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The enterohepatic circulation of amanitin: Kinetics and therapeutical implications

Christian Thiel[a], Karolin Thiel[a], Wilfried Klingert[a], Andreas Diewold[a], Kathrin Scheuermann[a], Elmar Hawerkamp[a], Johannes Lauber[a], Johannes Scheppach[a], Matthias H. Morgalla[b], Alfred Königsrainer[a], Martin Schenk[a,*]

[a] Department of General, Visceral and Transplant Surgery, Tuebingen University Hospital, Hoppe-Seyler-Strasse 3, Tuebingen 72076, Germany
[b] Department of Neurosurgery, Tuebingen University Hospital, Hoppe-Seyler-Strasse 3, Tuebingen 72076, Germany

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ABSTRACT

Background: Amatoxin poisoning induces a delayed onset of acute liver failure which might be explained by the prolonged persistence of the toxin in the enterohepatic circulation. Aim of the study was to demonstrate amanitin kinetics in the enterohepatic circulation.
Methods: Four pigs underwent α-amanitin intoxication receiving 0.35 mg/kg ($n = 2$) or 0.15 mg/kg ($n = 2$) intraportally. All pigs remained under general anesthesia throughout the observation period of 72 h. Laboratory values and amanitin concentration in systemic and portal plasma, bile and urine samples were measured.
Results: Amanitin concentrations measured 5 h after intoxication of 219 ± 5 ng/mL (0.35 mg/kg) and 64 ± 3 (0.15 mg/kg) in systemic plasma and 201 ± 8 ng/mL, 80 ± 13 ng/mL in portal plasma declined to baseline levels within 24 h. Bile concentrations simultaneously recorded showed 153 ± 28 ng/mL and 99 ± 58 ng/mL and decreased slightly delayed to baseline within 32 h. No difference between portal and systemic amanitin concentration was detected after 24 h.
Conclusions: Amanitin disappeared almost completely from systemic and enterohepatic circulation within 24 h. Systemic detoxification and/or interrupting the enterohepatic circulation at a later date might be poorly effective.

1. Introduction

Over 95% of fatal mushroom poisoning in the world occurs after ingestion of Amanita species, primarily "death cap" (*Amanita phalloides*). Amatoxins (Faulstich and Wieland, 1996; Karlson-Stiber and Persson, 2003), contained in these poisonous mushrooms, has well-known effect on humans (Vetter, 1998) by binding to and inhibiting nuclear RNA polymerase in eukaryotic cells. Lesions are found particularly in hepatocytes but also in kidney tubular cells. Hepatocytes incorporate the toxin fast and excrete them into the bile, so that amatoxins could be detected in the gastrointestinal fluid and faces (Jaeger et al., 1993), but toxin removal is mainly based (>85%) on renal elimination.

The clinical course of amatoxin poisoning (Faulstich, 1979) is characterized by a asymptomatic incubation delay from 6 to 12 h following gastrointestinal syndromes, such as vomiting, diarrhea, abdominal pain, hypoglycaemia and dehydratation after 6–12 h. Hepatocellular damage will become evident clinically and biochemically leading to progressive coagulopathy on day second or third. In fatal cases patients develop acute liver failure including haemorrhages, encephalopathy and coma following renal and/or multiorgan failure at around 6–8 days. Mortality ranges from about 10%–20% in adults to 22%–50% in children (Enjalbert et al., 2002; Jander and Bischoff, 2000) reported by different authors. Amatoxin kinetics could yet not be clearly demonstrated in human poisoning because of the delayed clinical presentation of most intoxicated patients.

It has first been postulated from results of animal studies with beagle dogs that amanitin appears in the bile fluid after intravenous administration (Faulstich and Fauser, 1973). They concluded that the biliary excretion of amanitin prolong their presence in systemic and enterohepatic circulation by intestinal reuptake. Therefore the clinical course of poisoning in humans and animals could be significantly influenced by this mechanism. These results were confirmed by further experimental animal studies (Faulstich et al., 1980a; Faulstich et al., 1985; Faulstich and Fauser, 1973) and transferred into clinical practise, although it has never been shown that amanitin kinetics in portal plasma confirm the theory of relevant or

* Corresponding author. Tel.: +49 7071 2986722; fax: +49 7071 29 4395.
E-mail address: Martin.Schenk@med.uni-tuebingen.de (M. Schenk).

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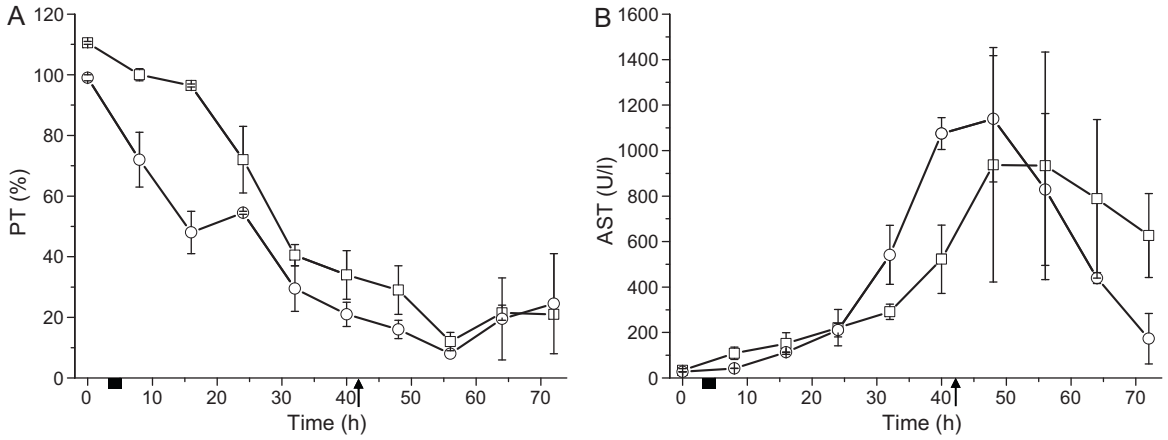

Fig. 1. Profile of prothrombin time (A), aspartate aminotransferase (B). (□) Indicates the 0.35 mg/kg group, (○) indicates 0.15 mg/kg group. Black bar indicates intoxication period over 120 min; black arrow indicates onset of ALF.

delayed intestinal reuptake. Based upon this theory, the attempt of interrupting the enterohepatic circulation through enteral administration of activated charcoal or other medications has become a commonly accepted substantial part of detoxification strategies. Extra-corporal blood purification through hemoperfusion as well as blocking the reuptake of amatoxins into hepatocytes using various chemotherapies was introduced to detoxification management. More recently, endoscopic nasobiliary drainage was performed to remove bile fluid completely in the case of a 18 year old patient who ingested extremely high doses of amatoxin (Madhok et al., 2006). A total amatoxin concentration of 2.5 mg could be removed through bile sampling on day second after poisoning. But the clinical relevance remained unclear. Large animal models evaluating the significance of the enterohepatic amanitin reuptake still do not exist.

Aim of our study was to evaluate amanitin kinetics in the enterohepatic circulation representing the clinical relevance of enteral detoxification. Simultaneous measurements of amanitin concentrations in systemic, portal, bile and urine samples have not been reported previously in a pig model and the hypothesis of relevant enterohepatic circulation of amanitin via the bile could thereby be tested.

2. Methods

2.1. Animals

After approval by the institutional review board for animal experiments, four female German landrace pigs weighing 34 ± 1 kg underwent α-amanitin (AppliChem GmbH, Darmstadt, Germany) intoxication after overnight fasting. All experiments were performed according to the international principles governing research on animals and under the supervision of a veterinarian, who set the guidelines for minimizing the suffering of the pigs. The aimed observation period in this study was 72 h. Pigs were euthanized by a single intravenous bolus of 10 mL T 61 (Intervet, Unterschleißheim, Germany).

2.2. Anesthesia and surgical procedures

Intramuscular premedication consisted of atropine 0.1% (0.05 mg/kg), ketamine (7 mg/kg), azaperone (10 mg/kg) and diazepam (1 mg/kg). Continuous infusion of ketamine (15 mg/kg/h), fentanyl 0.02 mg/kg/h and midazolam 0.9 mg/kg/h was administered to maintain anesthesia during the experiment. After oral intubation, animals remained in deep general anesthesia receiving pressure-controlled ventilation (KION, Siemens Medical, Sweden) until conclusion of the study protocol. Character of respiration, heart rate, eye movement and pain stimulus were used to confirm the depth of anesthesia; if any of these parameters indicated a lessening of anesthesia, infusion rates of anaesthetic agents were increased.

A stomach tube was placed for intestinal drainage. Adequate temperature 38–39 °C was maintained with a warming mat. An antibiotic prophylaxis of 2 g ceftriaxon (Rocephin®, Hoffmann-La Roche, Basel, Switzerland) was given every 24 h. The neck vessels were instrumented to measure arterial (Leadercath, Vygon, Écouen, France) and central venous pressure (Multi-Lumen Central Venous Catheter, Arrow International, Reading, PA, USA). Animals were laparotomized and a urinary catheter was placed to measure urinary output. An 18-gauge catheter (Cavafix®, Braun Melsungen, Germany) was inserted into the portal vein via cannulation of a small mesenteric vein for amanitin intoxication and portal blood sampling. A 2.5 mm Kehr T-tube (Ruesch GmbH, Kernen, Germany) was inserted into the common bile duct. The T-tube remained clamped so that no bile fluid was drained externally over the observation period. The t-tube was only opened every 8 h for the sampling of 500 μL bile fluid aliquots. Liver biopsies were sampled every 24 h.

2.3. Amanitin intoxication, acute liver failure and intensive care monitoring

0.35 mg/kg or 0.15 mg/kg α-amanitin were dissolved in 25 mL saline and administered intraportally over 120 min via the implanted portal vein catheter. Acute liver failure was defined by a decline of prothrombin time below 30% confirmed by the clinical presence of hemodynamic changes and histology.

Monitoring included electrocardiogram, mean arterial, central venous and intracranial pressure, oxygen saturation and core body temperature. Urinary output, arterial blood gas analysis (ABL 800, Radiometer Copenhagen, Denmark) including haemoglobin, lactate, serum electrolytes, acid base balance and blood glucose levels were monitored hourly and immediately corrected as required. Hydroxyethylstarch 6% (Voluven® HES 130/0.4, Fresenius, Bad Homburg, Germany) and sodium chloride solution 0.9% were used for fluid management to stabilize the hemodynamic parameters such as mean arterial pressure within a range of 60–70 mmHg and central venous pressure within 6–12 mmHg. Norepinephrine was used to ensure hemodynamic stability in the end-stage of acute liver failure. Blood glucose levels were maintained >100 mg/dL by glucose 20% solution, haemoglobin values remained stable within the range of 8.5–11.5 g/dL.

2.4. Biochemical analysis

All blood samples were measured by the certified central laboratories of the university hospital Tuebingen (Zentrallabor, Innere Medizin IV, Universitätsklinikum Tuebingen, Germany). Prothrombin time, aspartate aminotransferase, total plasma protein, albumin, bilirubin, creatinine and ammonia were analyzed in systemic blood samples. Amanitin concentration measurements from systemic, portal, bile and urine samples were performed before starting surgical procedures and every 8 h until conclusion of the study protocol. α-amanitin concentration measurements were performed by an ELISA-kit (Buehlmann, Basel, Switzerland).

2.5. Statistical analysis

Results in the manuscript are reported as mean ± standard deviation (SD). Figures are presented as mean ± standard error of mean (SEM).

3. Results

3.1. Clinical course and laboratory values

All pigs developed ALF within 39 ± 10 h. Due to standardized intensive care therapy (Thiel et al., 2010) in pigs, vital and ventilation parameters could be stabilized in acute liver failure until the end of the observation period of 72 h. Relevant laboratory parameters such as blood count, prothrombin time, aspartate aminotransferase, total plasma protein, albumin, ammonia, biliru-