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Pulmonary Epithelial Lining Fluid Concentrations after Use of Systemic Amphotericin B Lipid Formulations▽

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Amphotericin B (AMB) concentrations were determined in pulmonary epithelial lining fluid (ELF) of 44 critically ill patients, who were receiving treatment with liposomal AMB (LAMB) ($n = 11$), AMB colloidal dispersion (ABCD) ($n = 28$), or AMB lipid complex (ABLC) ($n = 5$). Mean AMB levels (± standard errors of the means) in ELF amounted to 1.60 ± 0.58, 0.38 ± 0.07, and 1.29 ± 0.71 μg/ml in LAMB-, ABCD-, and ABLC-treated patients, respectively (differences are not significant).

Invasive pulmonary mycoses exhibit a high mortality, particularly in critically ill patients (19). Amphotericin B (AMB) lipid formulations—liposomal AMB (AmBisome; Gilead) (LAMB), AMB colloidal dispersion (Amphotec [Three Rivers] and Amphocil [Torrex-Chiesi]) (ABCD), and AMB lipid complex (Abelcet; Zeneus) (ABLC)—display differences in plasma pharmacokinetics and tissue distribution (15, 26, 28). During treatment with AMB lipid formulations, AMB concentrations were investigated in epithelial lining fluid (ELF), which is a well-established model for pulmonary drug penetration (1–4, 6–10, 12, 17).

This study was approved by the local ethics committee. Patients on lipid-formulated AMB requiring bronchoalveolar lavage (BAL) were enrolled (Table 1). AMB concentrations were assessed in 8-ml aliquots of BAL samples obtained by a standard procedure (16). BAL fluid was concentrated by evaporation, and AMB was quantified by high-performance liquid chromatography as described previously, with modifications for BAL samples (13). The concentrations were assessed by using a linear standard curve (R between 0.995 and 0.999), obtained from standards comprising 0.9% saline solution spiked with AMB. The lower detection limit of AMB in BAL fluid was 0.005 μg/ml. The assay has been found to be linear over the concentration range of 0.005 to 2.5 μg/ml for AMB in BAL fluid. The intraday and interday precisions were 3.2% and 4.7%, respectively. AMB concentrations in ELF were calculated by the urea dilution method (23), $AMB_{ELF} = AMB_{BAL} \times (urea_{PLA}/urea_{BAL})$, where AMB_{ELF} is the AMB concentration in ELF, AMB_{BAL} is the AMB concentration in BAL fluid, $urea_{PLA}$ is the urea concentration in plasma, and $urea_{BAL}$ is the urea concentration in BAL fluid (23). Two ml of the BAL fluid was separated for urea quantification, which was performed using an enzymatic assay (urea/blood urea nitrogen; Roche) as with plasma.

Arterial blood samples were simultaneously taken for measurement of plasma AMB and urea concentrations. In patients on LAMB or ABCD therapy, the lipid-associated fractions were separated from AMB that had been liberated from its lipid encapsulation. AMB was measured with high-performance liquid chromatography as described previously (13).

Statistical analysis was performed using the Statistica software program, version 5. The differences between total AMB concentrations in plasma and in ELF were analyzed by using the Wilcoxon matched pairs test. For comparisons between the lipid formulations, the Mann-Whitney U test was applied.

Forty-four patients were enrolled: 11 patients on LAMB, 28 on ABCD, and 5 on ABLC. Table 2 displays the ELF and plasma concentrations of AMB and the penetration ratios. In the entire study population and in LAMB-treated patients, ELF concentrations correlated with plasma levels ($r = 0.68$, $P < 0.001$, and $r = 0.66$, $P = 0.04$, respectively). In the LAMB group, this correlation was even more significant when liberated AMB was considered ($r = 0.89$; $P < 0.001$). A positive correlation between the time from last infusion to sampling and the penetration ratio was found during LAMB ($r = 0.75$; $P = 0.01$) and ABLC ($r = 0.95$; $P = 0.01$) treatments.

Inhalation of fungal conidiae is the most common route of infection with molds. During treatment with AMB lipid formulations at standard doses, mean AMB levels in ELF were below 2 μg/ml. For *Aspergillus* species, the MIC of AMB has been reported to range from 0.25 to 4 μg/ml (14). Thus, in some cases, the MIC exceeds the AMB concentration in ELF. This may contribute to unsatisfying responses sometimes observed, though the impact of target site concentrations in relation to MICs is controversial. ELF concentrations are markedly lower than AMB levels in whole lung tissue (32.6 μg/g after ABCD treatment) (26). Whole tissue samples, however, comprise various compartments and potential targets of fungal invasion, such as different cells, extracellular matrix, and blood vessels.

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TABLE 1. Demographic and clinical characteristics of patients[a]

Characteristic	Value for treatment group		
	LAMB	ABCD	ABLC
Total subjects	11	28	5
Mean age in yr	46 ± 4	50 ± 3	55 ± 5
Sex			
Male	9	14	4
Female	2	14	1
Mean wt (kg)	68 ± 4	63 ± 2	71 ± 8
Main diagnosis			
Hematological disorder	6	18	2
Acute myeloid leukemia	2	3	
Other hem. malignancy	2	8	2
Lymphoma	2	7	
Solid-organ transplantation	2	4	1
Liver	2	3	
Heart			1
Kidney		1	
Solid tumor	2	1	1
Carcinoma of lung			1
Brain tumor	1		
Skin tumor	1		
Pharynx cancer		1	
Liver cirrhosis		4	1
Other	2	1	
Laboratory values			
Creatinine (mg/dl)	0.94 ± 0.10	1.33 ± 0.17	1.06 ± 0.37
Bilirubin (mg/dl)	6.51 ± 2.81	10.56 ± 2.56	11.65 ± 6.26
Prothrombin time (%)	76 ± 7	62 ± 4	73 ± 10
AMB treatment			
Duration (days)	6.1 ± 0.9	8.8 ± 1.5	5.6 ± 2.7
Daily dose (mg)	309 ± 22	279 ± 16	300 ± 47
Daily dose (mg/kg)	4.55 ± 0.23	4.46 ± 0.19	4.25 ± 0.58
Cumulative dose (mg)	1,688 ± 285	2,176 ± 340	2,061 ± 1,259
Time from start of last infusion to sampling (h)	22.0 ± 12.7	12.6 ± 2.5	7.3 ± 3.1

[a] Means ± standard errors of the means. Creatinine, plasma creatinine; normal range, 0.70 to 1.20 mg/dl. Bilirubin, plasma bilirubin; normal range, 0.00 to 1.28 mg/dl. Prothrombin time, normal range, 70 to 130 %. Duration, duration of treatment with lipid-formulated AMB. The time from start of last infusion to sampling was variable, since BALs were scheduled according to clinical requirements. The infusion time was 4 h. When AMB treatment was started at the intensive care unit, the choice of AMB formulation was made by randomization. In patients already on AMB at admission, the respective therapy was continued. Hem., hematological.

The differences in the underlying diseases, the limited number of patients that differed between the groups, slight differences in doses, and various intervals between AMB infusion and BAL are limitations of our study. In the LAMB group and in the ABLC group, penetration of ELF increased with this interval. Similarly, a slow increase in concentrations in lung tissue over 25 h was observed after LAMB infusion (11).

A study of rabbits revealed ELF concentrations comparable to our human data (2.28, 0.68, and 0.90 μg/ml after LAMB, ABCD, and ABLC treatment, respectively) (17).

In pleural effusion and ascites, where mainly liberated AMB is found, concentrations were even lower than those in ELF (27, 28). In vitro investigations suggest an influence of phosphatidylcholine liposomes within ELF on membrane oxidation and nitration that could potentially affect the activity of lipid-associated antimicrobial agents in vivo (25). Unlike the case with plasma and with body fluids, separation of liberated and lipid-encapsulated AMB was not feasible in ELF. For LAMB and ABCD, the penetration ratios of liberated AMB were similar, suggesting that mainly liberated AMB penetrates ELF.

Lung transplant recipients on prophylaxis with nebulized LAMB (several 25-mg doses) displayed concentrations in ELF of ~10 μg/ml 2 days after inhalation and 3 to 4 μg/ml after 2 weeks. AMB was undetectable in plasma of all but one patient, suggesting a poor systemic absorption and penetration into deeper lung compartments (20).

Penetration of ELF by voriconazole was studied for lung transplant recipients on prophylactic treatment, revealing various concentrations (0.29 to 83.32 μg/ml; mean penetration ratio, 1,100%) (5). In healthy volunteers who had received

TABLE 2. Concentrations of AMB in plasma and in ELF[a]

Parameter	Value for treatment group[b]		
	LAMB	ABCD	ABLC
Mean concn in ELF ± SEM (μg/ml)	1.60 ± 0.58**	0.38 ± 0.07*	1.29 ± 0.71
Mean concn in plasma ± SEM (μg/ml)			
Liberated	1.08 ± 0.31	0.57 ± 0.09	NA
Lipid associated	4.11 ± 1.61‡	0.54 ± 0.15‡	NA
Total	5.17 ± 1.89**	1.12 ± 0.21*	0.48 ± 0.18
Mean penetration ratio ± SEM (%)			
ELF/total plasma	61 ± 25†	125 ± 52†	447 ± 224†
ELF/liberated plasma	154 ± 44	153 ± 53	NA
Highest ELF concn (μg/ml)	6.01	1.70	6.97
Respective penetration ratio (%)	70	1,371	942
Respective time from start of AMB infusion to BAL (h)	6.25	24.00	5.50
Respective cumulative dose (mg)	2,600	900	11,700
Concn measured at maximum time from start of AMB infusion to BAL	0.35	0.28	0.84
Time from start of AMB infusion to BAL (h)	146.00	48.00	19.50
Penetration ratio (%)	242	180	1,276
Cumulative dose (mg)	1,775	1,375	150

[a] NA, not available. For the ABLC group, the chromatographic separation of lipid-associated and liberated AMB in plasma was not feasible. For patients who underwent more than one BAL, the mean concentration in ELF and penetration ratio were applied for statistical calculations. A P value of <0.05 was regarded as statistically significant. The penetration ratio was defined as the AMB concentration in ELF/simultaneous total AMB plasma level (%). The differences in concentrations in ELF between the treatment groups did not reach significance (LAMB vs. ABCD, $P = 0.21$; ABCD vs. ABLC, $P = 0.08$; LAMB vs. ABLC, $P = 0.95$).

[b] **, concentrations in ELF in were significantly lower than the respective total levels in plasma ($P = 0.001$); *, AMB concentrations in ELF were significantly lower than the respective total levels in plasma ($P = 0.01$); ‡, in LAMB therapy, the levels of the lipid-encapsulated AMB fraction exceeded those in the ABCD group highly significantly ($P < 0.001$); †, the penetration ratio was significantly higher for patients on ABLC therapy than for those in the ABCD and LAMB groups ($P < 0.05$).

posaconazole at the standard dosage for 8 days, a mean concentration in ELF of 1.86 μg/ml was measured (10). Treatment with the high-molecular-weight lipopeptide micafungin (150 mg daily for 3 days) resulted in concentrations in ELF of ~0.5 μg/ml and an accumulation in alveolar macrophage cells (8.4 to 14.6 μg/ml) (21). Similarly, AMB in either a deoxycholate or a lipid formulation accumulates in cells of the reticuloendothelial system, particularly in alveolar macrophage cells, as shown in animal and in vitro experiments (17, 18, 22, 24). In the present study, AMB was not separately quantified in alveolar macrophages.

In conclusion, treatment with AMB lipid formulations at standard doses yields ELF concentrations moderately above or even below MICs of relevant fungal pathogens. ELF levels are much lower than AMB concentrations in lung tissue samples. Further investigations should address the impact of target site penetration of antifungals on the therapeutic outcome in invasive pulmonary mycoses.

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REFERENCES

1. **Allegranzi, B., A. Cazzadori, P. G. Di, S. Bonora, M. Berti, L. Franchino, A. Biglino, A. Cipriani, and E. Concia.** 2000. Concentrations of single-dose meropenem (1 g iv) in bronchoalveolar lavage and epithelial lining fluid. J. Antimicrob. Chemother. **46:**319–322.
2. **Boselli, E., D. Breilh, M. Cannesson, F. Xuereb, T. Rimmele, D. Chassard, M. C. Saux, and B. Allaouchiche.** 2004. Steady-state plasma and intrapulmonary concentrations of piperacillin/tazobactam 4 g/0.5 g administered to critically ill patients with severe nosocomial pneumonia. Intensive Care Med. **30:**976–979.
3. **Boselli, E., D. Breilh, T. Rimmele, S. Djabarouti, J. Toutain, D. Chassard, M. C. Saux, and B. Allaouchiche.** 2005. Pharmacokinetics and intrapulmonary concentrations of linezolid administered to critically ill patients with ventilator-associated pneumonia. Crit. Care Med. **33:**1529–1533.
4. **Boselli, E., D. Breilh, M. C. Saux, J. B. Gordien, and B. Allaouchiche.** 2006. Pharmacokinetics and lung concentrations of ertapenem in patients with ventilator-associated pneumonia. Intensive Care Med. **32:**2059–2062.
5. **Capitano, B., B. A. Potoski, S. Husain, S. Zhang, D. L. Paterson, S. M. Studer, K. R. McCurry, and R. Venkataramanan.** 2006. Intrapulmonary penetration of voriconazole in patients receiving an oral prophylactic regimen. Antimicrob. Agents Chemother. **50:**1878–1880.
6. **Chono, S., T. Tanino, T. Seki, and K. Morimoto.** 2008. Efficient drug targeting to rat alveolar macrophages by pulmonary administration of ciprofloxacin incorporated into mannosylated liposomes for treatment of respiratory intracellular parasitic infections. J. Control Release **127:**50–58.
7. **Conte, J. E., Jr., J. Golden, S. Duncan, E. McKenna, E. Lin, and E. Zurlinden.** 1996. Single-dose intrapulmonary pharmacokinetics of azithromycin, clarithromycin, ciprofloxacin, and cefuroxime in volunteer subjects. Antimicrob. Agents Chemother. **40:**1617–1622.
8. **Conte, J. E., Jr., J. A. Golden, M. G. Kelley, and E. Zurlinden.** 2005. Intrapulmonary pharmacokinetics and pharmacodynamics of meropenem. Int. J. Antimicrob. Agents **26:**449–456.
9. **Conte, J. E., Jr., J. A. Golden, J. Kipps, M. McIver, and E. Zurlinden.** 2004. Intrapulmonary pharmacokinetics and pharmacodynamics of itraconazole and 14-hydroxyitraconazole at steady state. Antimicrob. Agents Chemother. **48:**3823–3827.
10. **Conte, J. E., Jr., J. A. Golden, G. Krishna, M. McIver, E. Little, and E. Zurlinden.** 2009. Intrapulmonary pharmacokinetics and pharmacodynamics of posaconazole at steady state in healthy subjects. Antimicrob. Agents Chemother. **53:**703–707.
11. **Demartini, G., C. Lequaglie, P. P. Brega Massone, F. Scaglione, and F. Fraschini.** 2005. Penetration of amphotericin B in human lung tissue after single liposomal amphotericin B (AmBisome) infusion. J. Chemother. **17:**82–85.
12. **Drusano, G. L., S. L. Preston, M. H. Gotfried, L. H. Danziger, and K. A. Rodvold.** 2002. Levofloxacin penetration into epithelial lining fluid as determined by population pharmacokinetic modeling and Monte Carlo simulation. Antimicrob. Agents Chemother. **46:**586–589.
13. **Egger, P., R. Bellmann, and C. J. Wiedermann.** 2001. Determination of amphotericin B, liposomal amphotericin B, and amphotericin B colloidal dispersion in plasma by high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. **760:**307–313.
14. **Espinel-Ingroff, A., K. Boyle, and D. J. Sheehan.** 2001. In vitro antifungal

activities of voriconazole and reference agents as determined by NCCLS methods: review of the literature. Mycopathologia **150:**101–115.
15. **Frothingham, R.** 2002. Lipid formulations of amphotericin B for empirical treatment of fever and neutropenia. Clin. Infect. Dis. **35:**896–897.
16. **Goldstein, R. A., P. K. Rohatgi, E. H. Bergofsky, E. R. Block, R. P. Daniele, D. R. Dantzker, G. S. Davis, G. W. Hunninghake, T. E. King, Jr., W. J. Metzger, H. Y. Reynolds, and G. M. Turing.** 1990. Clinical role of bronchoalveolar lavage in adults with pulmonary disease. Am. Rev. Respir. Dis. **142:** 481–486.
17. **Groll, A. H., C. A. Lyman, V. Petraitis, R. Petraitiene, D. Armstrong, D. Mickiene, R. M. Alfaro, R. L. Schaufele, T. Sein, J. Bacher, and T. J. Walsh.** 2006. Compartmentalized intrapulmonary pharmacokinetics of amphotericin B and its lipid formulations. Antimicrob. Agents Chemother. **50:**3418–3423.
18. **Hiemenz, J. W., and T. J. Walsh.** 1996. Lipid formulations of amphotericin B: recent progress and future directions. Clin. Infect. Dis. **22**(Suppl. 2)**:**S133–S144.
19. **Meersseman, W., K. Lagrou, J. Maertens, and E. van Wijngaerden.** 2007. Invasive aspergillosis in the intensive care unit. Clin. Infect. Dis. **45:**205–216.
20. **Monforte, V., P. Ussetti, R. Lopez, J. Gavalda, C. Bravo, A. de Pablo, L. Pou, A. Pahissa, F. Morell, and A. Roman.** 2009. Nebulized liposomal amphotericin B prophylaxis for Aspergillus infection in lung transplantation: pharmacokinetics and safety. J. Heart Lung Transplant. **28:**170–175.
21. **Nicasio, A. M., P. R. Tessier, D. P. Nicolau, R. F. Knauft, J. Russomanno, E. Shore, and J. L. Kuti.** 2009. Bronchopulmonary disposition of micafungin in healthy adult volunteers. Antimicrob. Agents Chemother. **53:**1218–1220.
22. **Perkhofer, S., G. Blum, C. Speth, A. Mayr, M. P. Dierich, and C. Lass-Florl.** 2007. Influence of amphotericin B and amphotericin B colloidal dispersion on the functions of human phagocytes in defence against Aspergillus species. Eur. J. Clin. Microbiol. Infect. Dis. **26:**413–417.
23. **Rennard, S. I., G. Basset, D. Lecossier, K. M. O'Donnell, P. Pinkston, P. G. Martin, and R. G. Crystal.** 1986. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. J. Appl. Physiol. **60:**532–538.
24. **Smith, P. J., J. A. Olson, D. Constable, J. Schwartz, R. T. Proffitt, and J. P. Adler-Moore.** 2007. Effects of dosing regimen on accumulation, retention and prophylactic efficacy of liposomal amphotericin B. J. Antimicrob. Chemother. **59:**941–951.
25. **Velsor, L. W., C. A. Ballinger, J. Patel, and E. M. Postlethwait.** 2003. Influence of epithelial lining fluid lipids on NO_2-induced membrane oxidation and nitration. Free Radic. Biol. Med. **34:**720–733.
26. **Vogelsinger, H., S. Weiler, A. Djanani, J. Kountchev, R. Bellmann-Weiler, C. J. Wiedermann, and R. Bellmann.** 2006. Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion. J. Antimicrob. Chemother. **57:**1153–1160.
27. **Weiler, S., R. Bellmann-Weiler, S. Dunzendorfer, M. Joannidis, and R. Bellmann.** 2008. Levels of amphotericin B lipid formulations in ascites. J. Antimicrob. Chemother. **62:**1163–1164.
28. **Weiler, S., R. Bellmann-Weiler, M. Joannidis, and R. Bellmann.** 2007. Penetration of amphotericin B lipid formulations into pleural effusion. Antimicrob. Agents Chemother. **51:**4211–4213.