Treatment of Amanita Phalloides Intoxication by Fractionated Plasma Separation and Adsorption (Prometheus®)

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Abstract

Objective: To investigate the effectiveness and safety of extracorporeal detoxification using the fractionated plasma separation and adsorption system (FPSA, Prometheus® 4008H, Fresenius Medical Care, Germany) in patients suffering from acute liver failure due to intoxication with Amanita phalloides (AP) toxin. Methods: The study population consisted of 20 patients with proven AP intoxication (FPSA treatment group n=9, control group n=11). Urinary amanitin toxin concentration was measured by the Amanitin ELISA Kit (Bühlmann Laboratories, Germany, cut off level 1.5ng/ml). All patients received standard medical treatment with activated charcoal, i.v. crystalloid fluids, siliibinine and N-acetylcysteine. Additionally 9 patients underwent treatment with FPSA until undetectable amanitin levels. Results: Mean urinary amanitin levels were significantly reduced by FPSA with 42.5 ± 21.9 ng/ml before and 1.2 ± 0.31 ng/ml after treatment (p=0.04). No hemodynamic, respiratory or hematological complications were observed. None of the patients had to undergo liver transplantation. All patients in the treatment group survived and were discharged fully recovered. One patient in the control group died due to shock and lactic acidosis; one patient remained dialysis dependent. Mean duration of hospital stay was 7.1 days in the treatment group and 11.7 days in the control group (p=0.30). Conclusions: Use of liver support therapy by fractionated plasma separation and adsorption (Prometheus®) offers a safe way for elimination of Amanita toxin with the potential to avoid the need for liver transplantation.

Key words

Acute liver failure – extracorporeal liver support therapy – amanitin intoxication – liver transplantation.

Introduction

Mushroom poisonings are a common reason for hospital admission in Europe. More than 90% of intoxications that require medical treatment are caused by toxin of Amanita phalloides (AP, death cap). The death cap mushroom is widespread over all parts of Europe and is frequently picked especially by people from South- or Eastern Europe due to mistaking it with other nontoxic mushrooms, e.g. the common Champignon mushroom Agaricus bisporus or its green relative, Agaricus furcatus. Amanita species (e.g. Amanita, Lepiota and Galerina spp.) provide three different types of heat resistant amatoxin (α-, β- and γ-amanitin), whereas α-amanitin is the most toxic one [1-3]. After enteral absorption it underlies enterohepatic circulation and is actively taken up especially by hepatocytes and renal cells causing cellular necrosis and apoptosis [4]. Intracellularly it acts as a RNA II polymerase inhibitor blocking the transcription process which causes immediate termination of protein synthesis.

Clinical symptoms may vary depending on the amount of mushrooms eaten and their content of toxin which diversifies according to the region where they are picked. The lethal dose of amanitin for humans is 0.1mg/kg body weight [5] and may be contained in one single mushroom. After ingestion, AP intoxication typically presents with 3 clinical phases: an asymptomatic lag period of 5-24 hours (median 11 hours) is followed by a gastrointestinal phase with nausea, vomiting and severe watery diarrhoea which usually lasts for 12-24 hours. Subsequently the patient seems to recover while elevation of amino transaminases, drop of coagulation parameters, jaundice, hepatic encephalopathy and ultimately acute hepatic and renal failure occur within the hepatorenal phase after 2-3 days [6].

Standard medical treatment includes oral decontamination with activated charcoal to absorb remaining toxins and...
to interrupt enterohepatic circulation. Additionally, i.v. rehydration with crystalloid fluids is given. Hepatoprotective N-acetylcysteine (NAC) and silibinine (the main isomer contained in silymarin, extracted from the seeds of Silybum marianum or milk thistle) are commonly administered in many patients [7–9]. Despite optimal medical treatment intoxication with AP still has a high mortality of up to 20% [10, 11]. In fatal courses, fulminant hepatic failure occurs and immediately requires liver transplantation as the only curative treatment option.

Considering the organ shortage, which results in patients dying on the waiting list despite high urgency status, bridging therapies to transplantation using the extracorporeal Molecular Absorbents Recirculating System (MARS) have recently been under investigation. However, literature is limited to case reports and few retrospective analyses. Data provided presumes MARS to be safe in treatment of AP-associated acute liver failure, but mortality and need for transplant remain almost unaltered [12–14]. Using a different technique, benefit of fractionated plasma separation and adsorption in combination with high-flux dialysis (Prometheus®) has been examined especially in condition of chronic liver failure due to liver cirrhosis and may offer reduced mortality and better outcome to a certain group of patients (e.g. hepatorenal syndrome Type I, MELD score greater than 30) compared to standard treatment [15]. The use of Prometheus® as a purification technique for water soluble and protein bound toxins in acute liver failure has not been fully elucidated yet. Available data shows quick detoxification in patients suffering from various intoxications, e.g. such as acetaminophen or mushroom poisoning in general without testing for amatoxins [16, 17]. Yet, there is no data on AP intoxication and course of amanitin levels under treatment with FPSA.

In the present study we investigated the safety and effectiveness of the fractionated plasma separation system in combination with high-flux dialysis (FPSA, Prometheus® 4008H, Fresenius Medical Care, Germany) in addition to standard medical treatment in order to achieve rapid detoxification from amanitin and to prevent the need for liver transplantation.

**Patients and methods**

**Study population**

The study population consisted of 20 patients with proven AP poisoning admitted to 6 hospitals of standard care or tertiary care in the German Rhein-Main area. Patients with mushroom poisoning other than AP or patients with negative amanitin levels were excluded. Urinary amanitin toxin concentration was measured by the Amanitin ELISA Kit (Bühllmann Laboratories, Germany, cut off level 1.5ng/ml). Nine consecutive patients admitted to ICU of Frankfurt University Hospital between August 2008 and September 2010 were included in the treatment group. Symptomatic patients (e.g. diarrhoea, nausea, vomiting) with positive Amanitin levels and elevated transaminases received standard medical treatment and fractionated plasma separation and adsorption (Prometheus®, Fresenius Medical Care) via a central-venous shaldon’s catheter until undetectable amanitin levels. Standard medical treatment included activated charcoal, i.v. rehydration with crystalloid fluids, silibinine at a dosage of 20 mg/kg daily and N-acetylcysteine at a dosage of 150mg/kg i.v.-bolus, followed by 50mg/kg within 4 hours followed by 100mg/kg within 16 hours. In FPSA citrate was used for anticoagulation. The treatment was administered in 1 with a maximum of 2 cycles. Duration of one treatment cycle in all patients was 6 hours. Written informed consent was obtained from all patients before treatment was started.

Amino transferases, bilirubin, prothrombine index, antithrombin III, blood urea nitrogen, creatinine, factor V, ammonia and Amanitin levels were assessed daily.

Patients’ data in the treatment group was matched with the data of the control group of 11 patients who had been tested positive for amanita toxin in our local laboratory between October 2004 and September 2010. Those patients were treated with the same medical therapy as mentioned above including oral charcoal, i.v.-fluids, N-acetylcysteine and silibinine. No extracorporeal detoxification method was used in those patients. Treatment, monitoring and taking of blood samples was equal in both groups except for the staff controlling the Prometheus machine.

Primary endpoint of this study was survival, secondary endpoints were the need for liver transplantation, length of hospital stay and need for haemodialysis due to renal failure.

**Statistics**

The results are presented in means ± standard error of the mean. A p-value of less than 0.05 was considered significant. All p-values are two-tailed. T-test was performed to compare independent means. Odds ratio was calculated to assess the risk of positive outcome. Data and figures were analyzed and compiled using MedCalc® Version 11.5.00 (2011, Mariakerke, Belgium).

**Results**

**Outcome in the study population**

At the end of the study period all patients in the Prometheus® group had survived, one patient in the control group had died from acute liver failure with lactic acidosis followed by refractory shock with consecutive cardiac arrest 3 days after admission. The patient was not listed for transplant due to his age of >70 years. Odds ratio for positive outcome in the treatment group compared to the control group was 2.71 (CI 0.09 - 74.98), but no statistical significance (p=0.55) was reached due to the small number of patients. No further renal replacement therapy than that applied by using FPSA was needed in any patient of the treatment group. A 62-year old woman in the control group had died from acute liver failure with lactic acidosis before treatment was started. A 62-year old woman in the control group had died from acute liver failure with lactic acidosis before treatment was started. A 78-year old developed progressive renal failure during her hospital stay and was on dialysis even after discharge, and a 78-year old
Amanita phalloides intoxication treated by FPSA

A man maintained elevated renal function parameters at the time of discharge from hospital. None of the patients in both groups had to undergo liver transplantation. Mean duration of hospital stay was 7.1 days in the treatment group and 11.7 days in the control group (p=0.30). All the patients in the treatment group were discharged completely recovered.

**Comparison of laboratory variables in both groups**

Patient characteristics on admission are shown in Table I. Characteristics were well balanced between both groups; no significant differences were seen in time from ingestion to diarrhoea (p=0.44), in time from ingestion to admission (p=0.82), in laboratory parameters AST (p=0.53), ALT (p=0.88) and amanitin levels (p=0.23). Three patients in both groups showed mild grade I-II hepatic encephalopathy.

Mean levels of AST in the treatment group were 1906 ± 880.3 IU/l on admission and declined to 290.8 ± 129.8 IU/l, whereas AST levels in the control group were 1109.2 ± 774.4 IU/l on admission, raised to 1430.5 ± 695.7 IU/l on day 2 and remained elevated on day 5 at 529.2 ± 390.5 IU/l. Mean ALT levels in the treatment group were 1882.2 ± 875.8 IU/l and declined to 1069.1 ± 343.1 IU/l after 5 days. In the control group mean ALT was 2104.6 ± 1283.7 IU/l on day 1 and 1197 ± 619.1 IU/l on day 5. As is depicted in Fig. 1 no further increase of AST/ALT was seen after initiation of Prometheus® treatment on day 2 and values kept dropping continuously until day 5. In contrast, liver enzymes in the control group peaked on day 2, then started to drop until day 4 and then slightly rose again on day 5. After 5 days, levels of ALT and AST were slightly higher in the control than in the treatment group whereas these differences were not statistically significant (p=0.43).

Liver synthesis was diminished on admission in both groups showing a mean prothrombine index 72.5 ± 6.91 % (treatment group) and 62 ± 19.43 % (control group), respectively (p=0.47). A further deterioration in both groups was seen on day 2; recovery to normal range was observed after 5 days (Fig. 2). The course of Factor V within 5 days was similar to prothrombin index in Prometheus® group. Factor V levels in the control group were not assessed.

<table>
<thead>
<tr>
<th>Table I. Patient characteristics on admission</th>
<th>Treatment group Patients (n=9)</th>
<th>Control group Patients (n=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male)</td>
<td>3</td>
<td>6</td>
<td>0.57</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.1 ± 5.39</td>
<td>50.8 ± 7.97</td>
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</tr>
<tr>
<td>Chronic liver disease</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Interval from ingestion to diarrhoea (h)</td>
<td>16.2 ± 4.63</td>
<td>12.1 ± 5.2</td>
<td>0.44</td>
</tr>
<tr>
<td>Time from ingestion to admission (h)</td>
<td>35.7 ± 6.37</td>
<td>39.0 ± 38.2</td>
<td>0.82</td>
</tr>
<tr>
<td>Hepatic encephalopathy (no. of patients)</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**Laboratory values on admission**

<table>
<thead>
<tr>
<th></th>
<th>Treatment group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/l)</td>
<td>1906 ± 880.3</td>
<td>1109.2 ± 774.4</td>
<td>0.53</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>1882.2 ± 875.8</td>
<td>2104.6 ± 1283.7</td>
<td>0.88</td>
</tr>
<tr>
<td>Prothrombin index (%)</td>
<td>72.5 ± 6.91</td>
<td>62 ± 19.43</td>
<td>0.37</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.0 ± 0.11</td>
<td>1.57 ± 0.45</td>
<td>0.21</td>
</tr>
<tr>
<td>Amanitin (ng/ml)</td>
<td>42.5 ± 21.9</td>
<td>15.4 ± 8.64</td>
<td>0.23</td>
</tr>
<tr>
<td>Factor V</td>
<td>33.2 ± 8.13</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: not available

Mean bilirubin levels were 1.03 ± 0.11 mg/dl in the treatment group on day 1, and 1.57 ± 0.45 mg/dl in the control group, respectively. A slight peak on day 3 was seen in the control group together with elevated results in both groups after 5 days (p=0.14).

For trend of further laboratory values see Figs 1-3.

**Treatment with FPSA**

Therapy with FPSA was generally well tolerated. No hemodynamic, respiratory or haematological complications were observed.

Mean urinary amanitin levels in the treatment group were significantly reduced by FPSA with 42.5 ± 21.9 ng/ml before and 1.2 ± 0.31 ng/ml after treatment (p=0.04; Fig. 4).

![Fig 1. Course of AST and ALT levels during 5 days after treatment with FPSA on day 1.](image-url)
A more constant decline of liver enzymes was seen in those patients treated with FPSA whereby patients’ values in the control group suggested a bimodal trend.

Coagulation parameters showed a similar course in both groups suggesting that these parameters are not influenced by FPSA.
A constant rise in bilirubin levels was observed in both groups with no significant difference after 5 days.

Grade I-II hepatic encephalopathy occurred in 3 patients, corresponding to elevated levels of ammonia (144, 106 and 101 pg/dl) that decreased after treatment with FPSA (90, 55 and 41 pg/dl, p=0.10).

Discussion

The present study was conducted to investigate the influence of FPSA on early toxin clearance from the blood in patients with acute AP poisoning. Little is known about kinetics of amanitin intoxication. As Jaeger et al have demonstrated in patients without extracorporeal detoxification, amanitin is still present in the liver and the kidney after 5 days. It is cleared from plasma within 36 hours which conversely means it is taken up by hepatocytes and renal cells, but it is detectable in urine until day 4 [19]. This underlines the importance of an early treatment strategy in order to avoid further cellular uptake and thereby minimize the extent of hepatic damage.

Typical procedure-related complications of extracorporeal liver support systems such as bleeding, haemodynamic instability or severe coagulation disorders that have been described in other studies [18] were not observed in our trial. The outcome of the treatment group was not significantly different from the control group mainly due to the small patients group, but a tendency to a favourable outcome cannot be disregarded since full recovery was given to all FPSA-treated patients whilst more secondary complications occurred in the control group.

Since Prometheus® is known to eliminate protein-bound and water-soluble toxin [21], detoxification from amanitin was expected and could be demonstrated by significantly reduced urinary toxin levels after one treatment cycle. But despite this being a positive result, the extent of effectiveness of FPSA can hardly be assessed properly at this time for several reasons. First, another toxin binding therapy known to interrupt enterohepatic circulation (activated charcoal) was given to all patients. Second, there is no data provided on follow-up amanitin levels in the control group. If amanitin was eliminated in a similar fashion in that group it is unlikely, since the toxin is known to be detectable in urine for 4 days, but this was not tested.

Furthermore, it remains unclear to which extent elimination of blood serum amanitin by extracorporeal detoxification contributes to the improvement of patients’ condition whereas intracellularly absorbed amanitin cannot be removed and keeps blocking protein synthesis irreversibly until the cell is replaced. This process takes several days and the effect can be observed clinically when the patient’s condition starts to improve after 4-5 days; it does not matter if they are treated with extracorporeal detoxification or not. Therefore, a crucial point that must be worked out in further studies is to screen patients that will potentially benefit from such treatment. As Ganzert et al have demonstrated, a more fatal outcome in AP intoxication can be predicted by using a combination of prothrombin index and serum creatinine [10]. In their study, prothrombin index below 21% plus creatinine over 1.2mg/dl was able to predict patient’s death with a probability of 92%. Prospectively, these may be the patients that benefit from extracorporeal detoxification, but the patients in our group did not fulfill these criteria. If that is the case, listing for transplantation should be considered, since sensitivity of these combined values was 100% and therefore seems more appropriate than criteria conventionally used (e.g. Clichy or King’s College criteria) that have been developed for acute liver failure for other reasons (e.g. viral, acetaminophen intoxication).

If the decision has been made to use extracorporeal toxin elimination the question which system to prefer – MARS or Prometheus® – can currently not be answered since large controlled randomized trials for both procedures are still lacking. According to a recently published review by Wittebole et al, the use of MARS in patients with acute liver failure shows clinical and biological benefit and is seen as a potentially promising treatment for patients with acute poisoning from drugs that have high protein-binding capacity and are metabolized by the liver [20]. Data on MARS treatment in acute liver failure due to AP intoxication described in the literature presumes the procedure to be safe and effective. Similar to the present study, no major complications were seen. Minor complications included thrombocytopenia, bleeding or problems regarding the vascular access. The number of treatments applied to patients was higher than in our study; patients usually received 2-3 cycles of MARS [12-14] compared to one cycle of Prometheus® in the present study.

To date, the main advantage of the Prometheus® system seems to be the better in vivo clearance. In a study conducted by Evenepoel et al, Prometheus® achieved significantly higher clearance rates of bilirubin, ammonia and urea. This resulted in higher reduction ratios of these substances. Unconjugated bilirubin, which is a marker of strong protein binding, was only removed by the Prometheus system and not by MARS [21].

Nevertheless, none of the trials concerning MARS or Prometheus could demonstrate a significant impact on the patient’s outcome and nor did our study, due to its limited number of patients.

Conclusion

In conclusion, the use of FPSA in AP intoxication provides a safe method to eliminate amanitin in acute symptomatic intoxication. Trends of shorter hospital stay and reduction of secondary complications such as renal failure were seen, but were not statistically significant. A clear and definite statement about the effectiveness of FPSA in AP intoxication cannot be made with the present study. Randomized controlled trials with a greater number of patients are needed to address this issue, to figure out the optimal detoxification system as well as the optimal number of treatment cycles and to identify patients who
will potentially benefit from extracorporeal detoxification in order to avoid costs and unnecessary treatment.

Conflict of interest statement
The authors have no conflicts of interest to declare.

References