Toxicon xxx (2015) 1–5

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Clinical report

Co-ingestion of amatoxins and isoxazoles-containing mushrooms and successful treatment: A case report

Juliana Garcia [a, *], Vera M. Costa [a], Ana Elisa Costa [b], Sérgio Andrade [c], Ana Cristina Carneiro [c], Filipe Conceição [c], José Artur Paiva [b], Paula Guedes de Pinho [a], Paula Baptista [d], Maria de Lourdes Bastos [a], Félix Carvalho [a, *]

[a] UCIBIO-REQUIMTE/Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua José Viterbo Ferreira nº 228, 4050-313 Porto, Portugal
[b] Internal Medicine Service, São João Hospital Center, 4200-319 Porto, Portugal
[c] Intermediate Care Unit of Emergency Service, São João Hospital Center, 4200-319 Porto, Portugal
[d] CIMO/School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-854 Bragança, Portugal

ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form
29 May 2015
Accepted 8 June 2015
Available online xxx

Keywords:
Wild mushrooms
Amanita pantherina
Amanita phalloides
Isoxazoles
Amatoxins
Intoxication

ABSTRACT

Mushrooms poisoning occurs when ingestion of wild mushrooms containing toxins takes place, placing the consumers at life-threatening risk. In the present case report, an unusual multiple poisoning with isoxazoles- and amatoxins-containing mushrooms in a context of altered mental state and poorly controlled hypertension is presented. A 68-year-old female was presented to São João hospital (Portugal) with complaints of extreme dizziness, hallucinations, vertigo and imbalance, 3 h after consuming a stew of wild mushrooms. The first observations revealed altered mental state and elevated blood pressure. The examination of cooked mushroom fragments allowed a preliminary identification of *Amanita pantherina*. Gas chromatography–mass spectrometry (GC–MS) showed the presence of muscimol in urine. Moreover, through high-performance liquid chromatography-ultraviolet detection (HPLC-UV) analysis of the gastric juice, the presence of α-amanitin was found, indicating that amatoxins-containing mushrooms were also included in the stew. After 4 days of supportive treatment, activated charcoal, silybin and N-acetylcysteine, the patient recovered being discharged 10 days post-ingestion with no organ complications. The prompt and appropriate therapy protocol for life-threatening amatoxins toxicity probably saved the patient's life as oral absorption was decreased and also supportive care was immediately started.

1. Introduction

An altered mental state is a very common occurrence in the emergency room and the diagnosis/treatment is highly challenging (Xiao et al., 2012). In fact, two or more etiologies may coexist, including pathological disorders, (e.g. hypoglycemia; cranial trauma; alcohol; infection; psychoses; stroke; hypertensive, metabolic, hepatic, or uremic encephalopathies), consumption of psycho-active substances; and others (Schwartz et al., 1992). Among these, possible accidental or intentional exposure to psycho-active substances present in some wild mushrooms species should not be neglected (Tsujikawa et al., 2006). In fact, consumption of wild mushrooms has increased substantially in the last decades (Thimmel and Kluthe, 1998). Mushrooms are appreciated worldwide as a delicacy, due to their exquisite palatability and texture. Moreover, their chemical, nutritional, and functional properties make them exceptional in the human diet (Cheung, 2010). Despite these benefits, the ingestion of wild mushrooms-containing toxins frequently occurs, which can put the consumers at life-threatening risk. Mushroom poisoning is usually accidental, rarely suicidal, and frequently occurs as a result of misidentification

Abbreviations: CNS, central nervous system; DAD, diode-array; EC, electrochemical coulometric detection; DNA, deoxyribonucleic acid; GC–MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography; RNA, ribonucleic acid; RNAP II, RNA polymerase II; TIC, total ion chromatogram; UV, ultraviolet.

* Corresponding authors.
E-mail addresses: jugarcia_18@hotmail.com (J. Garcia), felixdc@ff.up.pt (F. Carvalho).

http://dx.doi.org/10.1016/j.toxicon.2015.06.012
0041-0101/

of a toxic mushroom as an edible species (Barbato, 1993; Cochran, 1987). The severity of mushroom poisoning depends on the species of mushroom, the amount of mushroom consumed, the prompt therapy and the health status of the individual (Broussard et al., 2001; Durukan et al., 2007). The main toxins associated to mushroom poisoning are: cyclopeptides, orellanine, gyromitrin, isoxazoles, muscarine, psilocybin and gastrointestinal specific irritants (Koppel, 1993). Of the mentioned toxins, the most dangerous and potentially fatal poisonings are caused by cyclopeptides (mainly amatoxins) (Karlson-Stiber and Persson, 2003), while orellanine, isoxazoles, muscarine, psilocybin, and gyromitrin can cause a serious illness but are rarely fatal (Koppel, 1993). Amatoxins are natural toxic bicyclic octapeptides present in mushroom species from three different genera: *Amanita*, *Galerina*, and *Lepiota* (Vetter, 1998). The species *Amanita phalloides* is involved in the majority of human fatal cases of mushroom poisoning (Karlson-Stiber and Persson, 2003). Its main toxin is α-amanitin, which causes hepatic and kidney damage, often fatal (Faulstich, 1979; Klein et al., 1989; Santi et al., 2012). The mechanisms of toxicity of α-amanitin are complex, but its most known mechanism is the inhibition of RNA polymerase II (RNAP II) (Vetter, 1998).

Muscimol and ibotenic acid belong to the family of toxins known as isoxazoles. *Amanita pantherina* and *Amanita muscaria* are the most commonly known isoxazoles-containing mushrooms (Diaz, 2005). Data on the incidence of intoxication by isoxazoles-containing mushrooms are scarce. The severity of the symptoms is mild, thus intoxicated people often do not seek medical attention. In Portugal, very few cases of isoxazoles-containing mushrooms intoxications have been reported (Morgado et al., 2006). The American Association of Poison Control Centers annual report of 2012 presented 36 cases of isoxazoles-containing mushroom intoxications in the United States of America, with no related deaths (Mowry et al., 2013). Ibotenic acid is an agonist of glutamic acid receptors; its decarboxylated derivative, muscimol, is an agonist at gamma-aminobutyric acid (GABA) receptors. The central effects of these toxins are generally attributed to the previously mentioned pharmacological properties (Krogsgaard-Larsen et al., 1980; Michelot and Melendez-Howell, 2003; Snodgrass, 1978) and include hallucinations, confusion, dizziness, visual and auditory aesthesia (hypersensitivity), space distortion, and unawareness of time (Michelot and Melendez-Howell, 2003).

In this work, we describe a case of a 68-years-old patient with poorly controlled hypertension and altered mental state after eating wild mushrooms, and the respective clinical management of the patient. The suspicion of a hypertensive crisis and isoxazoles- and amatoxins-containing mushrooms poisoning were taken into consideration in the medical decisions.

2. Case report

A 68-years-old female with hypertension was admitted to the emergency room of São João Hospital (Porto, Portugal) after ingestion of mushrooms. After specific query, her son reported that the patient had ingested several wild mushrooms that were collected in a public yard by the patient. She stewed the mushrooms, and, immediately after ingestion, she started experiencing dizziness, vertigo and imbalance. She self-induced vomiting and had no symptoms of nausea, spontaneous vomiting or diarrhea. On admission, 3 h post-ingestion, the examination revealed an altered mental status including confusion and repetitive speech, only responsive to severe painful stimuli, and mydriatic pupils. These symptoms resolved in a few hours after admission. Initial emergency room vital signs were: blood pressure 230/108 mmHg, normal pulse and respiratory rate, temperature 96.8 °F, and oxygen saturation of 97.5 percent using an oxygen mask at 10 L/min. Physical exam demonstrated dry skin and dry mucous membranes. Additionally, she had clear breath and heart sounds, no abdominal tenderness, and no gross focal neurologic deficits. A brain computed tomography was immediately performed revealing no abnormalities. Additionally, electrocardiogram, funduscopy and urinary sediment were analyzed demonstrating no evidence of lesions. Her serial hematological parameters, coagulation profile, liver and renal function tests were normal.

As a standard procedure for intoxications with wild mushrooms and until species identification by the laboratory, the general detoxification treatment with oral-activated charcoal was immediately instituted on admission, and it was followed by the specific treatment for *A. phalloides* poisoning, through the administration of silybin and N-acetylcysteine. N-acetylcysteine and silybin were administered in a loading dose of 10.5 g (150 mg/kg) and 350 mg (5 mg/kg), respectively, followed by perfusion. The perfusion of silybin was maintained at a dosage of 58 mg/h (20 mg/kg/d) and N-acetylcysteine at 875 mg/h (12.5 mg/kg/h) for the following 4 h. Labetalol perfusion was also administered for blood pressure control. The patient was then transferred to intermediate care unit to continue with the treatment with continuous surveillance. A total of 300 mg/kg of N-acetylcysteine was administered in the first 21 h after admission. The perfusion of activated charcoal and silybin were kept until the fourth day (at the fourth day, normal coagulation profile and liver function tests were observed). Serum aminotransferases and coagulation profile were normal during all the time that the patient remained admitted, although an elevation of total bilirubin (twofold the normal value) was observed at day 2 (conjugated bilirubin was normal at that time). Two days after, total bilirubin level started to decline, and it normalized within one week. After labetalol perfusion, the blood pressure control was achieved by administration of sublingual captopril until the fourth day.

The patient kept some of the stew's leftovers, which were then provided for analysis. The identification of the mushrooms was firstly performed by 2 experts based on the macroscopic features of the fruiting body (Courtecuisse, 1999; Courtecuisse and Duhem, 2005). In the stewed mushrooms, the color of some of the cap fragments resembled *A. pantherina*, namely brown with whitish-brown warts (Fig. 1). The gills were free and whitish-brown color and the stipe was cylindrical and seemed to be white. Therefore, to

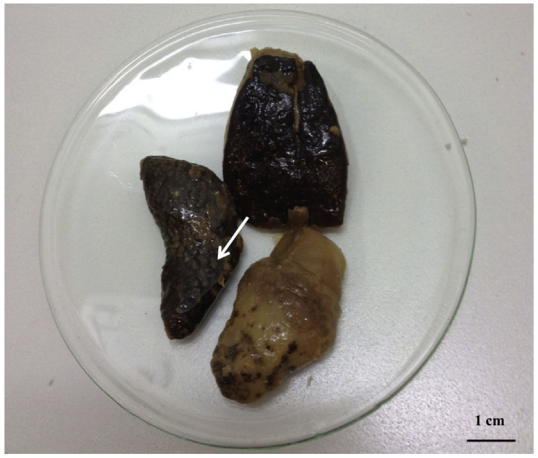

Fig. 1. Macroscopic identification of *Amanita pantherina* found in the stew, showing its characteristic features in a mushroom fragment (left). The cap fragments are brown with whitish-brown warts (white arrow). The stipe is cylindrical. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article in press as: Garcia, J., et al., Co-ingestion of amatoxins and isoxazoles-containing mushrooms and successful treatment: A case report, Toxicon (2015), http://dx.doi.org/10.1016/j.toxicon.2015.06.012