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CASE REPORT

A Case Study: What Doses of *Amanita phalloides* and Amatoxins Are Lethal to Humans?

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There are few data estimating the human lethal dose of amatoxins or of the toxin level present in ingested raw poisonous mushrooms. Here, we present a patient who intentionally ingested several wild collected mushrooms to assess whether they were poisonous. Nearly 1 day after ingestion, during which the patient had nausea and vomiting, he presented at the emergency department. His transaminase levels started to increase starting from hour 48 and peaking at hour 72 (alanine aminotransferase 2496 IU/L; aspartate aminotransferase 1777 IU/L). A toxin analysis was carried out on the mushrooms that the patient said he had ingested. With reversed-phase high-performance liquid chromatography analysis, an uptake of approximately 21.3 mg amatoxin from nearly 50 g mushroom was calculated; it consisted of 11.9 mg alpha amanitin, 8.4 mg beta amanitin, and 1 mg gamma amanitin. In the urine sample taken on day 4, 2.7 ng/mL alpha amanitin and 1.25 ng/mL beta amanitin were found, and there was no gamma amanitin. Our findings suggest that the patient ingested approximately 0.32 mg/kg amatoxin, and fortunately recovered after serious hepatotoxicity developed.

Key words: Amanita phalloides, amanitin, sublethal toxicity, high-performance liquid chromatography

Introduction

The *Amanitaceae* family is responsible for nearly 95% of all fatal mushroom poisonings.[1] From this family, *Amanita phalloides* often carries life-threatening risks for persons who ingest it. It is a very toxic and widespread species, and *A. phalloides* is often confused with edible mushrooms such as young *Agaricus* spp, *Lepiota naucina*, and *Leucoagaricus leucothites*.[2] The toxicity of the *A. phalloides* mushroom relates to its 2 major toxins, amatoxin and phallotoxin. Phallotoxins are limited poisons because they are easily degraded by heat or digestion.[3,4] Amatoxins are much more important agents, responsible for clinical poisoning[5,6] and 10 to 20 times more toxic than phallotoxins.[7] Toxin levels can vary among various *Amanita* species,[2,3,8,9] even among *Amanita* varieties.[9] More interestingly, this difference in the levels of amatoxins and phallotoxins can also be seen in different parts of a single mushroom, such as pileus, gills, stipe, and volva.[2,8,9] If the content of a mushroom is documented in detail, deadly mushroom poisoning can be prevented through early diagnosis and effective treatment methods. It is not known how much *A. phalloides* mushroom consumption and what doses of amatoxin are lethal to humans. Documenting these may provide benefits in managing the treatment of poisonings from this mushroom. We report a case of sublethal *A. phalloides* intoxication caused by ingesting 2 caps of the freshly collected mushrooms.

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Case Presentation

A 61-year-old man weighing 67 kg was admitted to the emergency room with fatigue, abdominal pain, nausea, vomiting, and diarrhea. In obtaining the patient's history, he said he had collected several mushrooms that differed from those he typically gathered, but he was not quite sure if they were edible. The patient tried a dangerous test on himself to determine whether the mushrooms he had collected were poisonous. He removed the stems of 2 mushrooms, cooked only the caps on the stove, and ingested them. He told the household that if nothing happened to him, they could eat the remaining mushrooms together the next day. At approximately

0400 hours, 8 to 9 hours after he ingested the mushrooms at approximately 1900 hours, he woke up with nausea, vomiting, stomachache, and diarrhea. The patient realized that he was poisoned from the mushrooms he had eaten; he drank some water and vomited a few times in an effort to clean his stomach. He thought that he did not need to go to a hospital, but approximately a day after the poisoning, he was persuaded by his family and brought to the emergency department.

When the patient was admitted to the emergency department, nearly 24 hours had passed since he had ingested the mushrooms. His initial vital signs and physical examination were normal, other than dehydration. His aspartate aminotransferase (AST) was 41 IU/L, alanine aminotransferase (ALT) 21 IU/L, total bilirubin 0.75 mg/dL, direct bilirubin 0.16 mg/dL, total protein 9.11 g/dL, albumin 4.4 g/dL, prothrombin time 11.6 seconds, and international normalized ratio 1.09. Hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, immunoglobulin M, and anti-hepatitis C antibody were nonreactive. His lactate level, complete blood cell count, electrocardiogram, posteroanterior lung radiograph, and complete urine test were normal.

Activated charcoal treatment was initiated and continued at a dose of 50 g every 6 hours for 3 days. The patient was rehydrated by intravenous administration of 0.9% sodium chloride and 5% dextrose to guard against the risk of hypoglycemia. Penicillin G in doses of 500,000 IU/h was started on a continuous infusion basis and continued for 72 hours.

The patient was then admitted to the internal medicine unit after having been diagnosed with mushroom poisoning. A consultation was requested from the clinical pharmacology and toxicology unit, which had experience with mushrooms. Following up the history taken by the specialist, the mushroom specimens at the patient's home were examined and identified as *A. phalloides* mushroom (Family: *Amanitaceae* R. Heim ex Pouzar; species: *Amanita phalloides* [Vaill. ex Fr.] Link) on the basis of their microscopic and macroscopic characteristics (Figure 1), as follows: cap is 70 to 100 mm across, ovoid to hemispherical at first, becoming convex and then planar; surface is smooth, satiny when dry, viscid and slightly shiny when wet; finely radially fibrillose; variable in color but usually greenish, olive-green, or yellowish green, sometimes with whitish veil remnants; margin is acute and smooth; flesh is white, yellowish under the cap cuticle; odor is sweetish, taste nutty; gills are white, free, and crowded; stem is 80 to 110 mm × 10 to 15 mm, cylindrical, thickening toward the base; white or tinged cap color and usually faintly banded; base of the stipe bulbous and enclosed in a large saccate whitish volva; ring is white, pendent, fragile, and superior; basidia is 50 to 55 μm × 15 μm, clavate and 4-spored; spores are 8 to 10 μm × 7 to 8 μm, subglobose to broadly ellipsoid, hyaline, and smooth; and ecology is widespread, late summer to autumn, on soil, usually in hardwood forest, rarely in coniferous forest or forest edge.[10]

Further samples were collected from the region described by the patient (Turkey-Duzce, on soil, under oak, 170 m, 40°53′45.83″ N, 31°7′10.99″ E, November 11, 2013, Akata 5999). These were shown to the patient to verify their species. The patient was asked to choose 2

Figure 1. *Amanita phalloides* (a) fruit body and amyloid spores; (b) spores.

Figure 2. *Amanita phalloides* mushrooms (ripe mature mushroom on the left, ripe young mushroom on the right) that are of similar size and ripeness as the mushrooms the patient said he ate.

that were similar in size and ripeness to the ones he had ingested (Figure 2). It was known that the amount of toxin was lower in young *A. phalloides* and higher in well-developed mushrooms.[5] The caps of these 2 mushrooms were removed, as the patient had done before ingesting them, and their wet weights were measured. After drying, their dry weights were measured. The amounts of toxin in the sample mushrooms were analyzed using the method described previously.[2,8,9] As a result of the analysis, we calculated that the patient may have ingested a total of 21.3 mg amatoxin from the mushrooms he had eaten, consisting of 11.9 mg alpha amanitin, 8.4 mg beta amanitin, and 1 mg gamma amanitin. The weights and toxin contents of the mushrooms are given in Table 1. The amatoxin analysis of the urine sample taken from the patient on day 4 revealed that he had 2.7 ng/mL alpha amanitin and 1.25 ng/mL beta amanitin but no gamma amanitin.

The AST and ALT values of the patient, who was being monitored in the internal medicine clinic, began increasing at hour 48 (AST 294 U/L, ALT 284 U/L) and increased at hour 72 and peaked (ALT 2496 IU/L, AST 1777 IU/L). At hour 96, the values began to decline (ALT 1904 IU/L, AST 719 IU/L) (Figure 3). The prothrombin time and international normalized ratio values of the patient reverted to normal on day 6, and his bilirubin values became normal on day 7. For the transaminases, AST returned to normal on day 8, but it took ALT until day 15 to normalize. The total protein and albumin values returned to normal on day 8. The patient was discharged at the end of day 9, with a recommendation that he come for outpatient follow-up every third day. The patient did not have any impairment in his renal function tests while he was hospitalized; he was considered fully recovered on day 15, and his follow-up ceased.

Discussion

In most mushroom poisoning cases, it is generally not possible to identify the species of the mushrooms eaten, largely because of the insufficient knowledge of patients

Table 1. Detailed amatoxin amounts in the mushrooms eaten by the patient

Mushroom part	*Fresh weight (g)*	*Dry weight (g)*	*Alpha amanitin (mg)*	*Beta amanitin (mg)*	*Gamma amanitin (mg)*	*Total amatoxin (mg)*
Young mushroom cap	25.9	2.1	6.5	4.5	0.6	11.6
Mature mushroom cap	17.5	2.2	5.4	3.9	0.4	9.7
Total mushroom	43.4	4.3	11.9	8.4	1.0	21.3

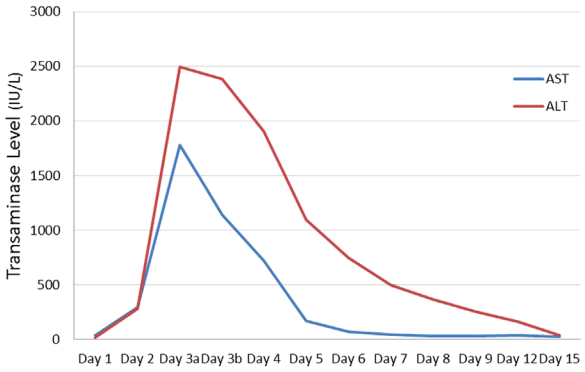

Figure 3. Progress of the patient's liver transaminase levels, aspartate aminotransferase (blue line), and alanine aminotransferase (red line).

or their relatives regarding mushrooms or insufficient or incorrect history given to the physician. Moreover, relatively few physicians are well acquainted with mushroom identification, especially for differentiating between poisonous and edible species.[11,12] The patient in our report was not experienced enough to identify whether the mushrooms he collected were poisonous, nor did he know their species. The mushrooms were identified by the clinical pharmacology and toxicology unit based on their microscopic and macroscopic characteristics, and the amount of toxin ingested was accurately analyzed, contributing to the positive management of the patient's treatment.

In clinical practice, only amatoxins are of interest for toxicological investigation. Alpha amanitin, beta amanitin, and gamma amanitin are some of the members of the amatoxins.[13] Although alpha amanitin in particular is held responsible for poisonings, the other amatoxins also have been reported to have a considerable share in toxicity.[14–16] The intraperitoneal lethal dose of alpha amanitin is determined as 0.1 mg/kg for mice,[4] but there is little information about the lethal dose for humans.

In case of poisoning, amatoxins can be analyzed in urine, blood, and various organs. Several methods can be used to measure the amatoxin and phallotoxin contents in a mushroom, but reversed phase high-performance liquid chromatography is the most widely used method. With this method, it is possible to measure amatoxin and phallotoxin contents by using 2 different ultraviolet wavelengths. The amatoxin and phallotoxin concentrations in *A. phalloides* and its varieties and the distribution of these in different parts of the mushroom have been shown in detail by our recent studies in which we used the high-performance liquid chromatography method. The gills and pileus contained the highest concentrations of amatoxin and phallotoxin.[8,9] The determination of the toxin content in parts and different species of *A. phalloides* mushroom is important in that it may serve as a guide to the amount of toxin ingested at times of poisoning.[3,8,17] Early and comprehensive treatment is very important in case of mushroom poisoning, making this information even more valuable.

When the toxin amounts in the cap (gills and pileus) of the mushrooms consumed by our patient were analyzed, it was found that he had ingested approximately 21.3 mg amatoxin from the mushrooms. Accordingly, taking this into consideration in our analysis, it is possible to say that oral intake of amatoxin of more than 0.32 mg/kg may be lethal. Correspondingly, the lethal dose of alpha amanitin, which is probably the most lethal toxin among amatoxins, becomes approximately 0.2 mg/kg taken orally. Our analysis suggests that consuming more than approximately 50 g fresh *A. phalloides* mushroom, which corresponds to roughly 2 *A. phalloides* mushrooms of medium size, can be deadly. The lethal dosage for humans probably depends on the health of the patient, his or her susceptibility or predisposition to liver injury, and ecotypic variation in the concentration of amatoxins in various locales. Our clinical and laboratory results showed that the amount taken by the patient remained below the hypothetical lethal dose. In our present study, the lethal dose was determined according to the data obtained from 1 patient, but these data are important for further investigations. Similar studies that include a greater number of patients may provide additional evidence for the quantification of potentially lethal doses of amatoxin.

With mushroom poisoning, if the toxic symptoms start within 2 hours after consuming the mushroom, the clinical prognosis is usually good. If the symptoms start after 6 to 7 hours, it should raise suspicion of poisoning from very toxic mushrooms (such as *A. phalloides*), and the clinical prognosis must be considered extremely bad and potentially fatal.[18] That symptoms appeared even later in our patient suggested that he was poisoned by a very toxic mushroom, as was confirmed by the history taken and the identification of the mushroom as *A. phalloides*. After amanitin poisoning, hepatotoxicity is the dominant clinical feature. The liver damage is characterized by liver necrosis, in many cases with acute hepatic failure with subsequent complications, including hepatic coma, coagulation disorders, and secondary renal failure.[1,6,19–22] The damage to the liver and kidneys depends on the dose of amanitin. The mortality rate can be very high, and irreversible liver failure may result in death in 3 to 7 days.[23]

Owing to the hepatocyte damage that developed in our patient in connection with *A. phalloides* poisoning, liver transaminases increased in his serum, but there was no impairment of his renal parameters. His ALT and AST levels began to rise at hour 48, reached 2496 IU/L and

1777 IU/L at hour 72, and then tended to decrease over subsequent days (Figure 3). In a recent study, Eren et al[24] showed that the patients who died had very high ALT levels of 2345 to 4048 IU/L and AST levels 2075 to 3464 IU/L. It was reported that hepatic coma developed after ALT and AST values rose, demonstrating a significant relationship between mortality and ALT and AST levels. Therefore, ALT and AST levels can be used as important and practical parameters that indicate the degree of mushroom poisoning. When transaminases were assessed in our case, the levels were observed to rise until, at hour 72, they were just below the very high levels reported to be associated with mortality.

The effects of the activated charcoal treatment—which is more important for early stage mushroom poisonings—were very limited for our patient because he came to the hospital after a significant delay after ingesting the mushrooms. In amanitin-containing mushroom poisonings, therapy options are confusing and controversial.[25] Intoxications caused by amanitin-containing mushrooms represent an unresolved problem in clinical toxicology as no specific and fully effective antidote is available.[26] There are very different therapy options, such as benzylpenicillin (penicillin G), silibinin, thioctic acid, antioxidant drugs, acetylcysteine, cimetidine, hormones, and steroids.[20,25,27,28] Of the therapy options, silibinin has been described as more effective than the others. Because silibinin was not available at our hospital at that time, we used penicillin G to treat our patient. This therapy administered to our patient was meant to inhibit amatoxin uptake by hepatocytes, and this effect is thought to be obtained in doses ranging from 300,000 to 1,000,000 IU/kg daily.[29]

Conclusion

Our patient had a severe case of mushroom poisoning, with an oral toxin intake of 0.32 mg/kg. Although treatment was started very late for our patient, treating a poisoned patient at an early stage is of utmost importance in cases of mushroom poisonings. Even more important is whether the patients or their relatives can provide a correct and sufficient history of the mushroom eaten; but often the only way to confirm the ingested species is by a qualified examination of a specimen. Healthcare sites that have teams with experience of mushrooms, especially in regions where many mushrooms grow naturally and are consumed frequently by local people, and have the facilities to identify and analyze the toxins involved will contribute greatly to clinicians' ability to treat patients with mushroom poisoning. Given this case of a sublethal poisoning, in which more serious events might have occurred, people should be better informed about wild mushrooms.

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