Identification of Taxine Derivatives in Biological Fluids from a Patient after Attempted Suicide by Ingestion of Yew (Taxus baccata) Leaves

Andrea Persico1, Giuseppe Bacis2, Francesca Uberti1, Claudia Panzeri2, Chiara Di Lorenzo1, Enzo Moro1, and Patrizia Restani1

1Università degli Studi di Milano, Pharmacological Sciences, Milano, Italy and 2Bergamo Poison Control Center, Bergamo, Italy

Abstract

The yew tree (Taxus baccata) is an evergreen conifer that is widespread over central and southern Europe. The toxic effects of this conifer and its leaves have been known since ancient times. The seeds are generally responsible for accidental intoxications in childhood, whereas the bark and the leaves are mainly used for homicidal or suicidal attempts. We investigated the metabolic pattern of taxines in a healthy 44-year-old male farmer who was admitted to Bergamo Emergency Department after attempting suicide. High-performance liquid chromatography was used to separate and identify taxine metabolites. Data reported in this paper confirmed that the patient attempted suicide by ingesting Taxus baccata leaves, which had been suggested by clinical examination. The most abundant free and conjugated taxine metabolites were characterized. The high concentration of conjugated metabolites found in urine underscores the critical role that conjugation in the liver plays in eliminating taxines and increasing the probability of the patient’s survival.

Introduction

The yew tree (Taxus baccata) also known as English yew, is an evergreen conifer with dark green needles grown in abundance in central and southern Europe. The Yew (Old Irish “Ibar,” genus Taxus) can grow to 60 feet tall and sometimes lives for 100 years. Yew is strongly associated with the history and legends of Great Britain, being found throughout Celtic mythology. The Druids thought as highly of the yew as they did the oak, preferring the yew for their wands. Considered a “guardian of mysteries,” an old grove of yews almost certainly signals the presence of a sacred spot. Symbolizing immortality, the yew was commonly planted in churchyards, although it is also known as the “death tree” because of the highly poisonous alkaloids contained in its needles and seeds. The cancer-fighting drug taxol is made from the bark of the relatively scarce Pacific yew tree.

The toxic effects of chewed yew seeds are often responsible for accidental intoxications in childhood, while the bark and the leaves are mainly used for homicidal or suicide attempts. Many cases of poisoning amongst animals have resulted from the ingestion of parts of the yew, although sensitivity to taxines differs among species: horses are more susceptible than cows, goats, and sheep (1,2). It is generally believed that wild deer can safely eat yew because their rumen fluid detoxifies taxines up to 88–96% (2,3). The leaves and seeds seem to be the most poisonous parts. Their toxicity is due to taxine alkaloids, which are present in all parts of the plant apart from the scarlet aril. Several taxine alkaloids have been isolated and characterized through the use of high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (1). The most poisonous compound is taxine B, approximately 30% of the total alkaloid fraction extracted from T. baccata. The main targets of toxic action of Taxus alkaloids are cardiac myocytes; poisoning often causes heart failure (1). This paper describes the analyses performed to confirm suspected intoxication by Taxus baccata in a case of attempted suicide.

Case History

A healthy 44-year-old male farmer was admitted to hospital with suspected myocardial infarction because of vomiting and loss of consciousness. The patient was examined for drug use/abuse, but laboratory tests for carbon monoxide, drugs of abuse (cocaine, opiates, amphetamines, cannabinoids), tricyclic antidepressants, digoxin, antiarrhythmic drugs, and pesticides gave negative results. Intensive treatment with amines, sodium bicarbonate and amiodarone infusions, repeated car-
dioversions, pacemaker, and intra-aortic balloon pump counter-pulsation (IABP), and extracorporeal support (ECLS) with membrane oxygenation (ECMO) were applied because there was an intractable severe hypotension and recurrent episodes of potentially lethal arrhythmias (4). The patient recovered completely, but some days after admission, large numbers of “rosemary” leaves were observed in his feces, and *Taxus* ingestion was suggested by botanical examination. Informed consent was obtained to perform analyses on the blood and urine samples.

**Methods**

**Reagents and chemicals**

HPLC-grade acetonitrile and water were purchased from Sigma Aldrich (Steinheim, Germany) and acetone from LAB-SCAN Analytical Sciences (Dublin, Ireland). Sodium acetate, acetic acid, ammonium carbonate, phosphoric acid, and hydrochloric acid, all analytical grade, were from Merck (Darmstadt, Germany). β-Glucuronidase, type B-1, from bovine liver, was from Sigma Aldrich.

The following authentic reference standards were used: 3,5-dimethoxyphenol, purity 98.5% (Fluka, Steinheim, Germany), stock solution 10 mg/25 mL water/acetone (1:1, v/v), corresponding to a final concentration of 400 mg/mL; 10-deacetylbaccatine, purity 98.5% (Indena, Settala, Italy), stock solution: 15 mg/25 mL water/acetone (1:1, v/v), corresponding to a final concentration of 600 mg/mL; and taxol (Paclitaxel®), purity 98.5% (Indena), stock solution: 11 mg/25 mL water/acetone (1:1, v/v), corresponding to a concentration of 440 mg/mL.

**Urine sample preparation**

The urine was collected at hospital admission of the patient (which was discovered later to be 30 h after the ingestion of the poisonous plant) and stored at −20°C until analyzed.

Unconjugated metabolites were analyzed by the direct injection of a urine

![Figure 1. HPLC chromatograms at 232 nm of a standard mixture (A), urine from the poisoned patient (B), and urine from a healthy subject (C). Peak identification: 1, 3,5-dimethoxyphenol; 2, 10-deacetylbaccatine; and 3, taxol.](image)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>RT (mean ± SD)</th>
<th>Range (µg/mL)</th>
<th>Linear Equation</th>
<th>Linearity (r²)</th>
<th>Recovery (%)</th>
<th>LOD (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-Dimethoxyphenol</td>
<td>11.17 ± 0.35</td>
<td>2–20</td>
<td>y = 207335x – 86747</td>
<td>0.998</td>
<td>98.9</td>
<td>0.20</td>
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<tr>
<td></td>
<td></td>
<td>20–200</td>
<td>y = 220897x – 32708</td>
<td>0.999</td>
<td>99.7</td>
<td></td>
</tr>
<tr>
<td>10-Deacetylbaccatine</td>
<td>13.14 ± 0.51</td>
<td>2–20</td>
<td>y = 210057x – 25844</td>
<td>0.999</td>
<td>100.2</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–200</td>
<td>y = 208159x – 37982</td>
<td>0.998</td>
<td>98.7</td>
<td></td>
</tr>
<tr>
<td>Taxol</td>
<td>28.98 ± 0.35</td>
<td>2.4–14.7</td>
<td>y = 60039x – 1593.7</td>
<td>0.999</td>
<td>99.3</td>
<td>0.35</td>
</tr>
</tbody>
</table>
sample that had been thawed and filtered through 0.45-μm membrane into the chromatograph.

For the analysis of glucuronated metabolites, 0.5 mL urine was added to an equal volume of acetate buffer (pH 4.7) containing 20 mg of β-glucuronidase and stored at 37°C for 24 h. After filtration using a 0.45-μm microfiltration system, samples were injected into the HPLC without further treatment. Urine from a healthy male volunteer was used as a control.

For the determination of total metabolites (free and conjugated metabolites), 0.5 mL urine was hydrolyzed with 0.5 mL of 2 N HCl and stored for 4 days in the dark at room temperature. The sample was filtered and injected into the HPLC (20 mL) without any further treatment.

**Blood sample preparation**

Blood was drawn from the patient at admission and stored at –20°C until it was analyzed. After thawing, 0.5 mL blood was mixed with 2 mL 0.01 M ammonium carbonate buffer pH 9.3. The mixture was vortex mixed for 30 s and centrifuged at 3000 × g for 10 min. The supernatant was separated and loaded onto an activated SPE C18 column (Merck). The column was pre-conditioned with 1 mL methanol, 1 mL water, and 1 mL 0.01 M carbonate buffer (pH 9.3). After sample loading, the column was washed with 3 mL of carbonate buffer, and the analyte was then eluted with 1.5 mL of methanol. The collected eluate was evaporated to dryness under nitrogen at 40°C, suspended in 0.5 mL of acetone/water (1:1, v/v), filtered through a 0.45-μm membrane and injected (20 mL) into the HPLC apparatus.

**HPLC conditions**

A P2000 Spectra System pump (Thermo Fisher Scientific, Waltham, MA) was connected to a diode-array detector model UV 6000 Spectra System (at 232 nm). Chromatographic data were analyzed using Chromatography Data System Chromquest software (Thermo Fisher Scientific) interfaced with the SN 4000 module. Samples were injected using the Rheodyne (Cotati, CA) injection valve equipped with a 20-μL loop.

Chromatography took place on a LiChrospher 100-RP8 (250 × 4 mm, 5 μm) analytical column (Merck) maintained at 40°C in the column heater module 7971 (Jones Chromatography), with gradient elution at a constant flow rate of 1 mL/min; solvent A was 0.085% o-phosphoric acid in distilled water and solvent B was pure acetonitrile. Initial conditions were 21.5% solvent B increased to 61.5% at 30 min, to 95% at 31 min, and held there for 19 min (total running time 50 min).

**Results and Discussion**

This poisoning case is very unusual because yew ingestion is usually fatal (5–9). After aggressive treatment and extracorporeal life support, this patient survived in spite of the number of needles found in the feces, which exceeded the usual fatal dose of 50 leaves (10). The clinical outcome allowed the collection of biological fluids for biochemical analysis, approximately 30 h after exposure.

The retention times of each urine peak were compared with those of standard taxine standards shown in Figure 1A. These metabolites were selected because of their use in taxus poisoning identification and their commercially availability (11). In the chromatographic conditions described, the three standards are well separated as symmetrical peaks. The injected concentrations and average elution times were 13.33 μg/mL and 11.17 min for 3,5-dimethoxyphenol, 20 μg/mL and 13.14 min for 10-deacetylbaccatin, and 14.66 μg/mL and 29 min for taxol. Elution times were calculated on 10 repeated injections, and showed a variability below 5% (Table I).

These compounds are also clearly visible in the chromatogram of the patient’s urine (Figure 1B), whereas no compound with a similar elution time was present in the urine of a healthy subject (Figure 1C). The UV spectrum of each peak was compared to those of purified standards, and each yielded similar profile and maximum of absorbance (not shown). Each peak was also subjected to the DAD software procedure testing purity, results in urine always being above 95% (taxol 0.997; 3,5...
dimethoxyphenol 0.992; 10-deacetylbaccatine 0.997).

Linear regressions were calculated by injection of control urine samples containing increasing concentrations of each metabolite, ranging between 2 and 20 mg/mL for urine itself and between 20 and 200 mg/mL for hydrolyzed urine. The parameters are reported in Table I.

The patient’s urine metabolite concentrations were 5.6 μg/mL of 3,5-dimethoxyphenol, 16.5 μg/mL of 10-deacetylbaccatine, and 4.10 μg/mL of taxol.

Because yew taxines are transformed in vivo into several conjugated metabolites (9,12), the patient’s urine was also submitted to acid and enzymatic hydrolysis.

After enzymatic hydrolysis with β-glucuronidase, there was a significant increase of the peaks corresponding to 3,5-dimethoxyphenol, 10-deacetylbaccatine, taxol, and an unidentified substance (Figure 2A). The concentrations of the three known metabolites increased to 8.40 μg/mL for 3,5-dimethoxyphenol, 20.8 μg/mL for 10-deacetylbaccatine, and 7.66 μg/mL for taxol, showing them to have been excreted in urine as glucuronides.

After acidic hydrolysis the increased concentrations of 3,5-dimethoxyphenol and 10-deacetylbaccatine were even more evident (Figure 2B): 50.3 and 134 μg/mL for 3,5-dimethoxyphenol and 10-deacetylbaccatine, respectively. The taxol concentration did not increase. These data indicate that these two substances are excreted largely as conjugated forms including glucuronates, glycosides, and/or sulfates. Unfortunately, the small amount of urine did not allow further investigation.

HPLC of the blood sample (not shown), revealed 5-dimethoxyphenol and taxol concentrations of 651 and 52 ng/mL, respectively. These values were very low because of the 30-h delay between the calculated time of poison ingestion and the blood sampling, but values were greater than those found in five lethal cases (6).

Conclusions

Data reported in this paper confirmed that the patient attempted suicide by ingesting of Taxus baccata leaves, as hypothesized during clinical examination and treatment. The high concentration of conjugated metabolites found in the urine underlines how critical urinary excretion is in eliminating the toxic Taxus compounds. We hypothesize that the probability of survival for patients is directly related to the intensive treatment and the efficiency with which the toxic compounds are conjugated in the liver.

References


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