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PHARMACOLOGY

Anidulafungin Pharmacokinetics in Ascites Fluid and Pleural Effusion of Critically Ill Patients

R. Welte,[a] P. Eller,[b] I. Lorenz,[c] M. Joannidis,[d] R. Bellmann[a]

[a]Clinical Pharmacokinetics Unit, Division of Intensive Care and Emergency Medicine, Department of Internal Medicine I, Medical University of Innsbruck, Innsbruck, Austria

[b]Department of Internal Medicine, Medical University of Graz, Graz, Austria

[c]General and Surgical ICU, Department of General and Surgical Intensive Care Medicine, Center of Operative Medicine, Medical University of Innsbruck, Innsbruck, Austria

[d]Division of Intensive Care and Emergency Medicine, Department of Internal Medicine I, Medical University of Innsbruck, Innsbruck, Austria

ABSTRACT Anidulafungin concentrations were quantified with high-pressure liquid chromatography (HPLC) and UV detection of the ascites fluid and pleural effusion of 10 adult critically ill patients. Samples were collected from ascites fluid and from pleural drains or during paracentesis and thoracentesis, respectively. Anidulafungin levels in ascites fluid (0.12 to 0.99 μg/ml) and in pleural effusion (0.32 to 2.02 μg/ml) were below the simultaneous levels in plasma (1.04 to 7.70 and 2.48 to 13.36 μg/ml, respectively) and below the MIC values for several pathogenic *Candida* strains.

KEYWORDS echinocandins, antifungal target-site pharmacokinetics, fungal peritonitis, fungal thoracic empyema, invasive candidiasis, invasive fungal disease, tissue distribution

Fungal peritonitis and fungal thoracic empyema are life-threatening conditions which are difficult to treat (1–3). The echinocandin anidulafungin has shown efficacy against candidemia and is recommended for treatment of invasive candidiasis in adult patients (1, 4). Data on its target-site penetration are sparse. Therefore, we assessed anidulafungin concentrations and kinetics in ascites fluid and pleural effusion of critically ill patients.

This study on critically ill adults with an indication for anidulafungin therapy and for paracentesis (or peritoneal drainage) or thoracentesis (or pleural drainage) was approved by the local ethics committees (Internal Review Board no. AN2013-0079 332/2.3 and AN2013-0079 352/5.14 [3616a]; EudraCT no. 2013-005065-38). Written informed consent was obtained from competent patients, and *post hoc* consent was obtained from patients who were incompetent at the time of enrollment. One hundred milligrams of anidulafungin (Ecalta; Pfizer, Sandwich, Kent, UK) was administered once daily after a 200-mg loading dose. When ascites fluid or pleural effusion was sampled via drainage, the collection bags were changed before the infusion as well as at 1 h, 4 h, 8 h, 12 h, 18 h, and 24 h after the start of the infusion. Two-milliliter blood samples were taken from an arterial line simultaneously with the changes of the collection bags, paracentesis, or thoracentesis using heparinized vials (Sarstedt, Nümbrecht, Germany). All samples were stored at −80°C. For anidulafungin quantification, a method using high-performance liquid chromatography (HPLC) and UV detection at 306 nm (5) was modified and validated. Two hundred microliters of plasma, ascites fluid, or pleural effusion was treated with 300 μl of pure acetonitrile (Rotisolv; Carl Roth, Karlsruhe, Germany) and centrifuged at 14,600 × *g* for 5 min at 8°C. Gradient separation was done with 0.1% ammonium acetate in H_2O, pH 5.0, and with pure acetonitrile on a Zorbax

Received 10 November 2017 **Returned for modification** 21 December 2017 **Accepted** 5 February 2018

Accepted manuscript posted online 12 February 2018

Citation Welte R, Eller P, Lorenz I, Joannidis M, Bellmann R. 2018. Anidulafungin pharmacokinetics in ascites fluid and pleural effusion of critically ill patients. Antimicrob Agents Chemother 62:e02326-17. https://doi.org/10.1128/AAC.02326-17.

Address correspondence to R. Bellmann, romuald.bellmann@i-med.ac.at.

TABLE 1 Study population and anidulafungin treatment[a]

Patient	Sex	Age (yr)	Body wt (kg)	Main diagnosis(es)	CVVH	Day(s) of anidulafungin treatment	Cumulative dose(s) (mg)	Sampling procedure
1	F	28	44	Sepsis, CF, liver failure, peritoneal mesothelioma	+	1	200	Ascites drain
2	F	68	85	Ileus, NHL	−	11	1,200	Ascites drain
3	M	60	52	AIDS, sepsis, liver cirrhosis	+	2	200	Ascites drain
4	M	68	71	Liver failure, septic shock	−	3, 6	300, 700	Paracentesis, ascites drain
5	M	65	61	Liver cirrhosis, pneumonia	+	5	1,000	Paracentesis
6	M	60	61	Septic shock, MODS	+	2	200	Paracentesis
7	F	48	60	Sharp syndrome, sepsis	−	7, 21	600, 2,000	Paracentesis, thoracentesis
8	F	59	45	Liver cirrhosis	−	17	2,000	Pleural drain
9	M	73	69	Sepsis, pneumonia	+	4, 7	500, 800	Thoracentesis, pleural drain
10	M	75	80	AML, MOF	+	12	1,300	Thoracentesis

[a]CVVH, renal replacement therapy by continuous veno-venous hemofiltration; F, female; M, male; CF, cystic fibrosis; NHL, non-Hodgkin lymphoma; MODS, multiorgan dysfunction syndrome; AML, acute myeloid leukemia; MOF, multiorgan failure.

Eclipse XDB C_{18} column (250 mm by 4.6 mm, with a 5.0-μm particle size; Agilent Technologies, Vienna, Austria) at room temperature. Seventy percent ammonium acetate was applied for 6 min, followed by 35% ammonium acetate over 4 min. Interday and intraday variability were <15%, and the lower limit of quantification was 0.01 μg/ml. Process efficacy was 43% for 10.0 μg/ml and 30% for 0.1 μg/ml. For patients with drains, the penetration ratio is the ratio between the area under the anidulafungin concentration-time curve in ascites fluid over the sampling period ($AUC_{0-n\ \text{ascites fluid}}$) or in pleural effusion ($AUC_{0-n\ \text{pleural effusion}}$) and the plasma AUC over the sampling period (i.e., $AUC_{0-n\ \text{ascites fluid}}/AUC_{0-n\ \text{plasma}}$ or $AUC_{0-n\ \text{pleural effusion}}/AUC_{0-n\ \text{plasma}}$, respectively). For single samples obtained at paracentesis or thoracentesis, it is the ratio between the concentration in ascites fluid or pleural effusion and the plasma concentration ($C_{\text{ascites fluid}}/C_{\text{plasma}}$ or $C_{\text{pleural effusion}}/C_{\text{plasma}}$, respectively). Pharmacokinetics was calculated by a noncompartmental model using Kinetica 2000 (InnaPhase Corp., Champs-sur-Marne, France). AUC_{0-n} was computed using the log-linear method whenever the concentration in a trapezoid decreased or with the trapezoidal method when the concentration increased. The significance of the difference between concentrations in ascites fluid or pleural effusion and in plasma was assessed by the Wilcoxon matched-pairs test calculated with IBM SPSS Statistics 24.0.

Ascites fluid was taken from seven patients and pleural effusion from four patients (Table 1). Ascites drains had been inserted in patients 1 to 4 and pleural drains in patients 8 and 9. In these patients, anidulafungin kinetics was determined in ascites fluid and in pleural effusion, respectively, and in plasma (Table 2 and 3). Concentrations and penetration ratios obtained from paracentesis and thoracentesis are listed in Table 4.

TABLE 2 Anidulafungin pharmacokinetics in ascites fluid and pleural effusion with 24-h sampling[a]

Sample	Patient	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-24} (μg · h/ml)	$t_{1/2}$ (h)	PR
Ascites fluid	2	0.98	4	17.5	26	0.37
	3	0.34	12	7.0	162	0.07
Plasma	2	3.82	1	47.2	25	
	3	7.70	1	93.8	30	
Pleural effusion	9	0.99	4	21.4	362	0.18
Plasma	9	8.83	1	116.5	36	

[a]C_{max}, anidulafungin maximum concentration; T_{max}, time to C_{max}; AUC_{0-24}, area under the concentration-time curve from the start of the anidulafungin infusion to the last sampling, which was at 24 h; $t_{1/2}$ anidulafungin half-life; PR, penetration ratio, specifically, $AUC_{0-24\ \text{ascites fluid}}/AUC_{0-24\ \text{plasma}}$ for ascites fluid and $AUC_{0-24\ \text{pleural effusion}}/AUC_{0-24\ \text{plasma}}$ for pleural effusion.

TABLE 3 Anidulafungin pharmacokinetics in ascites fluid and pleural effusion[a]

Sample	Patient	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-n} (μg · h/ml)	Sampling period (h)	PR
Ascites fluid	1	0.58	4	5.6	12	0.12
	4	0.99	4	12.1	18	0.20
Plasma	1	5.51	1	44.1	12	
	4	4.51	4	64.5	18	
Pleural effusion	8	2.02	14	16.8	14	0.17
Plasma	8	13.36	1	98.5	14	

[a]Specimens were from patients sampled for <24 h. C_{max}, anidulafungin peak concentration; T_{max}, time to C_{max}; AUC_{0-n}, area under the concentration-time curve from the start of the anidulafungin infusion to the last sampling; sampling period, time from the first to the last sampling; PR, penetration ratio, specifically, $AUC_{0-n\ ascites\ fluid}/AUC_{0-n\ plasma}$ for ascites fluid or $AUC_{0-n\ pleural\ effusion}/AUC_{0-n\ plasma}$ for pleural effusion.

Anidulafungin levels in ascites fluid (0.12 to 0.99 μg/ml) and in pleural effusion (0.32 to 2.02 μg/ml) were lower than the simultaneous levels in plasma (1.04 to 7.70 and 2.48 to 13.36 μg/ml, $P < 0.001$). We found anidulafungin plasma pharmacokinetics comparable with previous data on critically ill adults, including patients with intra-abdominal candidiasis (6, 7).

Pfaller et al. found MIC values of 0.008 to 2.0 μg/ml for *Candida albicans* and *Candida tropicalis*, 0.015 to 4.0 μg/ml for *Candida glabrata*, and 0.25 to 4.0 μg/ml for *Candida parapsilosis* (8). Thus, the *in vitro* MICs for several relevant pathogens exceed anidulafungin concentrations in ascites fluid and in pleural effusion. The ratio between the AUC for the drug in plasma over 24 h and the MIC for the pathogen (AUC_{0-24}/MIC) appears to correlate with the antifungal activity of anidulafungin. Total anidulafungin AUC_{0-24}/MIC ratios of 2,780, 1,370, and 1,150 were fungistatic to *C. albicans*, *C. glabrata*, and *C. parapsilosis*, respectively (9). In critically ill patients, mean free (unbound) anidulafungin plasma AUC_{0-24}/MIC ($fAUC_{0-24}$/MIC) ratios of 229 and 118 (equivalent to the total AUC_{0-24}/MIC ratios of 22,900 and 11,800) were determined for *C. albicans* and *C. glabrata*, respectively, exceeding proposed target values for the $fAUC_{0-24}$/MIC of 36.7 and 18.3, respectively (6). In the ascites fluid of our patient 2, the total anidulafungin AUC_{0-24}/MIC ratio was 2,181 for an MIC of 0.008, 35 for an MIC of 0.5, and 9 for an MIC of 2.0 μg/ml. In pleural effusion, the respective values amounted to 2,674, 43, and 11 (patient 9). Thus, plasma target values were achieved only for highly susceptible strains in these two compartments. However, for ascites fluid and for pleural effusion, the relevance of *in vitro* MIC values and the extent of anidulafungin protein binding are unknown. Furthermore, steady state had probably not yet been reached in three patients, and only three patients had undergone sampling over 24 h. No patient in our small study population suffered from peritonitis or thoracic empyema. However, anidu-

TABLE 4 Anidulafungin concentrations in single samples obtained by paracentesis and thoracentesis[a]

Patient	Concn of anidulafungin (μg/ml) in:			Time from infusion (h)	Day of anidulafungin treatment	PR
	Ascites fluid	Pleural effusion	Plasma			
4	0.28		2.88	8.0	3	0.10
5	0.30		5.39	5.5	5	0.06
6	0.32		4.69	8.8	2	0.07
7	0.45		2.15	18.0	7	0.21
7		0.45	2.48	20.0	21	0.18
9		0.88	4.50	9.5	4	0.20
10		0.32	4.18	4.0	12	0.08

[a]Time from infusion, time from the start of the anidulafungin infusion to sampling; PR, penetration ratio obtained from single ascites fluid samples (for patients 4 to 7, we determined the concentration in ascites fluid/concentration in plasma) and pleural effusion samples (for patients 7 to 10, we determined the concentration in pleural effusion/concentration in plasma). Patient 4 underwent paracentesis on day 3 of anidulafungin treatment and ascites fluid drainage on day 7 because of recurrent ascites. Patient 7 underwent paracentesis on day 7 and thoracentesis on day 21 of anidulafungin treatment.

lafungin levels in thoracic empyema specimens and peritoneal concentrations of micafungin during peritonitis were ~1 μg/ml, resembling anidulafungin levels that we have measured in uninflamed body fluids (10, 11). Micafungin achieved mean concentrations of 0.7 and 1.0 μg/ml in pleural effusion and in ascites fluid, respectively (12–14).

Amphotericin B concentrations of <0.5 μg/ml were recovered from ascites fluid and from pleural effusion during treatment with different amphotericin B formulations (15–17). Flucytosine levels in peritoneal fluid were comparable to the simultaneous levels in plasma (15). Fluconazole reached a trough level of 9.6 μg/ml (85% of the simultaneous level in plasma) in ascites fluid (18). Voriconazole levels of 2.2 and 0.8 to 1.4 μg/ml (penetration ratio, 0.45 to 0.95) were measured in pleural effusion and in thoracic empyema, respectively (19, 20).

Probably, the success of anidulafungin treatment in *Candida* peritonitis or thoracic empyema depends largely on the susceptibility of the pathogen. Additional strategies, including drainage and surgery, are essential. Investigations on echinocandin activity at various target sites are required.

ACKNOWLEDGMENTS

This study was supported by IIR grant WI194757 from Pfizer. R.B. has received research support from Chiesi Pharmaceuticals and Merck Sharp & Dohme and lecture fees from Basilea Pharmaceutica Ltd., Chiesi Pharmaceuticals, Merck Sharp & Dohme, and Astellas, Austria. He is a member of an advisory board of Merck Sharp & Dohme. The other authors have no conflicts of interest to declare.

We thank the nurses of the participating ICUs for their support.

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