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CASE REPORT

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Confirmation of oleander poisoning by HPLC/MS

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Abstract A non-fatal case of *Nerium oleander* (common oleander) self-poisoning in a 45-year-old female is presented. Initial symptoms were nausea and vomiting, abdominal pain, phosphenes, cardiovascular shock and sinus bradycardia. Blood and urine were assayed for oleandrin, the major cardiac glycoside of *N. oleander*, using a highly specific HPLC/MS procedure. The blood concentration of oleandrin at admission was 1.1 ng/ml. This is the first report of an oleander intoxication ascertained by the mass spectrometric identification of oleandrin in blood. HPLC/MS appears to be the method of choice for the forensic-toxicological investigation of poisonings by cardiac glycosides.

Key words Oleander · Oleandrin · Poisoning · Liquid chromatography · Mass spectrometry

Introduction

The common oleander (*Nerium oleander*, Apocynaceae) is an ornamental evergreen shrub, ubiquitous in temperate and subtropical areas [1, 2]. Although dismissed from modern pharmacopoeias, preparations of oleander have been used for centuries as rodenticides and insecticides, as well as folk remedies for indigestion, malaria, leprosy, mental or venereal diseases, or as abortifacients [1–4]. Oleander extracts have also been medically used in the past for cardiac insufficiency (Oleander Perpurat, Knoll). This plant is highly toxic since all parts contain several potent glycosides with digoxin-like activity, the main one (and the only one available as a pure standard) being oleandrin. Leaves from *Nerium oleander* were shown to contain 0.018 to 0.425% oleandrin (weight/wet weight).

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Oleander poisoning is clinically well-documented, and has been profusely described in humans [3–11] as well as in various animals [12, 13]. Ingestion of a single leaf has been considered potentially lethal in children [3], but in most instances larger amounts are probably required to cause severe toxicity [1]. Symptoms closely resemble those seen in digitalis poisoning, and include gastrointestinal problems (nausea, vomiting, abdominal pain), neurosensorial symptoms (headache, dizziness, blurred vision, dyschromatopsia), and cardiovascular perturbations (bradycardia, heart block, ventricular arrhythmias) which are responsible for the seriousness of the intoxication.

Oleander ingestion however remains difficult to ascertain analytically, which might be detrimental in forensic situations [14]. We present here a case of non-fatal poisoning in which liquid chromatography coupled to mass spectrometry (HPLC/MS) was employed.

Case report

A 45-year-old Caucasian female, known to be depressive, was admitted to an intensive care unit after a suicide attempt involving the ingestion of a 'salad' made of five handfuls of oleander leaves (with cheese and vinegar dressing !) together with an undetermined amount of Lysanxia (prazepam, 10-mg tablets) and Rohypnol (flunitrazepam, 1-mg tablets). Vomiting occurred several times afterwards and the vomitus was found to contain pieces of oleander leaves. On admission she was conscious but drowsy, and complained of abdominal pain and phosphenes. Initial examination revealed a cardiovascular shock (blood pressure 70 mmHg) and a sinus bradycardia (heart rate 45–60 beats/min) with no other ECG abnormalities. She was placed under continuous ECG and diuresis monitoring. A vigorous gastric lavage (45 l) was carried out as long as oleander leaves were recovered, then activated charcoal was administered orally (25 mg/6 h); digoxin-specific Fab fragments (Digidot) were not employed. The haemodynamic status and general condition rapidly improved and the ensuing 2 days were uneventful, except for a mild and transient hypokaliaemia (3.3 mEq/l) on day 2. The patient was discharged on day 3. Blood and urine were sampled for toxicology on admission, and another blood sample was taken on day 2.

Toxicological analyses

The samples were assayed for oleandrin using an adaptation of an HPLC/MS procedure originally developed in our laboratory for the

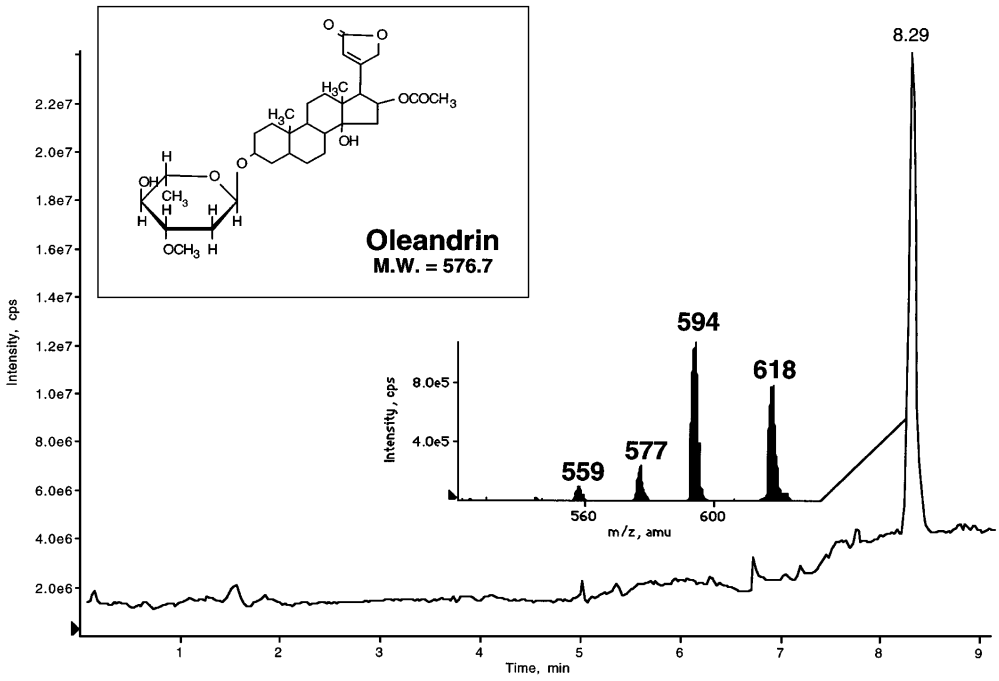

Fig. 1 TIC (*m/z* 520–630) from a blank plasma sample, spiked to contain oleandrin at 5 μg/ml. Peak at 8.29 min: oleandrin (with its typical ionspray spectrum). Inset: chemical structure of oleandrin

plasma determination of some therapeutic cardiac glycosides (digoxin, digitoxin, lanatoside C, proscillaridine, α- and β-acetyldigitoxin) [15, 16]. Briefly, blood and urine (3 ml) were extracted at pH 9.5 in a chloroform/2-propanol (95:5, v/v) mixture, using digoxin (40 ng) as the internal standard. After agitation and centrifugation, the organic phase was evaporated and the dry extract resuspended in 25 μl methanol. Separation was performed on a Waters Nova-Pak C18 column (150 × 2.0 mm, i.d.), using a gradient of acetonitrile/2 mM NH_4COOH buffer, pH 3.0 (acetonitrile, 20% at T_0 to 38% at 5.0 min; up to 65% at 6.0 min; up to 70% at 8.5 min then isocratic to 9.5 min; down to 20% at 10 min; all segments linear). Detection was done on a Perkin-Elmer API-100 mass analyser equipped with an ionspray atmospheric pressure interface. MS data were collected as either total ion chromatograms (TIC) over the mass range *m/z* 520–630 for oleandrin identification, or in single ion monitoring (SIM) at *m/z* 594 (oleandrin) and 799 (digoxin), for drug quantitation. Using these analytical settings, the average retention time for oleandrin is 8.30 min and the quantitation limit 0.4 ng/ml (Fig. 1). The extraction recovery for oleandrin was 86.8 ± 8.3% (six determinations on plasma spiked at 20 ng/ml); accuracy and precision were 4.9 and 12.7%, respectively (six determinations on plasma spiked at 20 ng/ml, using digoxin as internal standard). Oleandrin putative metabolites (glucuronosides or sulfates of oleandrigenin or gitoxigenin) were not searched for in the urine sample, since these compounds are not available.

The benzodiazepines were assayed using previously reported HPLC/DAD procedures [17].

Results and discussion

Based upon its specific fragmentation/cluster formation (generating typical ions at *m/z* 618, 594, 577, and 559), oleandrin could be identified in the blood sample taken at admission at a blood concentration of 1.1 ng/ml (Fig. 2). Both urine and the second blood sample (day 2) were negative for this compound. Nordiazepam (the circulating form of prazepam) and flunitrazepam were also present at supratherapeutic levels in the first blood sample (4.5 μg/ml and 27 ng/ml, respectively).

Toxicological measurements have been performed in only a few reports of oleander poisoning. Several authors took advantage of the fact that the cardiac glycosides from *N. oleander* partially cross-react with digoxin radioimmunoassays [1, 2, 7–11]. However the extent of this cross-reactivity has not been accurately established and is likely to change from one RIA kit to another. A positive result can therefore only indicate the presence of digoxin-related glycosides, but it is impossible to predict the degree of toxicity from the 'digoxin level' measured. In two cases of self-poisoning with *N. oleander* leaves resulting in mild symptomatology, apparent digoxin levels were 1.5 and 4.4 ng/ml at 2 and 8 h after the suicidal ingestion [10, 11]. On the contrary, an antemortem value of only 1.2 ng/ml was measured by RIA in a fatality due to the ingestion of an oleander herbal tea by a 30-year-old, healthy female [9]. In a non-fatal poisoning observed in a 13-month-old infant, an array of non-isotopic digoxin immunoassays (Abbott TDx, Roche On-Line, Baxter Stratus, Ciba-Corning ACS:180) was investigated by Jortani et al. [8]. The results also showed marked discrepancies from one assay to another, with 'digoxin levels' ranging from 0 to 1.3 ng/ml.

Chromatographic procedures have been more seldomly employed. In a fatality from oral and rectal administration of an oleander preparation, oleandrin was identified in autopsy samples by thin-layer chromatography (TLC), while separately quantified by fluorescence spectrophotometry on the dry extracts reconstituted in water/methanol [6]. A surprisingly high oleandrin concentration was measured in the post mortem blood sample (12 μg/ml), however the authors admitted that the fluorometric quantitation was of poor specificity and might have measured not only oleandrin, but also some of the closely related congeners of *N. oleander*.

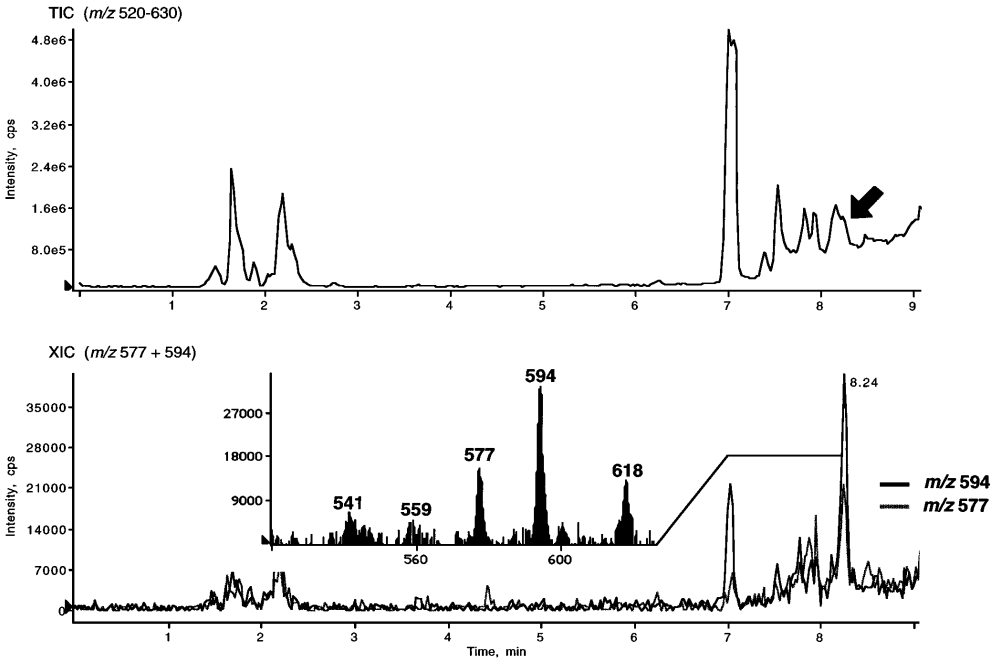

Fig. 2 Chromatogram from the patient's first blood sample. Upper graph: TIC recording (*m/z* 520–630); arrow: location of oleandrin. Lower graph: reprocessed chromatogram from the same sample, in selected ion monitoring at *m/z* 577 (gray line) and 594 (black line). Peak at 8.24 min: oleandrin, concentration 1.1 ng/ml

Whereas the thermolability and nonvolatility of cardiac glycosides precludes the use of GC/MS, HPLC/MS proved to be the method of choice for unequivocal identification of therapeutic compounds such as digoxin or digitoxin [15, 16, 18]. Likewise, this alternative approach has been previously employed to rule out the presence of oleandrin in human remains, in a death initially attributed to a criminal poisoning by *N. oleander* [14]. To our knowledge, our observation represents the first report of an oleander intoxication with conclusive identification of oleandrin in the patient's blood by mass spectrometry. This improvement may be of limited interest for emergency practitioners wishing to assess the severity of a poisoning, considering the existence in oleander of many other components, present at various concentrations and sharing oleandrin toxicity [1, 2, 19]. However HPLC/MS should be recognized as the reference method in all cases (e.g. forensic situations) requiring absolute identification of the toxin responsible for digoxin-like symptoms.

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