Role of the Clinical Laboratory in Guiding Treatment of Amanita virosa Mushroom Poisoning: Report of Two Cases
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Historically, mortality after Amanita mushroom ingestion has ranged from 50% to 90%. Prompt diagnosis is imperative, and aggressive therapeutic measures must be instituted quickly to improve the outcome. We report successful treatment of two cases of A. virosa poisoning by use of combined therapies, including thiotic acid and hemoperfusion.

Poisoning by ingestion of toxic mushrooms is increasing (1–3). About 100 cases are reported annually in the United States, although the actual incidence is probably higher. The state of Wisconsin had 25 confirmed cases of mushroom poisoning in 1981 (4). Mushroom poisoning is more common in Europe; in Germany alone, 200 cases are reported annually (5). Amatoxins are responsible for 95% of fatal mushroom poisonings. Historically, within four to seven days after ingestion, the mortality rate has been 50% to 90%, the highest rate being in children. Recent reports of combined-modality therapy describe better results, with 20% to 50% mortality (6–8). In North America, most (>90%) deaths are due to Amanita phalloides ("death cap") and only a few to A. muscaria or A. virosa poisoning (2, 5).

Here we present two cases of successful treatment of A. virosa poisoning and illustrate the role of the clinical chemical laboratory in following the clinical course of the toxicity on the various organ systems.

Case Reports

Case 1
A 46-year-old white woman, an immigrant from Eastern Europe, ingested 10 mushrooms that she had gathered from a local park. No alcohol was consumed with them. About 9 h later, she awoke with nausea, vomiting, diarrhea, severe abdominal cramps, and shaking chills. Twelve hours after ingestion, she went to her local hospital, where she was treated with 60 g/L magnesium citrate solution (300 mL, orally), nasogastric suction, furosemide (20 mg, intravenously), methylprednisolone (125 mg, intravenously), prochlorperazine, and diazepam. The remaining mushrooms from her meal were identified as A. virosa by examination of spores and by gross morphology, by the United States Department of Agriculture Forest Service, Forest Service Laboratory, One Gifford Pinchot Drive, Madison, WI 53705. She was then transferred to our hospital for further therapy. Her past medical history was significant for an unknown congenital heart lesion, stable angina treated with nitroglycerin whenever needed, cholecystectomy, and appendectomy.

She was admitted to the intensive-care unit 31 h after ingestion of the mushrooms, complaining of severe cramping, abdominal pain, nausea, and diarrhea. On physical examination she was alert. Her pulse was 150/min, respirations 24/min, blood pressure 160/112 mmHg, and temperature 37.1 °C. A Grade II/VI systolic ejection murmur was present. Her abdomen was soft, with hyperactive bowel sounds. There was tenderness in the upper right quadrant of the abdomen, but her liver was not enlarged. The feces gave a negative test result for occult blood. Results of the neurological examination were unremarkable.

Laboratory values for her blood, sampled at admission, were as follows: hemoglobin 150 g/L (normal for females, 120 to 150 g/L), hematocrit 48% (normal for females, 42 ± 5%), leukocyte count 24 100/mm³ (normal, 4800 to 10 800/mm³), platelet count 421 000/mm³ (normal, 150 000 to 450 000/mm³), glucose 2.4 g/L (normal, 0.65 to 1.1 g/L), creatinine 11 mg/L (normal, 6 to 10 mg/L), serum urea nitrogen 130 mg/L (normal, 70 to 200 mg/L), total bilirubin 11 mg/L (normal, 2 to 12 mg/L), amylase 89 U/L (normal, 33 to 110 U/L), prothrombin time 16 s (control = 11 s), alkaline phosphatase 60 U/L (normal, 48 to 125 U/L), ammonia 30 μmol/L (normal, 11 to 35 μmol/L), aspartate aminotransferase (EC 2.6.1.1) 239 U/L (normal, 11 to 47 U/L), alanine aminotransferase (EC 2.6.1.2) 60 U/L (normal for females, 5 to 32 U/L), lactate dehydrogenase (EC 1.1.1.27) 322 U/L (normal, 270 to 560 U/L), and creatine kinase (EC 2.7.3.2) 105 U/L (normal, 30 to 160 U/L). The urinalysis showed a relative density of 1.023, pH 7.0, protein 2+, occult blood 2+, 5 to 15 leukocytes per high-power field, and 50 to 75 erythrocytes per high-power field.

Her treatment was as follows: Through a catheter placed into the superior vena cava, 50 g/L dextrose in isotonic saline containing 20 mmol of KCl and 12.5 g of mannitol per liter was infused at a rate of 175 mL/h. Charcoal, 100 g in 300 mL of 60 g/L magnesium citrate solution, was instilled through a nasogastric tube. Continuous infusion of penicillin (1.1 x 10⁶ USP units/h) was begun 34 h after the mushroom ingestion. Six hours of hemoperfusion with an Amberlite XAD-4 resin cartridge (Extracorporeal Medical Specialists, Inc., King of Prussia, PA) was begun 35 h after the ingestion. Thiocitic acid, 150 mg intravenously every 6 h, was begun 44 h after the ingestion. Meperidine and hydroxyzine were administered for abdominal pain. Thirteen units of fresh frozen plasma was administered over three days to treat her increased prothrombin time. Prophylactic cefamandole nafate was begun 48 h after the ingestion. Oral prednisone, 60 mg per day, was given from day 14 through 21 after the ingestion. Figure 1 illustrates her clinical course in response to therapy.

The patient improved clinically after the total bilirubin reached a peak value of 60 mg/L (85 μmol/L) 120 h after the ingestion. Penicillin was discontinued on day 4; thiocitic acid was gradually decreased and discontinued at day 19. She was discharged to her home the 21st day after the ingestion.

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Received August 28, 1989; accepted November 28, 1989.
Case 2

The second patient was a 39-year-old white man, a friend of the first patient, who developed nausea, vomiting, and diarrhea 12 h after eating three of the mushrooms served by the first patient. No alcohol was consumed. He went to his local hospital 16 h after the ingestion and was treated with an intravenous infusion of 50 g/L solution of glucose in isotonic saline plus 20 mmol of KCl at 200 mL/h; furosemide (20 mg), methylprednisolone (125 mg), and diazepam (5 mg) intravenously; and 300 mL of 60 g/L magnesium citrate orally.

While hospitalized, this patient developed severe muscle spasms in his legs; the spasms were treated with meperidine and hydroxyzine. His past medical history was unremarkable.

This patient was admitted to our hospital 31 h after the ingestion. He was drowsy but easily aroused and complained of mild abdominal cramps and diarrhea. His vital signs were stable. His abdomen was soft but not tender, with increased bowel sounds. His liver was nontender and normal in size. The feces gave a negative test result for occult blood. Results of the neurological examination were unremarkable.

Laboratory values for his blood, sampled at admission, were as follows: hemoglobin 167 g/L (normal for males, 130 to 170 g/L), hematocrit 50% (normal for males, 47 ± 5%), leukocyte count 18 200/mm³, glucose 1.95 g/L, serum urea nitrogen 270 mg/L, creatinine 14 mg/L, alkaline phosphatase 60 U/L, ammonia 32 mmol/L, prothrombin time 13 s, aspartate aminotransferase 60 U/L, alanine aminotransferase 34 U/L (normal for males, 8 to 45 U/L), lactate dehydrogenase 171 U/L, and creatine kinase 983 U/L. The urinalysis showed a relative density of 1.018, pH 6.0; glucose and protein tests were negative; and results of microscopic examination were unremarkable.

His treatment was as follows: Dextrose infusion, treatment with charcoal and penicillin, and hemoperfusion were performed as for the other patient. Thiocatic acid, 150 mg, was given intravenously 44 h after the ingestion, then decreased to 75 mg every 6 h for seven days. Prednisone, 100 mg, was given intravenously daily from the second through the fourth day after the ingestion.

The clinical course and therapy are summarized in Figure 1.

The patient improved more rapidly than his companion, probably because he ate fewer mushrooms. His high value for creatine kinase may have been due to the severe muscle spasms, which were not seen in the first patient. He was discharged 10 days after the ingestion.

Discussion

Amatoxins

Mushrooms of the genus *Amanita* contain amatoxins, thermostable bicyclic peptide toxins comprised of eight amino acids with a molecular mass of about 900 Da. Amatoxins, especially amanitin, cause cellular destruction by inhibiting RNA polymerase (9–13). The greatest damage is to cells with rapid rates of turnover, such as gastro-
intestinal mucosa cells, hepatocytes, and renal tubular cells (9-14). Amatoxins are taken up by hepatocytes, excreted into the bile, and reabsorbed (15, 16). This enterohepatic circulation prolongs the presence of the toxin in the serum, but rapid binding to proteins causes the concentration of "free" amatoxins to decline rapidly after absorption (17). Eighty-five percent of an administered dose of alpha-amatoxin to dogs is excreted in the urine in 6 h (17). Amatoxins possess a high affinity to surfaces of charcoal polymers in hemoperfusion cartridges (18, 19).

Clinical Picture
Symptoms of Amanita poisoning can be separated into three clinical stages (2, 5, 9, 10, 20). The initial stage of amanita poisoning, beginning 6-24 h after the mushroom ingestion, causes patients to experience nausea, vomiting, abdominal pain, cholera-like diarrhoea, and hematuria. In the second stage, 12 to 48 h after ingestion, there is apparent clinical recovery, with absence of symptoms. During this asymptomatic interval, subclinical hepatic and renal disturbances can be detected in the clinical laboratory by increases in the concentrations of aminotransferases, creatinine, and urea nitrogen in serum. Serum aminotransferase concentration is the most sensitive, constant, and specific indicator of hepatocellular damage, with alanine aminotransferase increasing before aspartate aminotransferase (17). During the third stage, 24 to 72 h after ingestion, there is progressive and symptomatic hepatic and renal failure, coagulopathy, cardiomyopathy, encephalopathy, convulsions, coma, and death. Aggressive treatment is indicated shortly after ingestion, during the apparent recovery stage, to prevent such progression to a fatal outcome.

Treatment
The major goals of therapy are to lower the serum amatoxin concentrations as soon as possible and to shorten the exposure time of certain susceptible cells, particularly hepatocytes, because, when the outcome is fatal, it is usually due to massive hepatic necrosis (8, 13, 15). A chromatographic assay (2) has been described. However, identification of the mushroom by examination of the implicated meal or gastric contents for typical morphology and spores is most commonly used to help predict the clinical course and lead to specific therapy. Attempts to decrease absorption of the amatoxin dose by inducing vomiting, followed by oral administration of activated charcoal slurry along with a cathartic are basic steps in the initial therapy (10, 17, 20). Attention to fluid and electrolyte abnormalities is critical, so that the decreased vascular volume associated with diarrhea and vomiting is replaced (20). Supportive measures of vital functions in patients with altered states of consciousness may be necessary. Hepatic clotting abnormalities must be corrected by using vitamin K and fresh-frozen plasma (15, 17). Dialysis may be necessary to treat acute renal failure, associated with acute renal tubular necrosis, which may last for as long as 31 days (14).

Compounds used to decrease the amount of toxin entering the cells include thiocytic acid, glucose, penicillin, steroids, ethanol, intravenous vitamin C, and silibinin. Thiocytic acid (alpha-lipoic acid), first used in the treatment of amanitin poisoning in 1959, reportedly decreases the mortality rate (2, 10, 15-17). It functions as one of the coenzymes in the oxidative decarboxylation of pyruvate and other alpha-keto acids of the Krebs cycle, where its protective effect is thought to take place. Hypoglycaemia is the only known side effect of therapy. Thiocytic acid is given intravenously with glucose in doses of 300 to 600 mg/kg of body weight per day in four divided doses. The doses are tapered as the patient improves, the usual duration of therapy being four to seven days. Penicillin may compete with amanitin for binding sites on serum proteins and has been used in high intravenous doses (250 mg/kg, daily) in combination with thiocytic acid (16-17, 21). Steroids (dexamethasone, 20 to 40 mg intravenously, daily) may have a beneficial role in therapy, but the results are controversial (16). Ethanol, ingested at the time of mushroom ingestion, may decrease hepatic damage by interfering with uptake of the toxin into liver cells (22).

In France, Bastien has advocated carrot broth along with intravenous vitamin C (3 g/day), oral nifuroxazole (1200 mg/day), and dihydrostreptomycin (1500 mg/day), with good (anecdotal) results (23). This has been adopted as the treatment of choice at some French centers (24).

Silibinin (silymarin), (not available in the U.S.A.), the active ingredient of milk thistle, given intravenously at 20 to 50 mg/kg per day for as long as 48 h after ingestion, has been said to inhibit amanitin uptake by hepatocytes in perfused rat liver. It inhibits enteric absorption of alpha-amanitin by interrupting the enterohepatic circulation of the toxin and prevents penetration of alpha-amanitin into the hepatocyte by stabilizing the cell membrane (16, 25-26).

Increasing the rate of removal of amatoxin from the circulation by forced diuresis, and by extracorporeal methods such as hemoperfusion and plasma exchange, reportedly lowers mortality (17, 18, 27, 28). These methods are most effective when instituted promptly when the concentrations of amatoxin in serum are high. Renal and liver transplants have been attempted in cases of massive cellular necrosis (29).

Susceptible patient groups are amateur foragers (e.g., Case 1, who had had previous mushroom-gathering activities while living in Eastern Europe) and those seeking mushrooms for their possible hallucinogenic properties. Patients with this complex of symptoms will be more common in the spring and fall, when mushrooms are the most plentiful.

When mushroom poisoning is suspected, attempts should be made to identify the offending mushroom by sending some of the meal and gastric contents to an experienced mycologist or toxicology laboratory to determine whether the ingested mushroom is toxic. Therapy should be started quickly, with close monitoring of vascular volume, electrolytes, and liver and renal function tests to guide the need for aggressive therapy, even if the patient is asymptomatic and reluctant to cooperate. Both of our patients had favorable responses to combined modality therapy with thiocytic acid, penicillin, and hemoperfusion. With prompt, aggressive therapy, victims of mushroom poisoning may recover from an otherwise often fatal illness.

We acknowledge the valuable discussions of Drs J. Lemann, Jr., and B. T. Doumas, Medical College of Wisconsin, and M. J. Dibben, Head, Section for Botany, Milwaukee Public Museum.

References
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CLINICAL CHEMISTRY, Vol. 36, No. 3, 1990 573