New Developments in Amatoxin Poisoning

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S Todd Mitchell MD, MPH
Principal Investigator:
Prevention and Treatment of Amatoxin Induced Hepatic Failure With Intravenous Silibinin (Legalon® SIL): An Open Multicenter Clinical Trial
Consultant: Madaus-Rottapharm
Amatoxin Poisoning: Overview

- 95%+ of all fatal mushroom poisonings worldwide are due to amatoxin containing species.
- 50-100 Deaths per year in Europe is typical.
- Growing Problem in North America, especially in Northern California

USA 1976-2005: 126 Reported Cases
  2006:  48 Reported Cases, 4 Deaths
  Summer 2008:  2 Deaths on East Coast

September/October 2012: 2 deaths, 1 transplant among 15 total cases on the East Coast.


Countless more in SE Asia, Indian Sub-Continent, South Africa

Assam, India  March 2008: 20 Deaths

Swat, Pakistan 2006:
Watsonville September 2006

• 57 yo former ER nurse, electrical contractor ingests 8 mushrooms from his property at 1800 on September 8.
• Onset of sx ~0200.
• Mushrooms identified as *Amanita Phalloides* by local expert amateur mycologist ~1500.
• Presented to ER 24 hours post ingestion:
  - BUN 37, Creat 2.0, Hgb 20.2, Hct 60.5, ALT 96.
• Transfer to UCSF 9/10. INR 2.2, ALT 869 after Rx with hydration, antiemetics, repeated doses of charcoal, IV NAC, and IV PEN G.
• 72 Hours: INR 4.5, ALT 2274, Bil 4.0.
• Liver Transplant 9/14.
2007 Santa Cruz Cohort

- EM age 82.
  ALT 12224, INR 5.4, Factor V 9% @ 72 hours.
  ALT 3570, INR 1.7, Factor V 49% @ 144 hours.
  Died from anuric renal failure 1/11.

- MN age 29.
  64 hours: ALT 18,073, PT>100, INR 14.9, Factor V 13%, Ammonia 129. Listed for transplant.
  108 hours: INR 2.2, Factor V 27%, ALT 4558.
  Discharged home on 1/12.

- First patients in North America treated with IV Silibinin via Emergency IND granted by FDA.

- Silibinin infusions start @ ~78 hours post ingestion.

- All survivors make full recovery with completely
FDA Open IND-Clinical Trial

Intravenous Milk Thistle (Silibinin-LegalonSIL) for Hepatic Failure Induced by Amatoxin/Amanita Mushroom Poisoning

http://clinicaltrials.gov/ct2/show/NCT00915681

- Enrollment began September 2009 with Newton, MA cohort.
- ~60 cases treated so far.
Amatoxin Mushrooms

- **Death Caps:** *Amanita phalloides*
- **Destroying/Death Angels (White)**
  - *A. ocreata* (CA native)
  - *A. bisporigera/virosa* (East Coast)
- Mixed coniferous and deciduous forest
- Mycorrhizal = symbiotic relation with tree roots.
- Often grow among edible mushroom species
  - *Lepiota*, *Galerina*, *Conocybe*. Smaller mushrooms, less commonly ingested, larger numbers needed for severely toxic dose.
A. phalloides – Death Cap Mushroom

- Native to Europe
- Introduced to US on imported trees
  - Oak, chestnut, and pine
- Most common on West Coast but also now found on East Coast.
Amanita Phalloides

- Most common culprit in Europe and California.
- Now proven by rDNA analysis to be European native & California exotic (A Pringle PhD, Harvard).
- Rapidly expanding habitat & thriving along West Coast from Vancouver to SoCal.
- Frequently mistaken for native mushrooms by Asian and Latin American immigrants.
- Occasionally misidentified and consumed by semi-well educated mycophiles.
- Said to be quite tasty.
Amanita bisporigera
Destroying Angels

- Extensive range in forests of North America. East Coast & Midwest.
- Responsible for multiple poisonings in 2011 and 2012.
Amatoxin

- Amanitins: Family of bicyclic octapeptides, primarily α, β, γ:
- Single cap of *Amanita phalloides* (10-15mg) contains a lethal (0.1mg/kg) dose.
- ~10-15% cited mortality is misleading as outcomes best correlated with amount consumed.
- Children more vulnerable with greater likelihood of a poor outcome (>mg/kg exposure).
-amanitin molecule
Amatoxin

- Most toxic to rapidly dividing cells: Intestine, Liver, Kidneys.
- Rapid GI absorption to liver (2/3rd) and general circulation (1/3rd).
- OATP1B3 is the primary human hepatic uptake transport protein for amatoxins.
- Rapid renal elimination as long as urine output is maintained.
- Centrilobular Hepatic Necrosis & Apoptosis
-amanitin molecule in RNA
Polymerase II
Amatoxin Kinetics

• Absorbed from GI tract while poisoning gastrointestinal epithelium.
• Rapid clearance from general circulation (~40%) by kidneys.
• Uptake by hepatocytes via portal circulation (~60%).
• High concentrations measurable in gastroduodenal fluid at 110 hours post ingestion.
Enterohepatic Circulation

- Circulation of bile from liver to small intestine and back. 95% bile acids recycled to liver after delivery to duodenum.

- **Bile salts** (H2O soluble) actively & passively reabsorbed into hepatic portal circulation. Venous blood from ileum collects into portal vein, then to hepatic sinusoids. Hepatocytes then efficiently extract bile acids.

- Each bile salt molecule reused ~20x. Allows for preservation & recycling of bile acids for digestion of fats.
Enterohepatic Circulation of Amatoxins
Enterohepatic Circulation of Amatoxin

- Beagles with a prior surgical biliary fistula survived lethal amatoxin exposure.
- 10 Fold lower transaminases along with slower rate of rise.
- Demonstrated that enterohepatic circulation enhances the effect of amatoxin by continuous reabsorption.
- Allows amatoxin to be re-concentrated in biliary tract providing depot for recurring hepatocyte exposure.

*Fauser and Faulstich, 1973. Biliary Drainage*
Clinical Presentation

- **Delayed Onset of Symptoms:** Minimum 6-24+ hours.
- **Gastrointestinal Phase:** 8-48 hours.
  - Sudden onset of severe colicky abdominal pain.
  - Severe Nausea & Vomiting
  - Severe “rice water” or “cholera-like” diarrhea.
  - Dehydration/Hypovolemia with Prerenal Azotemia
- **Honeymoon/Quiescent Phase:** 48-72 hours.
  - Relative Remission of Clinical Symptoms accompanied by insidious rise in transaminases, progressive coagulopathy, and slowly rising creatinine.
- **Hepatic/Cytotoxic Phase:** 72-96 hours.
  - Recurrence of GI symptoms: watery, bloody diarrhea. Possible renal failure.
Diagnosis

- Not difficult, especially with history of foraged mushroom ingestion and delayed onset of symptoms.
- Serial transaminases will rule in/out the diagnosis.
- Mixed mushroom ingestions can be misleading.
- Identification of leftovers by a mycologist can be either helpful or misleading. Should be neither relied upon for diagnosis, nor allowed to delay admission & aggressive intravenous hydration.
- Laboratory confirmation of amatoxin from urine is generally not available.
- Meixner Test not reliable.
Labs

- Initially order CBC, Comprehensive Metabolic Panel, LFTs, PT/INR.
- Hemoconcentration & Azotemia can be impressive at initial presentation.
- LFTs, PT/INR often normal at first but will rise 6-12 hours later.
- Follow serum phosphate, magnesium, and ammonia. Factor V’s can be useful if run in-house.
Prognosis

- **Dosage**
  
  *The more one eats, the sicker one gets.*

- **Age**
  
  Mg/kg exposure will be much greater in children. Elderly more susceptible to renal failure.

- **Length of Latency Period**
  
  The shorter the period before onset of symptoms following ingestion, the worse the prognosis. Onset at less than 10 hours, particularly at 8 hours or less is associated with poor outcomes.
Prognosis: Lab Parameters

- **Transaminases**: May reach 10’s of thousands after a large ingestion.
- Defects in protein synthesis, especially clotting factors are associated with poor outcomes. Significant elevation of **PT/INR** in first 48 hours or >100 seconds at anytime is especially bad. Cannot be more than partially corrected by Vitamin K or FFP. Return of INR toward normal is a good prognostic sign and the earliest manifestation of recovery.
- Persistent **hypoglycemia** is a particularly bad sign.
- **Metabolic acidosis** may be progressive, exacerbated by renal failure. Elevated lactic acid often seen at presentation.
- Rising serum **ammonia** correlates with onset of hepatic encephalopathy & coma.
- Bilirubin levels rise late. Not a reliable indicator of
Traditional Treatment of Amatoxin Poisoning
Management

- Aggressive IV rehydration with the rapid establishment of a brisk urine output is essential for the successful elimination of amatoxin from the general circulation, reversal of the prerenal azotemia and metabolic acidosis seen at presentation, and for the prevention of early acute renal failure secondary to ATN.

- Serial Labs (LFTs/INRs) are key to the confirmation of the diagnosis and predicting clinical course of hepatic failure.

- Serial doses of activated charcoal (M-DAC), 10 grams q 2-4 hours for binding of amatoxin traditionally used. No supportive data.

- Nasoduodenal Tube/Nasogastric Tube. Poorly tolerated. No supportive data.
Management

• Hepatic Phase
  Good Supportive ICU Care is key with ongoing aggressive fluid management; attention to glucose & phosphate to prevent hypoglycemia, hypophosphatemia.
  Early Transfer to Liver Transplant Program.
  Vitamin K & FFP for coagulopathy.
  Hepatic Encephalopathy: Lactulose & intraluminal antibiotics

Frequently Used but Not Helpful
  IV PEN G 40 million units/day. IV NAC.
  Thiocytic Acid- No efficacy in animal studies.
  Steroids, Cimetidine, hyperbaric oxygen.

**Extracorporeal Purification**: Hemoperfusion, Plasma Exchange, MARS (Molecular Absorbent Regenerating System)
Liver Transplant

- No precise criteria exist
- MELDs criteria not helpful as bilirubin peaks very late and not correlated with outcomes.
- Overall Clinical Assessment most important.
- List early, but some will recover with continued aggressive supportive care. Generally 2-3 days to locate donor.
- Life saving but risky, expensive, and survivors require life long immunosuppression.
- Some will survive without after prolonged hospital course and likely long-term morbidity.
Silibinin/Legalon Sil

- Approved in Germany since 1983 & 14 European countries.
- Extracted from seeds of *Silybum marianum*, the common milk thistle.
- Inhibits amatoxin uptake via inhibition of OATP1B3 & NTCP transport, interrupting enterohepatic circulation.
- Inhibits extrinsic (TNF alpha mediated) & intrinsic (p53 mediated) apoptosis.
Silibinin/Legalon-Sil

- The only substance proven effective for post exposure prophylaxis of liver failure (*in dogs when given 5 & 24 hrs after oral amatoxin ingestion*).
- Highly favorable results in clinical studies, but until now no prospective trials.
- Data suggests monotherapy is more effective and better tolerated than combining with Penicillin.
Silibinin/Legalon-Sil

• 20-50 mg/kg/day IV in 4 divided doses or by continuous infusion.
• Documented beneficial up to 48-54 hours post ingestion. Santa Cruz cohort received first doses ~ 78 hours post ingestion.
• Warmth/Flushing during infusion is the only commonly reported side effect.
Influence of the Addition of TNF & Silibin on the Cytotoxic Action of Amanitin in Rat Hepatocyte Culture
Intravenous Milk Thistle (Silibinin-LegalonSIL) for Hepatic Failure Induced by Amatoxin/Amanita Mushroom Poisoning

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- Enrollment began September 2009 with Newton, MA cohort.
- 60 cases now treated including 10 by prior Emergency IND
- SIL induces a predictable drop in INR by 48 hours following initiation of treatment heralding full recovery provided that renal function has been preserved.
- NAC, Pen G, MDAC all ineffective.
- Early development of ARF invariably associated with poor outcomes.
Nasobiliary Drainage

- 1973: Beagles undergoing pre-exposure placement of a surgical biliary fistula survived lethal amatoxin exposure.


Amatoxin & Bio-Chemical Terrorism

- Readily available for collection worldwide.
- Safe to handle; Easily transported; Virtually undetectable. No technical expertise required for preparation. Process in any kitchen.
- Amatoxin is not inactivated by drying, freezing, or cooking. Water insoluble.
- Savory delicious taste.
- Delayed onset of symptoms: Gastroenteritis like syndrome F/B honeymoon phase during which liver and renal failure may develop insidiously.
- No readily available assay for clinical detection
October 2001: 6 young men ingest presumed hallucinogenic mushrooms they obtain after breaking into an Illinois rural trailer-home where *Amanita bisporigera* mushrooms had been intentionally collected. Owner was concentrating mushrooms into syringes for “research purposes” but had no academic or institutional affiliation.

Several **pounds** of dried *Amanita bisporigera* confiscated by police.

Illinois Poison Control subsequently notified state, federal, and FBI Bioterrorism task forces.

*Veterinary and Human Toxicology, August 2003.*
Concluding Remarks

• Mortality following mild-moderate AMP remains extremely high in developing countries where access to care is limited, presentation is often delayed and *aggressive hydration may not be administered*.

• Mortality following a large ingestion remains extremely high even in Europe and North America.

• Rapid diagnosis, early admission and especially *aggressive intravenous hydration* are essential to preventing a bad outcome.

• Nasogastric/duodenal drainage and multidose activated charcoal (MDAC) are poorly tolerated and appear to be ineffective.
Concluding Remarks

- **Intravenous Silibinin** is now available in North America via an FDA sanctioned Open IND Clinical Trial. It is safe, well tolerated, and should be made available to all cases regardless of severity.
- **IV Penicillin G** had no demonstrable efficacy in the large French reviews of 2001 and 2010.
- Silibinin **monotherapy** is preferred to combination with Penicillin G.
- **NAC** raises the INR and thus can adversely affect the clinical decision making process. It has not been shown to be helpful in animal studies and should be avoided.
- Nasoduodenal drainage and multi-dose activated charcoal are poorly tolerated and appear to be ineffective.
- **Biliary drainage** should be considered for **sicker** patients particularly when silibinin is unavailable or the very sick patient in combination with silibinin.