Intoxication by *Cortinarius orellanus*: Detection and assay of orellanine in biological fluids and renal biopsies

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Abstract

A woman suffering from acute tubulo-interstitial nephritis was admitted to the hospital ten days after deliberate intoxication by ingestion of *Cortinarius orellanus*. Orellanine, the main toxin responsible for orellanine poisoning, was detected in biological fluids and renal biopsies. It was assayed by direct spectrofluorimetry on two-dimensional thin-layer chromatograms after specific photodecomposition into orelline. The orellanine concentration was 6.12 mg/l in the plasma (10 days after ingestion). Orellanine levels in renal biopsies were 7 µg per 25 mm² of the first biopsy (13 days after ingestion) and 24 µg per 8 mm² of the second biopsy (6 months later).

Introduction

Many cases of orellanine poisoning were described after several fatal intoxications in Europe. They were attributed to *Cortinarius orellanus* (Fr.) Fr. [5, 8, 10, 17, 18, 19, 32, 33] and *Cortinarius speciosissimus* Kühn & Romagn. [6, 9, 13, 14, 20, 22, 29, 30, 31, 38, 39]. Following the isolation of orellanine by Grzymala [11], Antkowiak & Gessner [4] reported a tetra-hydroxy bipyridine, N,N-dioxide structure for this molecule. This was confirmed by synthesis [7, 34].

We recently described [2, 26] the first case of deliberate intoxication by *C. orellanus*. This report was confirmed by assaying orellanine in the plasma and two renal biopsies of a patient after specific photodecomposition into a non-toxic metabolite called orelline [1, 3]. The originality of our work is primarily the reporting of the experimental conditions of detection and assay of orellanine in biological tissues [2, 26].

Materials and methods

Case report

A 31-year-old female psychiatric patient was brought to hospital ten days after deliberate ingestion of two fruit-bodies of *C. orellanus* (Novem-
ber, 1987). She experienced severe thirst, nausea and abdominal pains, and vomiting respectively eight and nine days after ingestion. On day 10 after ingestion, the woman had a high plasma creatinine level (1100 µmol/l) and a severe renal failure. She was afebrile and anicteric (SGPT: 5 UI/l; SGOT: 12 UI/l; LDH: 370 UI/l). Haemodialysis with femoral catheters (500 ml/min for 3 hours) and perfusion with furosemide (Lasilix), diltiazem (Tildiem), dopamine and vitamin C were started immediately on the night of admission. The treatment was repeated on the following five days (except for Tildiem up to nine days). On days 11 to 13 and 17 to 19 after ingestion (Table 1), haemodialysis was performed with Amberlite XAD-7 resin (ref. DHP-1, Kurakay Co., Japan and ref. Hemo France, France). On day 16, the patient’s plasma was exchanged for 3% albumin in saline combined with a haemoperfusion through Amberlite XAD resin for 90 min. Parenteral 30% glucose solution (60 ml), isotonic bicarbonate solution (2 x 500 ml) and Calciparine were administrated continuously during perfusion. On days 11 to 19 after ingestion, the patient was perfused with an amino acid mixture (Protinutril, Roger Bellon Laboratories, France) (500 ml/24 h) and given another amino acid mixture in the form of four tablets three times a day (Ketosteril, Fresenius Laboratories, FGR). Renal biopsies were performed on days 13 and 180 after ingestion.

Histologic failures were those of a marked tubular necrosis with scattered infiltration of lymphocytes and interstitial oedema and fibrosis. Plasma creatinine decreased to 350 µmol/l and clearance creatinine remained at about 10 to 20 ml/min for the next days. On day 27 after ingestion, the woman returned home with a sufficient renal function to maintain life without periodic dialysis (plasma creatinine: 383 µmol/l; uraemia: 24 mmol/l; red corpuscles: 2.81 x 10⁹/mm³; hemoglobin: 8 g/100 ml; hematocrit: 24¥%). Four months after ingestion, plasma creatinine (240 µmol/l) and uraemia (11.1 mmol/l) decreased and urinary excretion of proteins remained at about 0.44 g/24 h. Six months after ingestion, blood analysis gave the following results: plasma creatinine: 209 µmol/l; uraemia: 11.5 mmol/l; red corpuscles: 3.94 x 10⁹/mm³; hemoglobin: 10.9 g/100 ml; hematocrit: 32.5%; urinary creatinine: 4.1 mmol/l; clearance: 0.4541 ml/s.

Table 1. Chronology of biological samples.

<table>
<thead>
<tr>
<th>Days*</th>
<th>Plasma b</th>
<th>Urine</th>
<th>Renal biopsy</th>
<th>Haemodialysis fluid</th>
<th>Haemodialysis resin</th>
<th>Plasmapheresis resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>P10B +</td>
<td>-</td>
<td>-</td>
<td>HF10</td>
<td>HR10 +</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>P11B +</td>
<td>-</td>
<td>-</td>
<td>HF11</td>
<td>HR11 +</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>P11A +</td>
<td>-</td>
<td>-</td>
<td>HF12 +</td>
<td>HR12 +</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>P12B +</td>
<td>-</td>
<td>-</td>
<td>HF13 +</td>
<td>HR13 +</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>P12A +</td>
<td>-</td>
<td>-</td>
<td>RU13 +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
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<tr>
<td>16</td>
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<td>PR16 +</td>
</tr>
<tr>
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<tr>
<td>180</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>RB180 +</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Days after ingestion.

b A: after haemodialysis, B: before haemodialysis.

+ Analyzed samples.

- No sample.
Purification of orellanine in biological fluids and renal biopsies

Orellanine and its photodecomposition metabolites were purified by passage through a 22 × 200 mm column containing Amberlite XAD-4 resin (Rohn & Haas, ref. 2-6007) adjusted to pH 2 with hydrochloric acid. Elution was carried out by successive passages of following solvents: water pH 2 with hydrochloric acid, methanol-water (1:1) and methanol. The fractions were evaporated in vacuo to known volumes (about 0.5 ml).

Plasma samples (P10B to P13A) were loaded directly on the column. Urine samples (U15 and U16) and haemodialysis fluid samples (HF12 and HF13) were evaporated in a rotary vacuum evaporator to 5 ml before Amberlite XAD-4 resin treatment. Haemodialysis resin samples HR10 to HR13 and plasmapheresis resin sample PR16 were washed with water (30%, w/v). Orellanine was extracted by maceration of washed resin in methanol (12%, w/v) several times for periods of 15 min under magnetic agitation at room temperature. The methanolic extracts were evaporated in vacuo to 2 ml. Tissue biopsies (RB13 and RB180) were homogenized with 1 ml of methanol-water (1:1) in a Potter homogenizer and then sonicated for 20 min at room temperature. The hydromethanolic extract was centrifuged at 3000 g for 30 min. The supernatant was evaporated in vacuo to 0.2 ml. Because of the instability of orellanine in light, this study was carried out in the dark [1, 15, 25].

Detection and assay of orellanine

Orellanine was revealed by two-dimensional tlc on cellulose (Merck, ref 5716) developed in the following solvent: n-butanol-hydrochloric acid-chloroform-water (40:20:15:3.8). The assay used the specific photodecomposition of orellanine into oreline under UV light (366 nm) for 90 min through a quartz cell in a liquid environment. Fluorescence intensity of oreline was determined with a Farrand UV-Vis-2 chromatographic analyzer (Optical, New York, USA) by direct spectrofluorimetry on two-dimensional chromatograms (10 ng detection limit on chromatograms). We compared 5 or 10 µl of each extract, previously exposed to UV light, with an oreline solution prepared with an orellanine standard solution (0.002% in methanol-water 1:1), irradiated with UV light in the same conditions (Fig. 1).

Results and discussion

Many cases of orellanine poisoning have been described in various European countries since 1952. Two cases histories reported by Marichal [18, 19] and Brousse [5] illustrated such poisoning in France. In September 1987, the failure to identify Cortinarius mushrooms led a collective poisoning by C. orellanoides of 26 soldiers in survival training [23].

The primary originality of our work is firstly the reporting of the first case of deliberate orellanine poisoning with accurate knowledge of the name of the Cortinarius species, the amount of mushroom eaten and the date of ingestion. This is very important because the clinical and histological aspects of orellanine poisoning are notable by virtue of the long incubation period (10 days). They were characterized in particular by a predominantly renal involvement (plasma creatinine: 1100 µmol/l) with tubulo-interstitial failures, infiltration of lymphocytes and subsequent development of fibrotic tissue. These findings are the same as those described in animal tests [16, 24]. No glomerular damage such as that reported in the literature [5, 10, 13, 17, 18, 19] was noted. Liver function tests were normal [5, 6, 19, 22].

The tubular damage with oliguric nephritis necessitated symptomatic early treatment with haemodialysis-haemoperfusion combined with plasmapheresis and perfusion with furosemide, dopamine and vitamin C for five days. The patient was also treated with amino acid perfusion and per os mixture with good results with regard to kidney preservation. According to Toback [35], amino acid perfusion acts directly on the kidneys to increase the synthesis of phospholipids
Fig. 1. Two-dimensional TLC on cellulose for orellanine assay in biological tissues. a: before photodecomposition; b: after photodecomposition; I: first development; II: second development; RB13: renal biopsy on day 13 after ingestion of *C. orellanus.*
containing choline for new membranes in regenerating cells and also to decrease the level of renal functional insufficiency after tubular necrosis. The vasodilatation effects of parenteral Tildiem have been used as theoretical protective treatment in acute renal failures [36, 37]. With respect to restoration of renal function, treatment by amino acids combined with Tildiem, haemodialysis and plasmapheresis-haemoperfusion combination might have been beneficial for the patient. After that, plasma creatinine and uraemia declined from 383 to 209 \( \mu \text{mol/l} \) and from 24 to 11.5 \( \text{mmol/l} \) respectively for the six following months. Renal biopsy RB180 (six months after ingestion) showed tubular recovery but with intensive, evolutive interstitial fibrosis.

We were also able to detect and assay orel lanine, the main toxin responsible for orel lanine poisoning. The ability of Amberlite XAD resin (type 2, 4 or 7) to remove e.g. psychotropic drugs [12, 21] and phenolic type compounds [2, 26, 27, 28] from aqueous solutions led us to purify orel lanine from biological fluids and renal biopsies with exclusion chromatography carried out on Amberlite XAD-4 resin. On account of its specific macroreticular structure and its very high surface area, Amberlite XAD-4 resin has a greater adsorption capacity for compounds with low molecular weights (MW < 500) i.e. orel lanine than Amberlite XAD-2 or XAD-7 resins. According to Rosler & Goodwin [28], the initial acidification on the resin and the wash water-HCl may indirectly improve the capacity of the resin. Large molecules (i.e. creatinine) were excluded from the pore structure of the gel with water pH2 and passed straight through the column. On the other hand, small molecules (i.e. orel lanine and its compounds of photodecomposition) were eluted with methanol-water (1:1) and methanol.

Orel lanine was only partially separated by the mobile phase when a one-dimensional thin-layer chromatographic technique was used. We therefore employed a two-dimensional procedure in the same solvent for the present investigation. The identity of orel lanine was confirmed by its specific photodecomposition into orel line under UV light. The identity of orel lanine was confirmed by its fluorescence spectrum in the chromatogram spot (excitation wavelength: 399 nm; emission wavelength: 447 nm).

This procedure led us to detect and assay orel lanine (6.12 \( \mu \text{g/l} = 0.02 \text{mmol/l} \)) in plasma P10B (obtained before first haemodialysis). We did not detect orel lanine and its photodecomposition products in other plasma or in urine, haemodialysis fluid or haemodialysis and plasmapheresis resin samples. On the other hand, we detected high amounts of orel lanine and orel line and small amounts of its photodecomposition compounds in renal biopsy RB13 (13 days after ingestion). Orel lanine level was 7 \( \mu \text{g} \) (= 0.02 \( \mu \text{mol} \)) per 25 mm\(^3\) of first renal biopsy. Renal biopsy RB180 (six months after ingestion) contained mainly orel line; its amount was equivalent to 24 \( \mu \text{g} \) (= 0.09 \( \mu \text{mol} \)) of orel lanine per 8 mm\(^3\) of the second renal biopsy. Haemodialysis had thus cleared circulating toxin from the blood but had not been able to remove toxin bound to renal cells.

The characterization of orel lanine in plasma obtained ten days after ingestion and the detection of orel line in renal biopsy six months later proved a particularly slow release of orel lanine and its photodecomposition compounds from blood and renal cells. These metabolites may play a significant role in orel lanine poisoning and might form covalent bonds with glutathione and with numerous proteins causing kidney disturbance. These findings support our hypothesis of the phototoxicity of orel lanine [1]. The originality of our work is primarily related to the experimental conditions which made it possible to detect and assay orel lanine in complex biological fluids and renal biopsies for the first time. Using this easy analytical procedure, it is now possible to assay this toxin anywhere in the body and to make an accurate diagnosis of orel lanine intoxication.

References

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