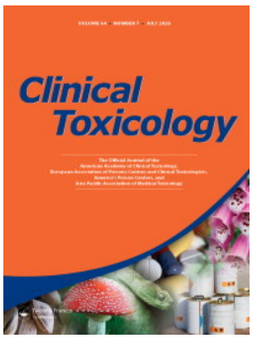

Clinical Toxicology

ISSN: 1556-3650 (Print) 1556-9519 (Online) Journal homepage: www.tandfonline.com/journals/ictx20

Serum benzylpenicillin levels during treatment for amatoxin poisoning: pharmacokinetic support for OATP1B3 inhibition

Andrea Missanelli, Giada Crescioli, Cecilia Lanzi, Francesco Gambassi, Alessandra Ieri, Anita Ercolini, Arianna Totti, Roberto Baronti, Nicoletta Cini, Francesca Luceri, Antonio Munafo, Guido Mannaioni & Alfredo Vannacci

To cite this article: Andrea Missanelli, Giada Crescioli, Cecilia Lanzi, Francesco Gambassi, Alessandra Ieri, Anita Ercolini, Arianna Totti, Roberto Baronti, Nicoletta Cini, Francesca Luceri, Antonio Munafo, Guido Mannaioni & Alfredo Vannacci (03 Jul 2026): Serum benzylpenicillin levels during treatment for amatoxin poisoning: pharmacokinetic support for OATP1B3 inhibition, Clinical Toxicology, DOI: 10.1080/15563650.2026.2688233

To link to this article: https://doi.org/10.1080/15563650.2026.2688233

Published online: 03 Jul 2026.

CLINICAL TOXICOLOGY
https://doi.org/10.1080/15563650.2026.2688233

Taylor & Francis
by informa

POISON CENTRE RESEARCH

Serum benzylpenicillin levels during treatment for amatoxin poisoning: pharmacokinetic support for OATP1B3 inhibition

Andrea Missanelli[a], Giada Crescioli[b,c], Cecilia Lanzi[a], Francesco Gambassi[a], Alessandra Ieri[a], Anita Ercolini[a], Arianna Totti[a], Roberto Baronti[d], Nicoletta Cini[e], Francesca Luceri[e], Antonio Munafo[f], Guido Mannaioni[a,b] and Alfredo Vannacci[b,c]

[a]Toxicology Unit and Poison Control Center, Careggi University Hospital, Florence, Italy; [b]Department of Neurosciences, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy; [c]Tuscan Regional Center of Pharmacovigilance, Florence, Italy; [d]Laboratory of Clinical Toxicology and Antidoping, Azienda USL Toscana Centro, Florence, Italy; [e]General Laboratory, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [f]Headache center and Clinical Pharmacology, AOU Careggi, Florence, Italy

ABSTRACT

Introduction: Benzylpenicillin has long been included in treatment protocols for amatoxin poisoning based on experimental evidence suggesting inhibition of hepatocellular amatoxin uptake via OATP1B3. However, no human pharmacokinetic data have confirmed whether therapeutic regimens achieve serum concentrations sufficient to inhibit this transporter *in vivo*.

Methods: We retrospectively analyzed serum benzylpenicillin concentrations in 16 patients with confirmed amatoxin poisoning treated at the Florence Poison Control Center between 2020 and 2024. All patients received continuous intravenous benzylpenicillin infusion (500,000 IU/kg/day for 48 h, followed by 250,000 IU/kg/day for 48 h) as part of a standardized multimodal protocol. Serum samples ($n = 44$) were collected during routine care and analyzed using LC–MS/MS. Concentrations were expressed in micromolar (μM) and compared with the reported OATP1B3 inhibitory concentration (IC_{50} = 25 μM).

Results: During the first 48h, all serum samples exceeded the IC_{50} threshold, with mean concentrations of 265 ± 87 μM at 24 h and 241 ± 74 μM at 48 h. Across all samples, the median concentration was 176 μM (range 45–430 μM), and 93% exceeded 25 μM. Following dose reduction, mean concentrations declined but remained above the IC_{50} in 66% of samples. No adverse events attributable to benzylpenicillin were observed, and all patients survived to discharge without liver transplantation.

Discussion: Although benzylpenicillin's clinical efficacy has yielded conflicting results across retrospective series, variability in dosing regimens and timing likely accounts for much of this inconsistency. The present findings demonstrate that continuous infusion maintains pharmacologically relevant exposure throughout the critical window of amatoxin hepatocellular uptake, supporting target engagement *in vivo*. The reduced-dose regimen sustained inhibitory concentrations in most samples, suggesting dose optimization is feasible without compromising pharmacological activity.

Conclusion: Continuous intravenous infusion of benzylpenicillin achieves serum concentrations exceeding the *in vitro* OATP1B3 inhibitory threshold in patients with amatoxin poisoning, providing human pharmacokinetic support for its proposed antidotal mechanism and its continued inclusion in multimodal treatment protocols.

ARTICLE HISTORY
Received 4 March 2026
Revised 26 May 2026
Accepted 8 June 2026

KEYWORDS
Amanitin; antidotal therapy; benzylpenicillin; pharmacokinetics; poisoning

Introduction

Amatoxin-containing mushrooms, *Amanita phalloides*, *Lepiota brunneoincarnata* and *Galerina marginata*, are responsible for the majority of fatal mushroom poisonings worldwide [1,2]. Amatoxin-related cyclopeptides selectively inhibit RNA polymerase II, disrupting mRNA synthesis and causing hepatocellular necrosis, apoptosis, and oxidative stress [3,4]. The clinical course is characterized by a latency period followed by gastrointestinal symptoms, hepatic dysfunction, and potentially acute liver failure with high mortality rates [5,6]. Despite decades of clinical experience, the optimal therapeutic strategy for amatoxin poisoning remains debated.

CONTACT Alfredo Vannacci alfredo.vannacci@unifi.it Department of Neurosciences, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Viale G. Pieraccini, 6, 50139, Florence, Italy.

Several antidotal/supportive therapies have been investigated: silibinin, acetylcysteine (NAC), and benzylpenicillin (penicillin G), which was introduced in the 1950-60s based on experimental evidence suggesting a protective effect on hepatocytes and interference with amatoxin uptake [7,8]. The hypothesized mechanism involves competitive inhibition of the organic anion-transporting polypeptide OATP1B3, that mediates the hepatic uptake of α-amanitin [9]. *In vitro* data also suggest antioxidant properties and membrane stabilization effects [10,11].

However, despite widespread historical use and reported clinical success [12], the efficacy of benzylpenicillin remains debated. Retrospective studies and case series have yielded conflicting results, some suggesting improved survival while others found no significant benefit compared with silibinin or NAC. A comparative clinical study by Ganzert et al. evaluating a relatively large cohort of patients with amatoxin poisoning found no clear additional benefit of combining benzylpenicillin with silibinin compared to silibinin alone, further highlighting the ongoing uncertainty regarding the clinical role of benzylpenicillin [3,4,13,14]. Systematic reviews and comparative analyses generally favor silibinin as the most effective antidote, with a mechanism of action comparable to that of benzylpenicillin, largely because of its better tolerability. Several international guidelines currently recommend its use as first-line therapy where available [4,15].

Within this framework, benzylpenicillin has persisted mainly on pharmacological plausibility rather than proven clinical superiority. It remains a mechanistically justified and potentially complementary component of combination therapy, whose role may have been limited more by the lack of *in vivo* pharmacokinetic data than by evidence of inefficacy. Letschert et al. [9] demonstrated that α-amanitin uptake into hepatocytes is mediated by the OATP1B3 transporter, using transfected cell models expressing human OATP isoforms. Importantly, this study showed that several compounds, including benzylpenicillin, can inhibit this transporter and reduce intracellular toxin uptake, providing a mechanistic basis for their potential antidotal effect. Authors reported an *in vitro* IC_{50} of ~25 µM for benzylpenicillin on OATP1B3-mediated amatoxin uptake, but whether standard dosing achieves comparable systemic exposure remains unclear. However, this estimate is derived from a single *in vitro* model and should be interpreted cautiously, as comparable IC_{50} data for benzylpenicillin in this context are limited. Given its extensive tissue penetration and close serum–extravascular correlation, circulating levels can reasonably be considered a surrogate of tissue exposure [9]. On this basis, it can be inferred that sustained serum concentrations above the *in vitro* inhibitory threshold are likely to translate into effective hepatic exposure during continuous infusion. Yet no human studies to date have confirmed that therapeutic regimens of benzylpenicillin achieve serum concentrations within or above this inhibitory range. This absence of *in vivo* pharmacokinetic evidence represents a critical gap, especially considering the high doses (up to 1,000,000 IU/kg/day i.v. continuous infusion) administered in clinical protocols [3,11,12,16–18].

The Florence poison control center (PCC) has extensive experience in the management of amatoxin poisoning. Over a 15-year period, the authors' research group analyzed 105 confirmed cases and identified key prognostic and therapeutic patterns [12]. On this basis, the current protocol includes continuous intravenous benzylpenicillin at a reduced dosage (500,000 IU/kg/day for 48 h, followed by 250,000 IU/kg/day). However, it remains unclear whether this regimen achieves plasma concentrations sufficient for OATP1B3 inhibition. Data from veterinary models indicate rapid equilibration between serum and tissue concentrations, suggesting that circulating levels may serve as a surrogate for hepatic exposure [19]. Accordingly, assessing *in vivo* serum concentrations in humans is essential to determine the pharmacological plausibility of penicillin's proposed antidotal mechanism.

The present study aimed to evaluate the pharmacokinetics of intravenous benzylpenicillin in patients treated for amatoxin poisoning, and to determine whether achieved serum concentrations exceed the *in vitro* inhibitory threshold for OATP1B3, validating the pharmacological basis for penicillin use in clinical practice and supporting evidence-based revision of the current treatment protocols for amanitin poisoning.

Methods

Study design and treatment protocol

This retrospective, non-interventional study did not involve prospective recruitment; analyses were performed on biological samples collected during routine clinical care. It was conducted at the Florence PCC, in collaboration with the Clinical Toxicology and Anti-Doping Laboratory of the Local Health Authority. Inclusion criteria were: (1) clinical presentation consistent with amatoxin poisoning (gastrointestinal symptoms after >6 h latency) and (2) laboratory confirmation of urinary amatoxins (>10 µg/L). Urinary α-amanitin was measured using the Amanitin ELISA kit (Bühlmann Laboratories AG, Switzerland) on the Personal Lab

system (Adaltis S.r.l., Italy) at the Careggi University Hospital General Laboratory. All cases had positive amanitinuria, with mycological confirmation when available. All patients were managed according to the Florence PCC therapeutic protocol for amatoxin poisoning, which includes:

- Multiple-dose activated charcoal (MDAC)
- Aggressive intravenous rehydration: administration of Ringer's lactate solution at 20 mL/kg/h for the first two hours, followed by maintenance hydration titrated to a urinary output of at least 1.5–3 mL/kg/h;
- Methylprednisolone: 40 mg/day orally;
- Proton pump inhibitors: intravenous pantoprazole 40 mg/day;
- NAC (300 mg/kg/day): diluted in 500 mL of 5% dextrose solution, administered as a continuous 24-hour intravenous infusion, maintained for at least 4 days or until clinical and biochemical resolution;
- Continuous intravenous infusion of benzylpenicillin.

Benzylpenicillin was administered at a dosage of 500,000 IU/kg/day for the first 48 h, followed by 250,000 IU/kg/day for the following 48 h This dosage protocol, lower than that described by Giannini et al. [12] was introduced in 2020 to minimize drug load while maintaining pharmacological efficacy.

Serum sample collection

Serum for benzylpenicillin quantification was obtained from residual blood after routine liver function testing. To prevent contamination, samples were drawn from the arm contralateral to the infusion line. The first sample was collected 6–12 h after starting continuous infusion, with subsequent samples every ~24 h for up to 4 days (44 total). All samples were analysed at the Clinical Toxicology and Anti-Doping Laboratory of the Local Health Authority.

Analytical quantification of serum benzylpenicillin

Serum benzylpenicillin concentrations were determined using a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method. Analyses were performed using a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, USA) coupled via an electrospray ionization (ESI) interface to an AB Sciex API 4000 Q-Trap triple quadrupole mass spectrometer (AB Sciex, Canada).

Chromatographic separation was achieved using a Poroshell 120 SB-C18 column (2.1 × 150 mm, 2.7 µm). The mobile phases consisted of:

- Phase A: 0.2% formic acid in water
- Phase B: 0.2% formic acid in acetonitrile

Column temperature was maintained at 45 °C. The flow rate and gradient program were as follows: 0 min, 0.3 mL/min, 5% B → 2 min, 0.5 mL/min, 50% B → 6.5 min, 0.5 mL/min, 95% B → 10 min, 0.5 mL/min, 95% B → 11 min, 0.3 mL/min, 5% B → 16 min, 0.3 mL/min, 5% B.

Transitions monitored were m/z 335.2 → 160.2 and 335.2 → 176.2 (DP 98 V, EP 8 V, CE 17). Injection volume was 5 µL. Calibration curves were prepared using penicillin standards (0, 100, 250, and 500 ng/mL) in normal saline. Samples were processed by protein precipitation: 200 µL of serum were mixed with 600 µL acetonitrile, vortexed for 20 s, centrifuged for 10 min, and 100 µL of the supernatant were injected for analysis.

Data analysis

All concentration data were expressed as micromolar (µM) values for direct comparison with the reported OATP1B3 IC_{50} of 25 µM [9]. Results were summarized as means, standard deviations, and proportions of samples exceeding the inhibitory threshold. Temporal trends in concentration were evaluated descriptively by treatment day. To assess whether serum benzylpenicillin concentrations exceeded the minimum IC_{50} required for OATP1B3 inhibition (25 µM), a one-sample Wilcoxon signed-rank test was performed against the predefined threshold.

Ethical considerations

All analyses were conducted in accordance with the principles of the Declaration of Helsinki and the institutional standards for handling anonymized patient data. The study protocol was approved by the local Ethics Committee of AOU-Careggi (Florence, Italy; protocol code AMAMIR1, registered number 21967_bio, date of approval April 26, 2022).

Results

Patients' characteristics

Between 2020 and 2024, sixteen patients (7 males and 9 females; age range 35–84 years) with confirmed

amatoxin poisoning were treated at the Florence PCC according to the institutional protocol. All patients presented with delayed-onset gastrointestinal symptoms (>6 h after ingestion) followed by positive urine amanitin testing, with initial concentrations exceeding 10 µg/L. Mycological confirmation of *Amanita phalloides* or related amatoxin-containing species was available in 14 of 16 cases; 13 patients presented laboratory evidence of hepatocellular injury. The median time from ingestion to hospital admission was 12 h (IQR 9–15 h), and from ingestion to initiation of antidotal therapy was 14 h (IQR 11–18 h). All patients were treated according to the Florence PCC protocol. No patient required liver transplantation, and all survived to discharge. Table 1 summarizes the main characteristics of the included patients, including demographic data (age and sex), clinical presentation (symptoms), and relevant laboratory findings.

Serum benzylpenicillin concentrations

A total of 44 serum samples were collected and analysed for benzylpenicillin concentrations. The mean number of samples per patient was 2.8 (range 2–4 samples). The first sample was collected 6–12 h after the start of the continuous infusion, followed by samples approximately every 24 h.

Figure 1 illustrates the time-course of benzylpenicillin serum concentrations (median and quartiles) during therapy, and Table 2 summarizes descriptive statistics (mean and SD) for each treatment day in relation to the OATP1B3 inhibitory threshold.

During the first two days of treatment, when benzylpenicillin was administered at 500,000 IU/kg/day, all collected serum samples (100%) displayed concentrations exceeding the OATP1B3 inhibitory threshold of 25 µM. Measured values ranged from 120 µM to 430 µM, with mean concentrations of 265 ± 87 µM at 24 h and 241 ± 74 µM at 48 h Across all analyzed samples ($n = 44$), the median serum benzylpenicillin concentration was 176 µM (range 45–430 µM), corresponding to a median excess of +151 µM above the IC_{50} threshold. Overall, 93% of samples exceeded 25 µM, and no patient at any time point exhibited concentrations below 10 µM, indicating stable pharmacokinetic exposure throughout treatment.

Following dose reduction to 250,000 IU/kg/day on days 3 and 4, serum benzylpenicillin concentrations showed a progressive decline, with mean values of 118 ± 55 µM on day 3 and 92 ± 47 µM on day 4. Despite this reduction, 66% of samples collected during the maintenance phase remained above the 25 µM inhibitory concentration, confirming that pharmacologically relevant levels were preserved in the majority of patients. In the subset of five patients with complete four-day sampling, serum benzylpenicillin concentrations decreased proportionally to the reduction in infusion dose but remained consistently above the IC_{50} threshold throughout the observation period. No values below 45 µM were recorded, and this value represents the lowest measured concentration in our cohort.

A linear mixed-effects model with patient as a random effect demonstrated a significant effect of treatment day on serum benzylpenicillin concentrations ($\chi^2(3) = 25.83$, $P < 0.001$), indicating a time-dependent decrease consistent with the modified dosing regimen. Importantly, a one-sample Wilcoxon signed-rank test confirmed that serum concentrations were significantly higher than the IC_{50} threshold of 25 µM ($z = 5.80$, $P < 0.001$), providing formal statistical evidence that inhibitory concentrations were systematically achieved *in vivo* at both tested dose levels.

No statistically significant differences in serum benzylpenicillin concentrations were observed between male and female patients or across age groups. Mild interindividual variability was noted in relation to hydration intensity, with patients receiving more aggressive intravenous rehydration displaying slightly lower serum levels, likely reflecting increased renal clearance. However, this variability did not result in subtherapeutic concentrations in any case.

Clinical course and safety

All patients experienced a favourable clinical course, with progressive biochemical improvement and normalization of liver function tests within 10–14 days after mushroom ingestion. No patient developed acute liver failure or required liver transplantation during hospitalization. No adverse events attributable to benzylpenicillin therapy were observed. In particular, no nervous system side effects (especially seizures, neurotoxicity) occurred, and no cases of hypersensitivity reactions, electrolyte disturbances, or renal impairment were recorded. Mild transient nausea was reported in three patients and was considered related to concomitant acetylcysteine administration rather than to benzylpenicillin infusion. No cases of secondary infection or antibiotic-associated diarrhea were documented. Exploratory analyses did not reveal any association between peak serum benzylpenicillin concentrations and markers of hepatic injury (ALT, AST, or bilirubin). These findings suggest that the serum concentrations achieved in this cohort were pharmacologically relevant but not hepatotoxically significant.

Table 1. Patients' characteristics.

N patient	Age	Sex	Symptoms	Latency of symptoms (hours)	Monitoring	Amanitin in urine mcg/L	Creatinine in urine mg/dl	ALT max U/L	PT % (quick)	INR max
1	35	M	Diarrhoea	24	1st day			5,000	50	
					2nd day	37.3	332	7,000	39	
					3rd day			5,900	45	
2	60	M	Vomiting and diarrhoea	14	1st day	18.6	463	<50	86	
					2nd day	1.5	31	<50	74	
					3rd day	2.2	67	<50		
					4th day	3.2	101			
3	68	M	Vomiting	9	1st day	>100	572	<50		1
					2nd day	56.6	724	190		1.1
					3rd day	<1.5	15			
					4th day	<1.5	65	120		1
					5th day			39	67	1.97
4	56	F	Vomiting	7	1st day	>100	504	<50		
					2nd day	27.4	281	<50		
					3rd day	1.9	22	<50		
					4th day	<1.5	34	<50		
5	65	F	Vomiting and diarrhoea	12	1st day	>100	406	70		1
					2nd day			1,083		1
					3rd day	<1.5	41	1,163	63	1.3
					4th day			862		1.26
					5th day			450		1.17
					6th day			247		1.1
6	66	F	Vomiting and diarrhoea	12	1st day			<50		3
					2nd day			946		2.7
					3rd day	>100	348	5,224		2.7
					4th day			4,300		2.7
					5th day			2,900		1.9
					6th day			1,274		1.8
					7th day			1,289		1.7
					8th day			800		1.4
7	65	M	Vomiting and diarrhoea	9	1st day	>100	300	54		
					2nd day	5.4	129	900		1.1
					3rd day			2,652		1.65
					4th day			1,682		1.44
					5th day			903		1.3
8	84	M	Vomiting and diarrhoea	NA	1st day	>100				
					2nd day			5,027		3.09
					3rd day			3,880		2.3
					4th day			2,550		1.97
					5th day			1,257		1.69
9	51	F	Vomiting and diarrhoea	NA	1st day	81		90		1.48
					2nd day			81		1.3
10	57	M	Vomiting and diarrhoea	NA	3rd day	>100				
					4th day			4,500		3
					5th day			1,052		1.81
11	74	F	Vomiting and diarrhoea	NA	1st day	>100		432		1.4
					2nd day			364		1.4
					3rd day			193		1.5
12	70	F	Vomiting and diarrhoea	NA	1st day	>100		378		1.4
					2nd day			1,066		1.2
					3rd day			703		
13	75	M	Vomiting	8	1st day	12				
14	72	F	Vomiting	8	1st day	20				
					5th day			180		1.27
15	26	F	Vomiting	NA	1st day	45.7				
16	52	F	Vomiting	NA	1st day	41.7				

ALT: alanine aminotransferase (reference values <40 U/L, 0.67 µkat/L); INR: international normalized ratio (reference values 0.8–1.2; normal range approximately 11–13 sec); NA: not available; PT: prothrombin time (reference values 70–120%).
To convert to SI units: creatinine in urine mg/dL x 0.084 = mmol/L.

Discussion

The present study provides the first *in vivo* pharmacokinetic evidence that benzylpenicillin, administered according to current treatment protocols for amatoxin poisoning, achieves systemic exposure consistent with concentrations required for OATP1B3 inhibition, addressing a longstanding gap in the mechanistic interpretation of its proposed antidotal action. Although competitive inhibition of α-amanitin uptake via OATP1B3 has been demonstrated *in vitro*, with an IC_{50} of approximately 25 µM [9], it has remained unclear whether clinically used regimens achieve pharmacologically relevant exposure in humans. While our findings demonstrate that serum concentrations frequently

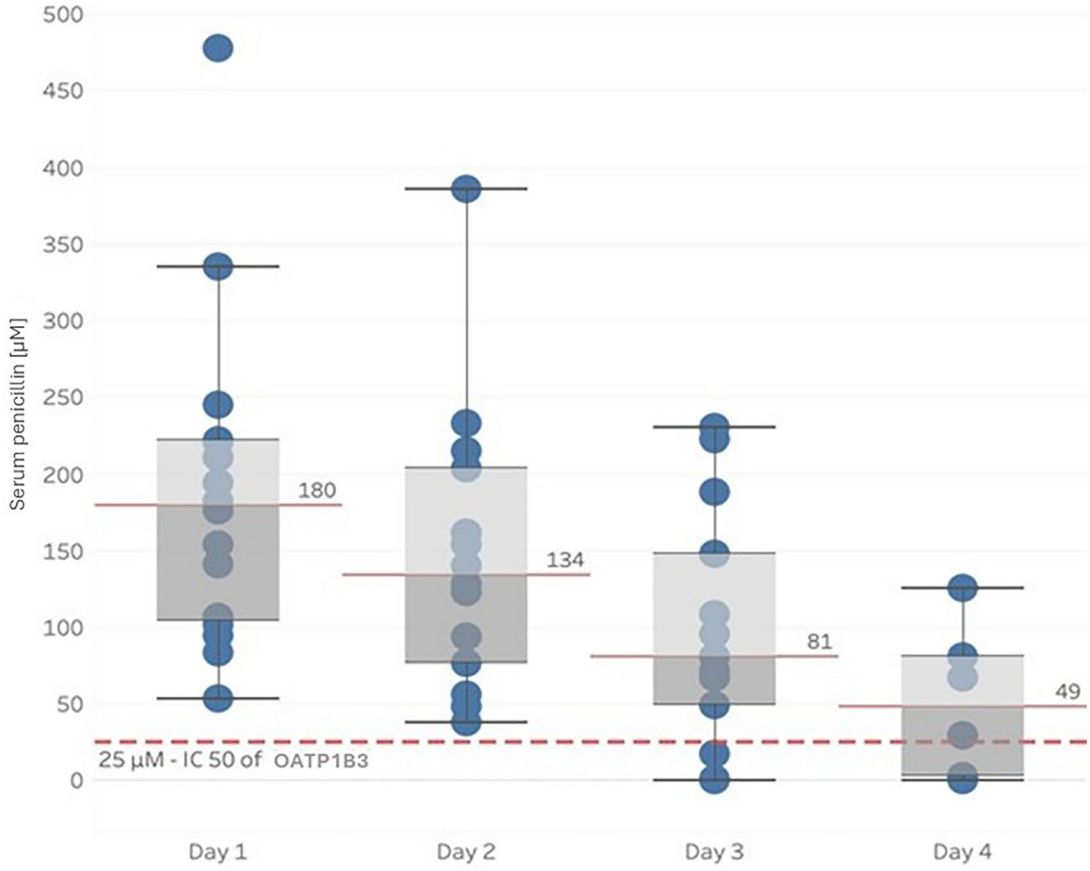

Figure 1. Serum concentrations of benzylpenicillin in the first four days of treatment. Boxplot of median (continuous red line) and quartile values (grey boxes). Dotted red line: OATP183 IC50 (25 µM).

Table 2. Mean serum benzylpenicillin concentrations during therapy in relation to the OATP1B3 inhibitory threshold.

Treatment day	Penicillin dose (IU/kg/day)	Mean concentration (µM ± SD)	Range (µM)	% samples >25 µM
Day 1	500,000	265 ± 87	120–430	100%
Day 2	500,000	241 ± 74	110–385	100%
Day 3	250,000	118 ± 55	48–240	68%
Day 4	250,000	92 ± 47	45–180	64%
Overall	—	176 ± 89	45–430	93%

Note: Concentrations were obtained from routine clinical samples collected at non-standardized time points; no predefined sampling protocol was applied.

exceed this threshold, actual transporter inhibition *in vivo* cannot be directly confirmed and remains inferred from pharmacokinetic considerations.

Importantly, prior pharmacokinetic studies indicate that serum concentrations represent a conservative estimate of tissue exposure for benzylpenicillin. Experimental data from animal models have shown that, following intravenous administration, tissue concentrations rapidly exceed plasma levels within the first few hours and remain higher for at least 20 h thereafter. Luthman and Jacobsson [19] demonstrated close equilibration between serum and extravascular fluid concentrations, with sustained tissue penetration after systemic administration, and subsequent work by Bergtsson, Bredberg, and Luthman further confirmed this distribution pattern under steady-state conditions [20].

In this study, benzylpenicillin was administered by continuous intravenous infusion rather than bolus, likely achieving hepatic concentrations comparable to or higher than serum levels. Thus, even on days 3–4, when serum concentrations fell below the *in vitro* IC_{50}, hepatic exposure was plausibly maintained within the inhibitory range, making serum levels a conservative surrogate of tissue exposure. However, this pharmacokinetic consideration does not necessarily translate into clinical efficacy, which remains influenced by multiple factors and has shown inconsistent results across studies. These findings support the mechanistic plausibility of earlier clinical observations, showing that sustained systemic concentrations above the inhibitory threshold are achievable *in vivo* and provide a pharmacological rationale for including penicillin in combination therapy protocols.

Benzylpenicillin was historically introduced in amatoxin poisoning after early clinical observations suggested improved survival with supportive care [7,8,13].

Although later studies supported inhibition of hepatic amatoxin uptake as a pharmacological rationale [9,10], protective effects in animal models have been inconsistent, possibly due to suboptimal dosing, as bolus or intermittent regimens may not sustain plasma levels required for transporter inhibition [11,21]. The present findings address this discrepancy, showing that continuous infusion, as used clinically, maintains concentrations sufficient for sustained transporter inhibition, unlike most experimental models.

Clinically, penicillin's efficacy remains controversial. Retrospective studies show conflicting results: Enjalbert et al. [3] (>2000 cases) found no specific benefit, whereas Tan et al. [4] (877 cases) reported higher survival with penicillin, not significant after severity adjustment; similar conclusions were reached by Choi et al. and Ganzert et al. [13,14]. Overall, silibinin appears the most effective single agent, but combination therapy may provide additive effects, as both drugs inhibit OATP1B3 at distinct sites and exert hepatoprotection [10,22], potentially enhancing inhibition of amatoxin uptake despite pharmacokinetic variability. Although retrospective series report favorable outcomes with penicillin–silibinin co-administration, the absence of randomized controlled trials prevents definitive conclusions. In fact, early clinical reports, such as the study by Floersheim et al. described mortality rates exceeding 20%, largely in patients treated with penicillin-based regimens, highlighting the limitations of penicillin as a standalone therapy [4,7,13,14].

In this context, present results provide pharmacokinetic validation for the continued use of penicillin as part of combination regimens. The concentrations measured in all patients during the first two days of full regimen treatment consistently exceeded the *in vitro* inhibitory threshold for OATP1B3, indicating systemic exposure compatible with transporter inhibition. Even after dose reduction, concentrations remained above this threshold in the majority of samples, suggesting that pharmacologically relevant exposure may be maintained during the early critical phase of toxicity. The effectiveness of this mechanism is inherently time-dependent, as amatoxins persist in the systemic circulation and undergo enterohepatic recirculation for up to 48–72 h after ingestion [23]. Clinically, prognosis is strongly influenced by the type and amount of mushrooms ingested and, critically, by the time to initiation of care; when treatment is started promptly, mortality is uncommon and remains below 5% [24]. Consequently, delayed initiation of benzylpenicillin therapy may substantially reduce its ability to limit hepatocellular uptake, providing a plausible explanation for the inconsistent efficacy reported in retrospective studies. In our protocol, early initiation and continuous infusion over 48 h were adopted to align with this pharmacokinetic window. Importantly, the lower dose regimen introduced in 2020 appears sufficient to maintain inhibitory exposure while reducing the overall drug load, supporting the rationale for dose optimization without loss of efficacy.

Our findings are consistent with previous clinical observations from the Florence PCC, where multimodal therapy including benzylpenicillin has been standard for decades. In a 15-year retrospective analysis of 105 confirmed cases, Giannini et al. [12], reported improved survival and reduced hepatic failure with early combined therapy including penicillin and NAC. Although the individual contribution of each drug could not be determined, that study established the clinical foundation of the current protocol. The present pharmacokinetic analysis builds directly on this experience by demonstrating that penicillin achieves pharmacologically relevant systemic concentrations in humans. Together, these studies provide complementary clinical and mechanistic evidence, reinforcing the biological plausibility of the observed outcomes.

The evidence also helps explain why comparative studies have yielded divergent conclusions. When used in appropriate doses and via continuous infusion, penicillin achieves plasma levels adequate for transporter inhibition; when administered intermittently or at lower doses, as in some earlier reports, these concentrations are likely not achieved, leading to apparent inefficacy. This interpretation reconciles the modest results from older multicenter reviews [3,15] with the improved outcomes seen in single-center experiences with aggressive dosing regimens [12,25]. It also emphasizes the importance of pharmacokinetic adequacy rather than drug selection alone in determining therapeutic success.

From a clinical standpoint, these findings have several implications. They support the continued inclusion of benzylpenicillin in multimodal therapy for amatoxin poisoning, providing pharmacokinetic evidence of systemic exposure exceeding the OATP1B3 IC_{50}. They also justify the use of continuous infusion at low dosages over bolus dosing to ensure stable plasma concentrations, with potential benefits in terms of safety and tolerability. Consistently, no penicillin-related adverse events were observed in our series.

Limitations

This study has some limitations. The sample size was modest, mainly given the rarity of confirmed amatoxin intoxications, and all patients received combination

therapy, precluding evaluation of penicillin monotherapy. An additional limitation relates to the non-standardized timing of serum sampling, as samples were collected during routine clinical care rather than according to a predefined pharmacokinetic schedule. This limits detailed characterization of inter-individual pharmacokinetic variability and direct kinetic comparisons between patients. However, this aspect does not preclude the primary objective of the study, namely the assessment of whether clinically used dosing regimens achieve serum concentrations exceeding the OATP1B3 inhibitory threshold. An additional limitation relates to the known limited stability of benzylpenicillin in blood samples [26]. Although samples were processed as rapidly as possible in routine clinical practice, the observational design and emergency setting did not allow strict control of pre-analytical timing. Consequently, some degree of analyte degradation before analysis cannot be excluded, potentially leading to underestimation of true serum concentrations. However, if present, this effect would suggest that the measured concentrations represent a conservative estimate of systemic benzylpenicillin exposure. Furthermore, while the pharmacological plausibility is now confirmed, definitive demonstration of clinical benefit would require prospective comparative studies, which remain ethically and logistically challenging in this context.

Conclusion

This study provides *in vivo* pharmacokinetic evidence that therapeutic intravenous benzylpenicillin achieves plasma concentrations exceeding the *in vitro* IC_{50} for OATP1B3. Thus, these findings support the pharmacological plausibility of penicillin therapy and its proposed mechanistic rationale, although direct demonstration of transporter inhibition *in vivo* was beyond the scope of this study. The results also suggest that the reduced continuous-infusion regimen adopted at our centre maintains concentrations within a pharmacologically relevant range while limiting overall drug exposure.

Although silibinin remains the preferred antidote, benzylpenicillin may represent a justified and accessible component of combination therapy, particularly in settings where alternatives are unavailable or more expensive. In this context, our findings contribute to addressing a longstanding lack of *in vivo* pharmacokinetic data and may help inform further optimization of treatment protocols in amatoxin-induced hepatotoxicity.

Author contributions

Dr. Andrea Missanelli, Dr. Cecilia Lanzi, Dr. Francesco Gambassi, Dr. Alessandra Ieri, Dr. Anita Ercolini, Dr. Arianna Totti, Dr. Antonio Munafo, and Prof. Guido Mannaioni contributed to patient care and clinical data collection. Dr. Roberto Baronti, Dr. Nicoletta Cini, and Dr. Francesca Luceri performed and supervised the laboratory analyses of hematological samples. Dr. Giada Crescioli, Dr. Andrea Missanelli, and Prof. Alfredo Vannacci analyzed the data and drafted the primary version of the manuscript, which was critically revised by Prof. Guido Mannaioni and Dr. Roberto Baronti.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was not funded.

ORCID

Andrea Missanelli http://orcid.org/0009-0008-3877-1165
Giada Crescioli http://orcid.org/0000-0002-1071-6120
Cecilia Lanzi http://orcid.org/0000-0002-2230-3729
Francesco Gambassi http://orcid.org/0000-0001-6095-770X
Alessandra Ieri http://orcid.org/0000-0002-7413-4293
Anita Ercolini http://orcid.org/0000-0001-9620-2755
Francesca Luceri http://orcid.org/0009-0001-5669-8984
Antonio Munafo http://orcid.org/0000-0003-1370-2880
Guido Mannaioni http://orcid.org/0000-0002-8273-7377
Alfredo Vannacci http://orcid.org/0000-0001-5259-2026

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, AV.

References

[1] Diaz JH. Syndromic diagnosis and management of confirmed mushroom poisonings. Crit Care Med. 2005;33(2):427–436. doi: 10.1097/01.ccm.0000153531.69448.49.
[2] Vetter J. Amanitins: the most poisonous molecules of the fungal world. Molecules. 2023;28(15):5932. doi: 10.3390/molecules28155932.
[3] Enjalbert F, Rapior S, Nouguier-Soulé J, et al. Treatment of amatoxin poisoning: 20-year retrospective analysis. J Toxicol Clin Toxicol. 2002;40(6):715–757. doi: 10.1081/clt-120014646.
[4] Tan JL, Stam J, van den Berg AP, et al. Amanitin intoxication: effects of therapies on clinical outcomes – a review of 40 years of reported cases. Clin Toxicol (Phila).

2022;60(11):1251–1265. doi: 10.1080/15563650.2022.2098139.
[5] Santi L, Maggioli C, Mastroroberto M, et al. Acute liver failure caused by Amanita phalloides poisoning. Int J Hepatol. 2012;2012:487480–487486. doi: 10.1155/2012/487480.
[6] Escudié L, Francoz C, Vinel J-P, et al. Amanita phalloides poisoning: reassessment of prognostic factors and indications for emergency liver transplantation. J Hepatol. 2007;46(3):466–473. doi: 10.1016/j.jhep.2006.10.013.
[7] Floersheim GL, Weber O, Tschumi P, et al. Clinical death-cap (Amanita phalloides) poisoning: prognostic factors and therapeutic measures. Analysis of 205 cases. Schweiz Med Wochenschr. 1982;112(34):1164–1177.
[8] Hruby K, Csomos G, Fuhrmann M, et al. Chemotherapy of Amanita phalloides poisoning with intravenous silibinin. Hum Toxicol. 1983;2(2):183–195. doi: 10.1177/096032718300200203.
[9] Letschert K, Faulstich H, Keller D, et al. Molecular characterization and inhibition of amanitin uptake into human hepatocytes. Toxicol Sci. 2006;91(1):140–149. doi: 10.1093/toxsci/kfj141.
[10] Magdalan J, Ostrowska A, Piotrowska A, et al. Benzylpenicillin, acetylcysteine and silibinin as antidotes in human hepatocytes intoxicated with alpha-amanitin. Exp Toxicol Pathol. 2010;62(4):367–373. doi: 10.1016/j.etp.2009.05.003.
[11] Magdalan J, Ostrowska A, Piotrowska A, et al. Comparative antidotal efficacy of benzylpenicillin, ceftazidime and rifamycin in cultured human hepatocytes intoxicated with α-amanitin. Arch Toxicol. 2009;83(12):1091–1096. doi: 10.1007/s00204-009-0466-3.
[12] Giannini L, Vannacci A, Missanelli A, et al. Amatoxin poisoning: a 15-year retrospective analysis and follow-up evaluation of 105 patients. Clin Toxicol (Phila). 2007;45(5):539–542. doi: 10.1080/15563650701365834.
[13] Ganzert M, Felgenhauer N, Schuster T, et al. Amanita poisoning–comparison of silibinin with a combination of silibinin and penicillin. Dtsch Med Wochenschr. 2008;133(44):2261–2267. doi: 10.1055/s-0028-1091268.
[14] Choi MW, Ko DR, Kong T, et al. Comparison of silymarin, penicillin, N-acetylcysteine in patient with amatoxin poisoning: a systematic review. JKSCT. 2018;16(1):33–41. doi: 10.22537/jksct.16.1.33.
[15] Mengs U, Pohl R-T, Mitchell T. Legalon® SIL: the antidote of choice in patients with acute hepatotoxicity from amatoxin poisoning. Curr Pharm Biotechnol. 2012;13(10):1964–1970. doi: 10.2174/138920112802273353.
[16] Magdalan J, Piotrowska A, Gomułkiewicz A, et al. Influence of commonly used clinical antidotes on antioxidant systems in human hepatocyte culture intoxicated with alpha-amanitin. Hum Exp Toxicol. 2011;30(1):38–43. doi: 10.1177/0960327110368418.
[17] Mushrooms SS. In: Ford MD, Delaney KA, Ling, LJ, Erickson WB, editor. Clinical toxicology. Philadelphia: W.B. Saunders Company; 2001. p. 899–909.
[18] Yilmaz I, Ermis F, Akata I, et al. A case study: what doses of Amanita phalloides and amatoxins are lethal to humans? Wilderness Environ Med. 2015;26(4):491–496. doi: 10.1016/j.wem.2015.08.002.
[19] Luthman J, Jacobsson SO. Distribution of penicillin G in serum and tissue cage fluid in cattle. Acta Vet Scand. 1986;27(3):313–325. doi: 10.1186/BF03548146.
[20] Luthman J, Dall V, Jacobsson SO, et al. Influence of the local tolerance on the pharmokinetics of two penicillin G preparations in cattle and swine. Acta Vet Scand. 1988;29(2):199–206. doi: 10.1186/BF03548371.
[21] Tong TC, Hernandez M, Richardson WH, et al. Comparative treatment of alpha-amanitin poisoning with N-acetylcysteine, benzylpenicillin, cimetidine, thioctic acid, and silybin in a murine model. Ann Emerg Med. 2007;50(3):282–288. doi: 10.1016/j.annemergmed.2006.12.015.
[22] Horowitz BZ. Silibinin: a toxicologist's herbal medicine? Clin Toxicol (Phila). 2022;60(11):1194–1197. doi: 10.1080/15563650.2022.2128815.
[23] Kayes T, Ho V. Amanita phalloides-associated liver failure: molecular mechanisms and management. Int J Mol Sci. 2024;25(23):13028. doi: 10.3390/ijms252313028.
[24] Horowitz BZ, Moss MJ. Amatoxin mushroom toxicity. StatPearls, Treasure Island (FL): StatPearls Publishing; 2026.
[25] Dluholucký S, Snitková M, Knapková M, et al. Results of diagnostics and treatment of amanita phalloides poisoning in Slovakia (2004–2020). Toxicon. 2022;219:106927. doi: 10.1016/j.toxicon.2022.09.013.
[26] Bahmany S, Ewoldt TMJ, Abdulla A, et al. Stability of 10 beta-lactam antibiotics in human plasma at different storage conditions. Ther Drug Monit. 2023;45(5):606–615. doi: 10.1097/FTD.0000000000001100.