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CASE REPORT

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A non-fatal oleander poisoning

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Abstract The study presents a case of non-fatal poisoning with oleander blooms in a 47-year-old female, with emphasis on the importance of toxicological service in a clinical emergency. After repeated vomiting at home, the patient was admitted at the hospital with cardiac symptoms more than 18 h after the ingestion. Serum samples were assayed immunochemically for digitoxin-related compounds by electrochemiluminescent immunoassay, and using HPLC/MS/MS analysis for oleandrin, the main cardiac glycoside of *Nerium oleander*. Confirming the non-specific immunoassay results, which are often clinically over-interpreted, oleandrin was detected by HPLC/MS/MS in the serum sample in a concentration of 1.6 ng/ml upon admission. Comparison with previous reports indicates that single compound analysis only permits a toxicological assessment for oleander poisoning and results in the proposal to classify an oleandrin level between 1.0 and 2.0 ng/ml as toxic blood plasma/serum concentration.

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Introduction

Nerium oleander (Apocynaceae), an evergreen ornamental plant native only in the Mediterranean, is cultivated worldwide particularly in warm temperate and subtropical areas. Elsewhere, where the shrub is not frost-tolerant, e.g. in Central and Western Europe, it may be grown as a conservatory plant [1]. The use of oleander as a pharmaceutical product, rodenticide, and insecticide has been recognised for centuries. However, the whole plant, including its sap, is toxic containing several cardiac glycosides (e.g. oleandrin, oleandrigenin, desacetyloleandrin, glucosyloleandrin, gentiobiosyloleandrin, nerigoside, odorosides, oleasides) at various concentrations and sharing toxicity [1, 2]. A lethal dose of 5 to 15 leaves of oleandrin is described for adults [1].

Parts of the plant can be ingested accidentally or used in suicide attempts, leading to oleander poisoning, with the following symptoms: gastrointestinal signs (nausea, vomiting, abdominal pain, diarrhoea) as well as neurological (tremor, drowsiness, ataxia) and cardiovascular symptoms [sinus bradycardia, atrioventricular (AV) block, fibrillation]. Decontamination by gastric lavage and charcoal, corrections of the electrolyte imbalance and the bradycardia, as well as the administration of digoxin-specific Fab antibodies are reported as treatment principles [1, 3, 4].

Recently, methods for the identification and quantification of oleandrin in biological matrices have been described. These include immunoassays using chemiluminescent (ACS), radio labelling (RIA), and fluorescence polarisation (FPIA) as detection system [5–9], as well as thin layer chromatography (TLC) [10, 11] and high-performance liquid chromatography (HPLC) combined with derivatisation and fluorescence detection (FLD) [12, 13] and with mass spectrometry (MS) [14–17], a very sensitive and specific method for the analysis of plant toxins [18, 19].

This study presents a clinical emergency case of a non-fatal oleander poisoning, comparing the analytical results observed from electrochemiluminescent (ECL) immunoassay and HPLC/MS/MS measurements.

Case history

A 47-year-old Caucasian female, known to be depressive, was admitted to the intensive care section of a hospital by an emergency physician more than 18 h after she ingested of a bowl of *N. oleander* blooms for the purpose of suicide. Relatives reported that the subject repeatedly vomited after the ingestion. On admission she showed obvious signs of slowness and drowsiness. Her heart rate was 40 beats/min and her blood pressure was 100 mmHg. Initial electrocardiography (ECG) revealed a bradycardic sinus rhythm including AV block I° and an intermittent AV block III°. Tachycardic rhythm disturbances were not obtained. The patient was treated by oral administration of charcoal combined with sodium sulfate as well as electrolyte solutions for the correction of her potassium level. Due to the lengthy period of remote ingestion and the circulatory stability, a gastric lavage and administration of digoxin-specific Fab antibodies were not carried out. A temporary external cardiac pacemaker was attached because of the intermittent AV block III°. After showing improvements in her haemodynamic status and general condition, the patient was discharged on day 6. Venous blood and urine were sampled for toxicology upon admission. Further venous blood samples were taken 16 and 36 h after admission. Ethanol, antidepressants, barbiturates, and benzodiazepines were not obtained in the sample taken upon admission.

Material and methods

Reagents and chemicals

Purified oleandrin was provided by the Institute of Pharmacognosy, University of Vienna, Austria. Acetonitrile and methanol, both of HPLC quality, were purchased from Merck (Darmstadt, Germany). Ammonium acetate, Fractopur, and formic acid, 89–91%, GR for analysis, were also obtained from Merck (Darmstadt, Germany). Water of HPLC quality was purchased from Mallinckrodt Baker (Deventer, Holland).

Extraction of serum and urine

Preparation of serum and urine samples was performed with a Gilson Automatic Sample Processor for Solid Phase Extraction ASPEC XL and sampler software 735 (ABIMED, Langenfeldt, Germany). The frozen samples (−30°C) were thawed at room temperature and mixed. Volumes of 1.0 ml serum or urine were mixed with 1.0 ml formic acid (9%) and extracted using SPE cartridges OASIS-HLB from Waters (Milford MA, USA) according to the following programme conditioning: 1.0 ml methanol, 0.3 ml air, 0.9 ml water, 0.3 ml air; loading: 2.0 ml sample, 0.8 ml air; washing: 1.2 ml water, 2.0 ml air; elution 1.0 ml methanol,

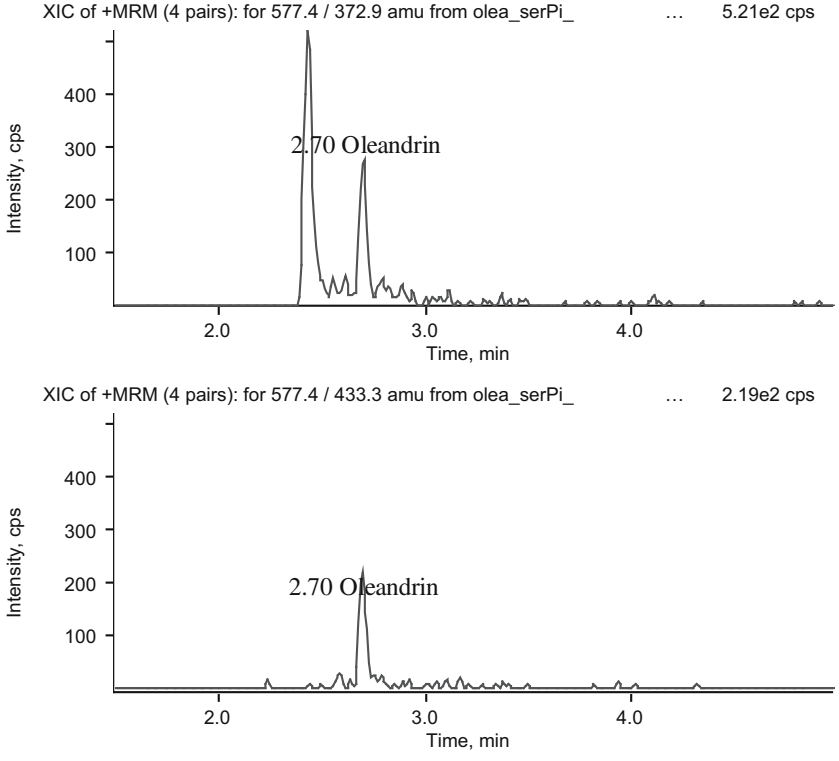

Fig. 1 HPLC/MS/MS chromatogram of a blank serum sample spiked with 2.0 ng/ml oleandrin. *Top*: specific transition *m*/*z* 577.4–372.9, *bottom*: qualifier transition *m*/*z* 577.4–433.3

Table 1 Results of the immunochemical and HPLC/MS/MS analysis of serum and urine samples

	Sample description	ECL immunoassay digitoxin equiv. (ng/ml)	HPLC/MS/MS oleandrin (ng/ml)
Serum	On admission	80.0	1.6
Urine	On admission	n.d.	1.2
Serum	16 h after admission	21.3	<1.0
Serum	36 h after admission	6.6	<1.0

equiv. Equivalents, *n.d.* not determined

0.3 ml air. The methanol eluates from SPE were evaporated to dryness in a stream of air, redissolved in 100 µl of mobile phase and 10 µl were injected for HPLC/MS/MS.

HPLC conditions and detector settings

A Perkin Elmer (Norwalk CT, USA) series 200 HPLC system including autosampler and binary pump was interfaced to an Applied Biosystems (Concord ON, Canada) HPLC/MS/MS system API 3000 with turbo ion spray interface (ESI+). The chromatographic separation was performed on a Synergy 4 µ Polar-RP 80A (150 mm×2 mm, i.d.) column with a Security Guard C_{18} (4 mm×2 mm, i.d.) both purchased from Phenomenex (Aschaffenburg, Germany). Gradient elution was applied at a constant flow of 0.5 ml/min using solvent A (5/95/0.2, v/v/v) and solvent B (95/5/0.2, v/v/v), each being mixtures of acetonitrile, ammonium acetate (2 mM), and formic acid. Initial conditions were 100% solvent A for 0.1 min, increasing to 100% solvent B at 1 min and hold for 2.5 min.

Full scan mass spectrum was acquired by continuous infusion of an oleandrin standard solution. The product ion mass spectra were obtained by choosing the molecular ion or the ammonium adduct as precursor ions, scanning Q2 in the range of *m*/*z* 100–650 with nitrogen as collision gas and a capillary voltage of +5,000 V. Oleandrin was measured using the multiple reaction monitoring (MRM) mode with the specific transition between *m*/*z* 577.4 (parent ion) and 372.9. Transitions between *m*/*z* 577.4 and 433.3, as well as between *m*/*z* 594.4 and 577.4 were used as qualifiers. Temperature in the turbo ion spray source was 400°C at a gas flow of 8 ml/min (N_2).

Using these analytical settings, the absolute limit of detection of oleandrin (retention time 2.6 min) was 10 pg. Experiments with oleandrin-spiked serum (20 ng/ml, n=6) resulted in SPE recoveries of ca. 62±6%. The limit of quantitation of oleandrin, determined by an external standard calibration using blank serum and urine samples as biological matrix, was calculated according to a German standard procedure (DIN 32645) with 1.0 ng/ml (serum, three samples of each concentration, r^2=0.9998, Fig. 1) and 1.2 ng/ml (urine, three samples of each concentration, r^2= 0.9999), respectively, linear in the range of 1–50 ng/ml. No matrix effects affecting the determination of oleandrin were observed.

Immunochemical analysis of oleandrin was carried out by using an electrochemiluminescent (ECL) immunoassay for digitoxin on a Modular Analytics E 170 system (Roche Diagnostics, Mannheim, Germany).

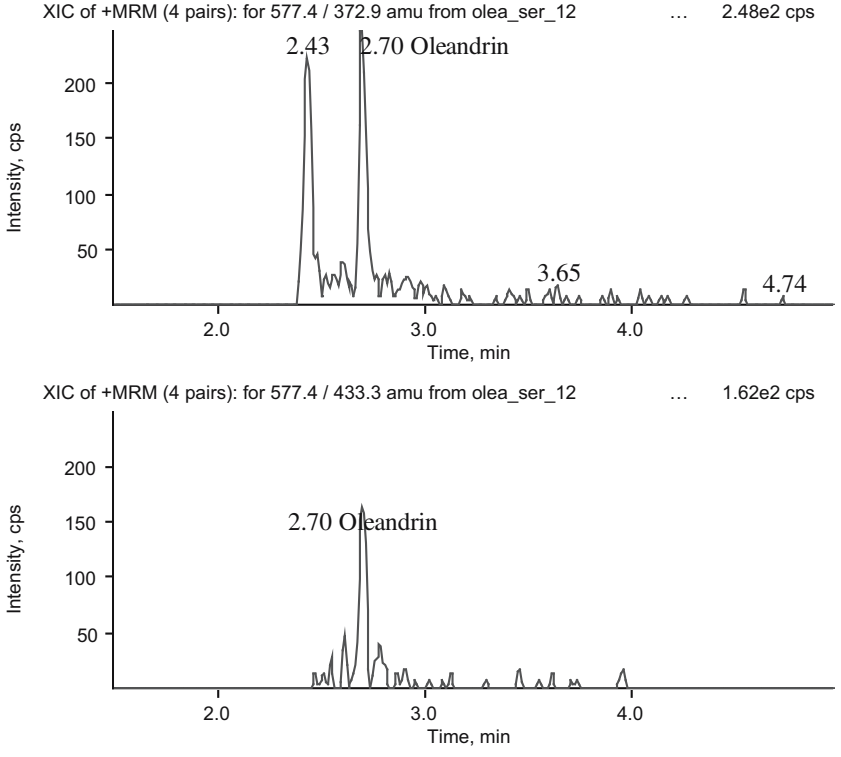

Fig. 2 HPLC/MS/MS chromatogram of the serum sample taken on admission (oleandrin 1.6 ng/ml). *Top*: specific transition *m*/*z* 577.4–372.9, *bottom*: qualifier transition *m*/*z* 577.4–433.3

Results and discussion

The results of the immunochemical (ECL immunoassay) and HPLC/MS/MS analysis of serum and urine samples are summarised in Table 1.

Obviously, the digitoxin equivalent determined immunochemically in the serum sample taken on admission significantly exceeded the common therapeutic range (10–25 ng/ml), but indicated the presence of digitoxin-related glycosides, only. HPLC/MS/MS analysis of the same serum sample identified oleandrin by specific mass transition (Fig. 2) and quantified the compound in a concentration of 1.6 ng/ml by external calibration.

From these results, an oleandrin cross-reactivity of ca. 5,000% can be derived indicating that the utilised digitoxin ECL immunoassay is unsuitable for toxicological oleandrin analysis.

In the urine sample taken on admission, oleandrin was also identified by HPLC/MS/MS (Table 1). The concentrations measured in the serum samples taken 16 and 36 h after admission were in the therapeutic range (immunoassay) as well as below the quantitation limit for oleandrin (HPLC/MS/MS, Table 1).

Summarising these results, the mild cardiac symptomatology could be confirmed analytically by HPLC/MS/MS, despite the long period that elapsed between oleander self-poisoning and admission at hospital and the repeated vomiting after ingestion, respectively.

To date, only few studies of oleander poisoning in humans have been published [8–10, 15, 17]. However, due to discrepancies in digitoxin/digoxin immunoassay results [7], the toxicological data reported are quite inhomogeneous and it appears that only the identification of oleandrin by chromatographic procedures permits a conclusion for the degree of toxicity. For example, in two cases of oleander poisoning in adults, comparable digoxin levels of 1.2 [8] and 1.5 ng/ml [9], respectively, in the blood samples were obtained; in these cases the immunoassays used yielded completely different results—mild cardiac symptoms [8] and death [9]. In contrast, well-defined toxicological data were published by Tracqui et al. [15], who reported 1.1 ng/ml oleandrin (venous blood) in a non-fatal case that resulted in mild cardiac symptomatology, and by Arao et al. [17], who reported 9.8 ng/ml oleandrin (heart blood) in a fatal poisoning.

The presented non-fatal case confirms the report of Tracqui et al. [15], both in the mild cardiac symptoms and the level of oleandrin measured in femoral blood. In conclusion, we propose to classify an oleandrin level between 1.0 and 2.0 ng/ml as toxic blood plasma/serum concentration. In contrast, the study of Wang et al. [16] reported an oleandrin plasma concentration of 7 ng/ml for a volunteer 3 h after an intramuscular dose of 15 mg oleander extract for cancer treatment. This value is remarkably high compared to this case as well as other case reports [15, 17], and can be explained only by an acquired tolerance of the volunteer.

Conclusion

The presented study of a non-fatal oleander poisoning underlines the importance of toxicological service in the case of clinical emergency. Comparison of immunochemical and single compound analysis by HPLC/MS/MS shows the inevitability of the clinical over-interpretation of non-specific immunoassay results of cardiac glycosides due to missing data of cross-reactivity. Single compound analysis only permits a toxicological assessment, resulting in adequate therapeutic concepts. Nevertheless, the ECL immunoassay seems to be applicable for a rapid screening of cardiac glycosides as well as for a time- and cost-effective monitoring of the therapy follow-up following the identification of the active agent.

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