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Benzylpenicillin, acetylcysteine and silibinin as antidotes in human hepatocytes intoxicated with α-amanitin

Jan Magdalan[a], Alina Ostrowska[a], Aleksandra Piotrowska[b], Agnieszka Gomułkiewicz[b], Marzena Podhorska-Okołów[b], Dariusz Patrzałek[c], Adam Szeląg[a], Piotr Dzięgiel[b,d,*]

[a]*Department of Pharmacology, Wrocław Medical University, Wrocław, Poland*
[b]*Department of Histology and Embryology, Wrocław Medical University, Wrocław, Poland*
[c]*Department of Vascular, General and Transplantation Surgery, Wrocław Medical University, Wrocław, Poland*
[d]*Department of Histology and Embryology, Poznań University of Medical Science, Poznań, Poland*

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Abstract

Fatalities due to mushroom poisonings are increasing worldwide, with high mortality rate resulting from ingestion of amanitin-producing species. Intoxications caused by amanitin-containing mushrooms represent an unresolved problem in clinical toxicology since no specific and fully efficient antidote is available. The objective of this study was a comparative evaluation of benzylpenicillin (BPCN), acetylcysteine (ACC) and silibinin (SIL) as an antidotes in human hepatocytes intoxicated with α-amanitin (α-AMA).

All experiments were performed on cultured human hepatocytes. Cytotoxicity evaluation of cultured cells using MTT assay and measurement of lactate dehydrogenase (LDH) activity was performed at 12, 24 and 48 h of exposure to α-AMA and/or antidotes.

The significant decline of cell viability and significant increase of LDH activity were observed in all experimental hepatocyte cultures after 12, 24 and 36 h exposure to α-AMA at concentration 2 μM. Exposure of the cells to α-AMA resulted also in significant reduction of cell spreading and attachment. However, addition of tested antidotes to experimental cultures significantly stimulated cell proliferation and attachment. In cell cultures exposed simultaneously to α-AMA and tested antidotes cytotoxic parameters (MTT and LDH) were not significantly different from control incidences. The cytoprotective effect of all antidotes was not dose-related, which reflects a high efficacy of all these substances. Administration of studied antidotes was not associated with any adverse effects in hepatocytes.

The administration of ACC, BPCN or SIL to human hepatocyte cultures showed a similar strong protective effect against cell damage in α-AMA toxicity.
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Keywords: Alpha-amanitin; Benzylpenicillin; Acetylcysteine; Silibinin; Amanita phalloides; Intoxication; Human hepatocytes

Introduction

Amatoxin poisoning is caused by mushroom species belonging to the genera *Amanita*, *Galerina* and *Lepiota* with the majority of lethal mushroom exposures attributable to *Amanita phalloides* and its subspecies

*Corresponding author at: Department of Histology and Embryology, Wrocław Medical University, Chalubinskiego 6a, PL 50-368 Wrocław, Poland. Tel./fax: +48 71 784 00 82.

E-mail address: piotr@hist.am.wroc.pl (P. Dzięgiel).

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(*Amanita virosa*, *Amanita vernalis*) (Enjalbert et al., 2002; Schneider, 2001). Amatoxins are heat-stable octapeptides. A wide variety of amatoxins have been isolated; however, α-amanitin (α-AMA) appears to be the primary human toxin (Schneider, 2001). Treatment of poisonings caused by amatoxin-containing mushrooms includes gastrointestinal decontamination, supportive measures, antidotes and, if liver failure occurs, liver transplantation (Escudié et al., 2007; Schneider, 2001; Yildiz et al., 2008). Various substances widely used in the past to treat *Amanita phalloides* poisonings (steroids, cimetidine, thioctic acid) are documented to be completely ineffective. Moreover, according to Enjalbert et al. (2002) the most often currently used antidote – benzylpenicillin (BPCN) – shows poor clinical efficacy. Experimental and clinical data of the silibinin (SIL) and acetylcysteine (ACC) efficacy as an antidote for amanitin toxicity are ambiguous (Enjalbert et al., 2002; Ganzert et al., 2008; Magdalan et al., 2009; Saller et al., 2008; Schneider et al., 1992; Vogel et al., 1984; Tong et al., 2007). However, some clinical reports indicated that SIL or ACC appear to be more effective in mushroom poisoning therapy than BPCN (Enjalbert et al., 2002).

The review of therapeutic procedures reported on patients intoxicated with amatoxin-containing mushrooms demonstrates a wide variability in medical treatment and overall response rate (Enjalbert et al., 2002; Erguven et al., 2007; Ganzert et al., 2008; Giannini et al., 2007; Kotwica and Czerczak, 2007; Krenova et al., 2007). Thus, evaluation of different therapies of intoxications caused by amatoxin-producing mushrooms seems to be extremely difficult. A number of clinical factors need to be taken into account when evaluating an effective treatment of mushroom poisoning, including but not limited to age and gender of the patient, health condition prior to intoxication, time from mushroom ingestion to onset of therapy and clinical condition at the time of therapy initiation. Moreover, using specific treatment regimens including combined pharmacotherapy in one patient significantly complicates such evaluation (Enjalbert et al., 2002; Ganzert et al., 2008; Giannini et al., 2007). Therefore, primary human hepatocyte cultures are a good model for objective analysis of different antidotes without the necessity of taking into consideration all the aforementioned clinical factors.

The objective of this study was a comparative evaluation of BPCN, ACC and SIL efficacy in human hepatocytes intoxicated with α-AMA.

Materials and methods

Hepatocyte isolation and culture

All experiments were performed after approval by the Local Ethics Commission at Wrocław Medical University (Wrocław, Poland), license no. 481/2006. Media, supplements and reagents used for hepatocyte isolation and culture, α-AMA and all tested antidotes were obtained from Sigma Poland Chem. Human liver was obtained through a network of organ procurement organization. At the time of referral the liver had been deemed unsuitable for organ transplantation. The liver was collected from brain-dead male donor and was protected from ischemic injury by flushing with ice-cold HTK solution immediately after vascular clamping. Hepatocytes were isolated from encapsulated left lateral segment of the liver as described previously (Ostrowska et al., 2000). After 4 h of initial incubation, the plating medium was substituted with defined culture medium (combination of EBSS and Waymouth's 752/1, supplemented with 10% FBS). After the next 12 h incubation the medium was exchanged and primary hepatocyte cultures were maintained for 48 h with 1 daily dose of α-AMA and/or antidotes (BPCN, ACC, SIL) at different final concentrations (Table 1).

ACC concentrations corresponded to its plasma levels obtained after the recommended dosage during the treatment in paracetamol toxicity (Prescott et al., 1989). BPCN concentrations corresponded to its plasma levels obtained after the dosage recommended in the therapy of the toadstool death cap poisoning, i.e. 300,000–1,000,000 U/kg/day (Heilmeyer et al., 1969; Schneider, 2001). SIL was used at concentrations corresponding to its therapeutic plasma levels (Kim et al., 2003; Lorenz et al., 1984).

Cytotoxicity evaluation of cultured cells was performed at 12, 24 and 36 h of exposure to α-AMA and/or antidotes.

Table 1. Final concentrations of α-AMA, ACC, BPCN and SIL administered to human hepatocyte cultures.

Group	α-AMA (μM)	ACC (mM)	BPCN (mM)	SIL (μM)
C				
AMA	2			
a		0.5		
A		1		
p			0.5	
P			1	
s				10
S				100
AMA-a	2	0.5		
AMA-A	2	1		
AMA-p	2		0.5	
AMA-P	2		1	
AMA-s	2			10
AMA-S	2			100

The cells were treated with 1 daily dose of antidote for 48 h.

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