myocytes [18] accompanied by nausea, vomiting [19], dizziness, seizures and abdominal pain. These initial symptoms are followed by cardiovascular effects such as bradycardia, and then ventricular tachycardia with severe ventricular arrhythmias and episodes of ventricular fibrillation, severe hypotension and respiratory distress.

As there is no known proved antidote management is essentially based on symptomatic and supportive: intensive treatment with antiarrhythmic drugs, temporary pacemaker, intra-aortic balloon pump, excessive diuresis, extracorporeal membrane oxygenation, and extracorporeal life support. The administration of atropine is effective for a short time in the control of bradycardia. Treatment with anti-digitalis Fab-Fragments may also be beneficial [20]. Death can occur as a result of respiratory arrest and diastolic cardiac failure [21–24]. In many cases, autopsy shows unspecific signs e.g. dilated cardiac ventricles or acute blood congestion of lungs, liver, kidney and brain [25].

Lethal intoxications in humans with yew plant material is a very rare event, but has been described in literature [19,21,25–28]. Accidental fatal poisoning in animals with yew plant material also occurred [29,30].

As a preliminary test or indirect evidence of yew ingestion in humans the determination of the aglycone of taxicatine, 3,5-dimethoxyphenol (3,5-DMP) by HPLC-DAD or GC–MS has been suggested [23,25,26,28,31,32]. Other authors described a method after isolating taxines from *Taxus* leaves and confirmed the ingestion by analyzing taxine B/isotaxine B from human body fluids and tissue samples using LC–MS [33] or LC–MS/MS [27,34,35].

The aim of our project was to apply a newly developed analytical LC–MS/MS method (for the simultaneous identification and quantification of paclitaxel, 10-desacetyltaxol, baccatin III, 10-desacetylbaccatin III, cephalomannine (taxol B) and 3,5-dimethoxyphenol and the identification of monoacetyltaxine (MAT), taxine B, monohydroxydiacetyltaxine (MHDAT), triacetyltaxine (TAT) and monohydroxytriacetyltaxine (MHDAT) when yew poisoning is suspected. We present here two fatalities involving suicide, three non-fatal intentional and three accidental oral ingestions of yew leaves.

2. Experimental

2.1. Materials and methods

2.1.1. Reagents and chemicals

Baccatin III (BAC III), 10-deacetylbaccatin III (10-DAB III), 10-deactetyltaxol (10-DAT), cephalomannine (taxol B) were obtained from LGC Standards (Wesel, Germany), paclitaxel, 3,5-dimethoxyphenol (3,5-DMP) were purchased from Sigma–Aldrich (Steinheim, Germany). LC–MS-grade methanol (>98%) was from Fluka (Buchs, Switzerland), acetic acid (96%), ethanol (EMPROVE®, 96%), ammonia solution (25%), ammonium acetate, dichloromethane (EMSURE) and TRIS (tris(hydroxymethyl)aminomethane) were from Merck (Darmstadt, Germany). The deionized water was produced by an in-house water purification system Aqua RO 5-20 by membraPure (Bodenheim, Germany). β-glucuronidase from *Escherichia coli* K12 (200 U/mL at 37 °C) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). All chemicals, reagents and solvents were of LC–MS/MS or analytical grade.

2.1.2. Sample preparation

To a sample volume of 0.5 mL, 0.2 mL TRIS-buffer (pH 9.1), 0.4 mL extraction reagent [50 μL trimipramine-d3 (c = 0.1 mg/mL in methanol) dissolved in 100 mL dichloromethane] were added and mixed in a 1.5-mL eppendorf cup for 5 min. The sample was centrifuged for 5 min at approx. 16,000 × g and 0.2 mL of the organic layer was evaporated to dryness under a stream of nitrogen at 30 °C. The residue was redissolved in 0.05 mL of methanol. *Hydrolysis:* Urine, diluted with water was incubated with 10 μL β-glucuronidase for 30 min at 37 °C prior to analysis.

2.1.3. LC–MS/MS conditions

The chromatographic separation was achieved within 15 min on a Kinetex C18 (100 mm × 3.0 mm, 2.6 μm) from Phenomenex (Aschaffenburg, Germany) using a gradient consisting of a mixture of solvent A (0.1% HAc with 5 mM NH4Ac:methanol (90:10) and solvent B (methanol) pumped at a flow rate of 0.30 mL/min (60 °C). Taxine related alkaloids which were described by Jenniskens et al. [36] and identified after precursor ion scan experiments using a 5500 QTrap® from AB Sciex (Darmstadt, Germany) [35]. After FIA optimization, the source temperature was set to 400 °C, curtain gas to 30 psi, GS1 to 45, GS2 to 65 psi, CAD gas to medium (all nitrogen) and ion spray voltage to 5500 V. The compounds were detected in positive-ion mode and *identified*/quantified by multiple-reaction monitoring following two mass transitions per analyte (Table 1).

2.1.4. Specificity and selectivity

Individual solutions of all analytes and the IS in mobile phase A at the concentration of 1000 μg/L were analyzed in order to investigate interference between the different mass transitions. By analyzing seven blank plasma and urine samples from different sources the absence of possible matrix effects was evaluated.

2.1.5. Precision and accuracy

Intra-day precision and accuracy of the method were tested by analyzing six quality control samples from each of the concentrations (3 and 12 μg/L). The measured concentrations were tested for outliers using the Grubbs test and eliminated when indicated. Afterwards, relative standard deviation (RSD) and bias values were calculated. Inter-day precision and accuracy of the method were determined by analysing six quality control samples from the two concentrations on different days.

2.1.6. Matrix effects

A possible effect of co-eluting matrix compounds on the ionization of the analytes was investigated by post-column infusion of a reference standard solution mix (c = 100 μg/L, speed = 7 μL/min)/the extract of *T. baccata* containing all analytes via a syringe pump (Harvard Apparatus, Holliston, MA, USA) while simultaneously analyzing a blank plasma/urine samples. No ion suppression or enhancement was detected in plasma samples from different origins and blood containers. In contrast, ion suppression was detected in two of three urine samples. Due to the detection of two transitions per analyte, the effect can be monitored.

2.1.7. Quantification

For quantification, drug-free serum was spiked at ten different concentrations of paclitaxel, 10-DAT, BAC III, 10-DAB, cephalomannine (taxol B), and 3,5-DMP (0.2, 0.5, 1.0, 2.0, 5.0, 10, 15, 20, 50, and 100 μg/L). Quantification was set up following the internal