Unexpectedly Dangerous Escargot Stew: Oleandrin Poisoning through the Alimentary Chain*

Cecilia Gechtman[1,†], Federico Guidugli[2], Alessandro Marocchi[1], Adriano Masarin[1], and Francesco Zoppi[1]

[1]Dipartimento di Medicina di Laboratorio, AO Ospedale Niguarda Cà Granda, Milano, Italy and [2]Thermo Electron Corporation, Rodano, Milano, Italy

Abstract

A female, aged 43 and a male, aged 66, experienced gastrointestinal and cardiovascular symptoms after a meal including snail stew. Twelve hours after the ingestion, they presented with nausea, vomiting, diarrhea, and cardiovascular symptoms typical of acute toxic digoxin ingestion and were hospitalized. The man's electrocardiogram was altered, and the woman's was normal. Serum digoxin levels, measured on a Roche COBAS Integra 800 with the Roche On-Line Digoxin reagent, were 1.14 and 1.00 nmol/L, respectively. Potassium levels were normal in both patients. The serum digoxin concentration decreased on the second day, and symptoms resolved on the third day with patients fully recovered (i.e., reversion to a normal sinus rhythm). Cardiac-glycoside-like intoxication symptoms follow the ingestion of leaves or flowers of *Nerium oleander*. The consumed snails were suspected to be responsible for the intoxication. In the homogenized snail tissue, the concentration expressed in digoxin equivalents was 0.282 nmol/g. The presence of oleandrin and oleandrigenin in the snails was confirmed by liquid chromatography–tandem mass spectrometry analysis, which was performed on a ionic-trap Finnigan LXQ instrument using an electrospray ionization interface. High-pressure liquid chromatographic separation was performed on a C18 column with a gradient of methanol/water. An extract of oleander leaves was used as reference.

Introduction

Nerium oleander, an evergreen flowering shrub belonging to the Dogbane family Apocynaceae, contains many bioctive constituents (1). They exhibit a wide range of activity, including insecticidal, cardiotonic, and antimitotic. This last characteristic has been recently exploited to produce a drug, Anvirzel™ (2). This is an extract of the plant, toxic, with an IC_{50} of 4.0 ng/mL, to human BRO melanoma cell lines by inducing a block in the G2/M phase of the cell cycle. Anecdotal reports from Europe of partial and complete remissions and symptomatic improvement in patients with advanced malignancy prompted formal evaluation of this agent (3). Some authors concluded that Anvirzel can be safely administered in doses up to 1.2 mL/m^2/d, with no dose limiting toxicity.

But the cardiotonic property, which is due to cardiac glycosides, which are of particular importance for the oleander toxicity, is more often studied (1,4–11) because of the cardic glycosides' ability to exert pharmacological effects on the cardiac muscle. The toxic constituents of oleander that have cardiotonic effects include oleandrin, folinerin, and digitoxigenin. Absolute and relative concentrations of these substances vary with the part of the plant and the season in which it is examined.

These molecules have a quite similar structure which is typically constituted by a lactone ring bound to a steroid backbone, the latter identified as "–genin" or "aglycone", which, in turn, is bound to a monosaccharidic or polysaccharidic moiety through a glycosidic link. The same structure and action is shared with foxglove cardiovascular glycosides. Cardiovascular glycoside-like intoxication following ingestion of *Digitalis purpurea* is a common clinical problem (12–16). Moreover, the therapeutic interval of digoxin and digitoxin—the most important cardiovascular glycosides present in foxglove and used as therapeutic drugs—is narrow, and an overlap of toxicity-pharmacological effect is common.

Because of the huge diffusion of oleander as an ornamental shrub, the ingestion of and intoxication from oleander in both humans and animals is common. Fortunately, mortality attributable to such incidents is low.

Modes of exposure to the plants include accidental ingestion as well as intentional administration in foods and drinks prepared from the leaves, but no report about poisoning through the alimentary chain has been published. We present such a case that was due to the ingestion of snails that had fed on plants containing cardiac glycosides.

Case History

Two subjects (a female aged 43 and a male aged 66), about

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† Author to whom correspondence should be addressed. E-mail: ceciia.gechtman@ospedaleniguarda.it.

8–10 h after a meal, which included an undefined amount of escargot (snail of the species *Helix pomatia*) stew, presented with gastrointestinal and cardiovascular symptoms. The subjects were promptly hospitalized, and cardiovascular symptoms similar to those observed after acute digoxin toxic administration were recognized. At the time of admission, about 10 h after ingestion, the patients presented with nausea, vomiting, and diarrhea. The man experienced electrocardiogram (ECG) changes, ectopic beats, and bradycardia (36 bpm), and the woman's ECG was normal. By the third day, the patients recovered fully with reversion to a normal sinus rhythm on the third day.

Materials and Methods

Raw materials

About 0.5 kg of frozen snails, collected by the patients, was brought to the laboratory. One-hundred grams of oleander leaves was collected from the patients' garden.

Solvents

All the solvents used were high-performance liquid chromatography (HPLC) grade and were purchased from Carlo Erba Reagenti (Rodano, Milano, Italy).

Automated aspecific measurements

The digoxin measurement in the different matrices used were performed on a COBAS Integra 800 automated biochemistry analyzer, with COBAS Integra Digoxin (Product No. 20737836, and system No. 07 3783 6) as a reagent (Roche Diagnostics, Mannheim, Germany). This reagent belongs to the Roche On-Line reagents family that exploit the KIMS technique. Its cross-reactivity versus oleandrin and oleandrigenin has been characterized by Jortani et al. (7).

Specific measurements

We used a Finnigan LXQ instrument coupled with a Surveyor MS pump and a Surveyor autosampler (Thermo Electron Corp., Walthman, MA). Separation was performed on a Waters (Milford, MA) XTerra C18 column (4.6 × 250 mm, 5 µ) using a gradient of methanol/water (methanol, 10% at T_0 to 95% at 10 min, then isocratic to 15 min; down to 10% at 17 min), pumped at a flow rate of 1000 µL/min. Detection was done using an electrospray ionization (ESI) interface.

Other machines

We used to disrupt the snails responsible of the intoxication and oleander leaves a Micro-Dismembrator II (B. Braun Biotech, Allentown, PA), operating with frozen (in liquid nitrogen) tissues. 500 mg of the pulverized material was extracted with 5 mL of methanol and/or in acetonitrile. The organic solutions were used in the LC–MS without further dilution. Aliquots of these solutions were diluted with saline (NaCl 0.9 g/L) to provide samples adequate for the immunochemical determination on the Integra.

Results and Discussion

At the admission, both patients presented cardiovascular symptoms similar to those observed after acute digoxin toxic administration. However, the same symptoms are present if leaves or flowers of *Nerium oleander* are ingested. The suspicion arose that the snails they had eaten were responsible for the intoxication. An exhaustive search of the most consulted medical databases—PubMed (17), Embase (18), and Scopus (19)—and chemical database—Scirus (20)—together with a search performed in Toxnet (4), did not retrieve any answer. The only two sites that correctly combined such terms as "oleander, digitalis, snail, toxicity" were an Italian blog (21)[1] and a note by Daniel F. Culbert, Indian River County Extension Agent, University of Florida, dated 23 August 2000 (22). From the last article, this phrase is extracted: "*...one suggestion sometimes made for management is to collect these snails and use them for a favourite French delicacy—escargot. The problem with using locally collected snails in this way is that they may have fed on poisonous plants such as oleander, and may lack the level of sanitation necessary for proper human consumption.*"

Therefore, the initial hypothesis was oleander intoxication because the patients had found the snails near the *Nerium oleander* in their garden.

Many papers have been published (7,8,11) about the possibility of determining the presence of oleandrin in the patient's serum or urine with an immunoassay because of the quite common cross-reactivity of this substance with digoxin in nearly all the available commercial kits.

The serum digoxin levels measured with the Roche kit for Digoxin on the Roche COBAS Integra 800 were within the therapeutic range for patients who regularly ingest digoxin.

The man's serum had a concentration of 1.14 nmol/L and that of the woman was 1.00 nmol/L (with a laboratory therapeutic range of 0.64–2.56 nmol/L). The urine digoxin concentrations were 0.37 and 0.95 nmol/L, respectively. The potassium levels were normal in both patients (4.8 mmol/L).

On the second day the serum digoxin concentration was 0.39 nmol/L in the man and 0.37 nmol/L in the woman, and those in urine were 0.94 and 0.88 nmol/L, respectively. No further therapy was performed. The symptoms resolved on the third day, with a serum digoxin of 0.37 nmol/L in both patients (urine concentrations were 1.97 and 1.07 nmol/L).

The leftover snails were brought to the laboratory. The digoxin concentration in the homogenized and methanol-extracted snail tissue was determined, after a proper dilution and with the same kit and machine used for sera and urine, to be 0.280 nmol/g.

Nevertheless, another hypothesis was put forward when a consulted entomologist declared that the *Nerium oleander* leaves are too hard to be eaten by snails. Therefore, it was sup-

[1] English translation of the blog entry is as follows: "Digitalis poisoning. In this last case, the poisoning came about in a rather strange way (see medical casuistry), that is, by ingesting snails (escargots) which had previously fed on oleander (rosebay) leaves. This plant is particularly rich in the bioactive molecules of digitalis. I agree that this case may seem absurd, but it must be kept in mind that 1. it is absolutely true (see also also R1) and 2. cases of digitalis poisoning after eating escargots are very frequent, especially in Brittany (France) where this dish is very common fare."

posed that snails could be fostered with foxglove leaves, also present in the patients' garden. In a recent paper (24), however, one of us (FZ) observed that healthy people treated with digoxin did not present any cardiac or gastrointestinal symptoms at levels of serum digoxin within the therapeutic range. Hence, we continued to follow the oleandrin hypothesis. In fact, peasant wisdom and experience suggest that snails generally cling to the shrub twigs, but eat the surrounding grass. The snails are contaminated by the plant juice adsorbed via their own slime.

The research of an oleandrin standard in many published or electronic catalogues did not give any result, even if all the authors who dealt with oleandrin determination (7,24) indicate Sigma as the provider of this substance. Hence to validate the MS–MS method we were compelled to resort to natural products, that is, oleander leaves.

The only available figures that could give a measure of oleandrin concentrations were those of the measured digoxin equivalent and those of the measured cross-reactivity of oleandrin in the kit we used (7). In Table I, we report the measured digoxin equivalents and the corresponding values of oleandrin and, if the case, of oleandrigenin concentration calculated on the basis of a linear behavior of their cross-reactivity (7) within a wide concentration interval. This value is 2.37% for oleandrin and 4.94% for oleandrigenin.

The recalculated concentrations (Table I) are extremely high, and only the low toxicity of oleandrin justifies the patients' survivals.

Toxnet (5) reports high values of LD in cat (LD50 = 0.43 µmol/kg, intravenous; LDLo = 0.42 µmol/kg, unreported route) and even higher in frog (LDLo = 3.90 µmol/kg, subcutaneous). Nevertheless, the data in Table I are astonishing, so we looked for confirmation of the oleandrin hypothesis and analyzed the snail tissue methanolic-extract on our LC–MS system and compared it with an oleander leaf extract in acetonitrile (25).

Our MS data were collected both as total ion chromatograms (TIC) in the mass range (*m/z*) 400–850 and in MS^2 full scan of the *m/z* 599.4 (Na^+ adduct of oleandrin) and 433.3 (oleandrigenin + H^+).

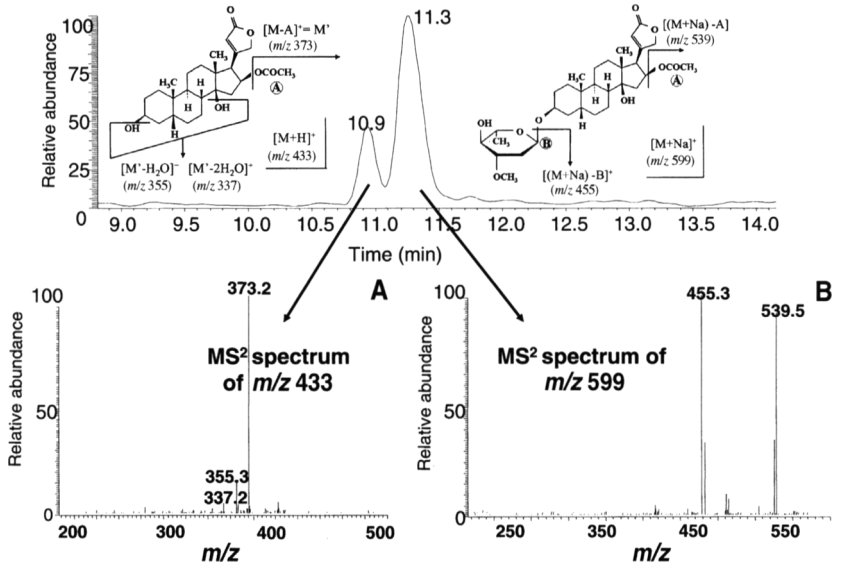

Figure 1. The upper part of the figure shows the full-scan chromatogram for the mass (*m/z*) range 400–850, together with the proposed fragmentation mechanism of oleandrigenin (left) and that of oleandrin (right). Retention times of oleandrigenin and oleandrin were 10.9 and 11.30 min, respectively. The lower part of the figure shows the MS^2 full scan of the *m/z* 599.4 (Na^+ adduct of oleandrin) (A) and the MS^2 full scan of the *m/z* 433.3 (oleandrigenin + H^+) (B).

Table I. Concentration in Sera and in Pulverized Snail Tissue Expressed as Digoxin-Equivalent and as Oleandrin or Oleandrigenin, Calculated from a Cross-Reactivity Value, versus Digoxin, of 2.37% for Oleandrin and 4.94% for Oleandrigenin (7)

	Man		Woman	
Approximate Time from Ingestion (h)	Digoxin-equivalent concentration (nmol/L)	Calculated oleandrin concentration (nmol/L)	Digoxin-equivalent concentration (nmol/L)	Calculated oleandrin concentration (nmol/L)
12	1.14	48	1.00	42
24	0.39	16	0.37	15
36	0.37	15	0.37	15

Disrupted snail tissue concentration	
Digoxin-equivalent concentration (nmol/g)	Calculated—on the basis of relative LC–MS spectrum area—Oleandrin concentration (nmol/g)
0.282	8.3
	Calculated—on the basis of relative LC–MS spectrum area—Oleandrigenin concentration (nmol/g)
	1.7

The retention time of oleandrigenin was 10.9 min, and that of oleandrin was 11.30 min.

Figure 1, in its upper and lower parts, shows all of the LC–MS–MS data obtained in our experimental conditions on both leaves and snails extracts

For both oleandrigenin and oleandrin, the two extracts produced superimposable chromatograms (Figure 1, upper part) and mass spectra (Figure 1A and 1B, lower part). The relative peaks' area is 30% oleandrigenin versus 70% oleandrin. Moreover, our mass spectra fit with those obtained by some authors (3,25), but differ from those shown in other papers (24,26–28) and were obtained with different instrumentation. The interpretation of our MS–MS peaks is shown in the upper part of Figure 1: a congruent comparison can be made with the full-scan CID mass spectra published by Rule et al. (25), where the derived ions of the protonated molecules are presented.

Hence, we believe that the case solution is unambiguous.

To our knowledge, this is the first case report about indirect cardiac glycosides intoxication, which involves poisoned food. The snails may acquire toxicity by absorbing toxic compounds from their surroundings, and the toxins may enter the alimentary chain and effect acute human poisoning.

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