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Nebulization of four commercially available amphotericin B formulations in persistently granulocytopenic rats with invasive pulmonary aspergillosis: evidence for long-term biological activity

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Abstract

The nebulization of amphotericin B desoxycholate (AMB-DOC), liposomal amphotericin B (L-AMB), amphotericin B lipid complex (ABLC) and amphotericin B colloidal dispersion (ABCD) has been investigated. Particle sizes of generated aerosol droplets were measured. Pulmonary amphotericin B deposition and amphotericin B concentration in blood directly after nebulization and at six weeks after nebulization was measured in healthy rats. The efficacy of nebulized amphotericin B formulations was evaluated in persistently granulocytopenic rats with invasive pulmonary aspergillosis. Treatment was given either after or before fungal inoculation. The endpoint was survival of animals. Aerosol particle sizes, expressed as the values for the mass median diameter were 1.38, 2.43, 0.90 and 2.29 μm for AMB-DOC, L-AMB, ABLC and ABCD, respectively. Amphotericin B concentrations in the lungs directly after nebulization exceeded the minimum inhibitory concentration of *Aspergillus fumigatus* and amphotericin B was still detected in lungs of rats at six weeks after nebulization. Treatment, started at 16 h after fungal inoculation, resulted in a significantly prolonged survival as compared with sham-treated rats for all four formulations. Prophylactic treatment at one week before fungal inoculation resulted in a significantly prolonged survival for all four formulations. Aerosol treatment given at two weeks before inoculation was effective only for AMB-DOC and L-AMB, whereas treatment given at six weeks resulted in a significantly prolonged survival for L-AMB only. All commercially available amphotericin B preparations could be nebulized efficiently and may be of value in the prophylactic treatment of invasive pulmonary aspergillosis.

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Introduction

An increase in the number of immunosuppressed patients in the last few decades has led to an increase in the number of invasive fungal infections such as invasive pulmonary aspergillosis (IPA) (Bodey et al 1988; Denning 1998). Although new non-polyene antifungal agents have been developed recently, amphotericin B, in the form of amphotericin B desoxycholate (AMB-DOC), remains an important drug for the treatment of IPA, due to its potent fungicidal activity (Gallis et al 1990). Its use, however, is limited by its narrow therapeutic index. A number of toxic side effects, especially nephrotoxicity, have made this drug difficult for patients to tolerate (Luber et al 1999). Lipid-based formulations of amphotericin B were developed to diminish side effects of the drug. Lipid-based amphotericin B formulations have a broader therapeutic index as compared with AMB-DOC, which permits higher dosing schedules. Currently, there are three marketed lipid formulations of amphotericin B. Liposomal amphotericin B (L-AMB) consists of small unilamellar vesicles with amphotericin B incorporated within the bilayer membrane of phospholipids. Amphotericin B lipid complex (ABLC) has amphotericin B complexed to two phospholipids in a ribbon-like structure. Amphotericin B colloidal dispersion (ABCD) is composed of disk-like structures of cholesteryl sulfate complexed with amphotericin B. In the empirical

treatment of systemic fungal infections, all three lipid-formulations of amphotericin B formulation are at least as effective as AMB-DOC and show less toxicity (Meis & Verweij 2001). Little direct comparative data are available on the relative efficacy of the different lipid formulations or on the comparative efficacy against AMB-DOC.

Despite the choice of several available amphotericin B formulations and new drugs such as voriconazole and caspofungin, mortality rates from IPA are still unacceptably high and most neutropenic patients with proven IPA die from this infection (Denning 1998; Leenders et al 1998). These severe life-threatening features of IPA stress the critical need for optimizing management of this infection. Failure of treatment of IPA with intravenous amphotericin B is probably the result of several factors. The fungus resides predominantly in the airways and can spread to other body tissues. Inadequate penetration of amphotericin B in the airways after intravenous administration of any amphotericin B formulation can contribute to failure of treatment. It has been shown that only a small percentage of intravenously administered amphotericin B is actually delivered to the lungs (Van Etten et al 1995; Lambros et al 1997). By administration of drugs via the inhalation route, the lungs are directly targeted which results in high pulmonary drug concentrations. Nebulized AMB-DOC, L-AMB, and ABLC in different animal models of IPA resulted in substantial, long-lasting pulmonary amphotericin B concentrations and significantly improved survival of rats (Allen et al 1994; Schmitt et al 1988; Cicogna et al 1997; Ruijgrok et al 2001). However, data on the differential value of the amphotericin B formulations are scarce.

Aerosol administration of amphotericin B formulations can be valuable in the optimization of the management of IPA. Which formulation is most suitable for this approach has not yet been investigated. This study presents a head-to-head comparison of the biodistribution, efficacy and toxicity of nebulized amphotericin B formulations.

Materials and Methods

Materials

Amphotericin B desoxycholate (AMB-DOC; Fungizone) was from Bristol Myers-Squibb (Woerden, The Netherlands), liposomal amphotericin B (L-AMB; AmBisome) was from Nexstar (San Dimas, CA, USA), amphotericin B lipid complex (ABLC; Abelcet) was from The Liposome Company (Princeton, NJ, USA) and amphotericin B colloidal dispersion (ABCD; Amphocil; Amphotec) was from Alza Corporation (Palo Alto, CA, USA). Cyclophosphamide was from Sigma Chemical Co. (St Louis, MO, USA). Sabouraud dextrose agar (SDA) was from Oxoid (Basingstoke, UK).

Animals

Female R-strain albino rats, specified pathogen free, 18- to 25-weeks-old (own breed) (185–225 g) were used for all experiments. Animals received a normal, pathogen free diet and water was freely available. Experiments were approved by the animal experiments ethical committee of the Erasmus University Medical Center Rotterdam.

Aspergillus strain

A clinical isolate of *Aspergillus fumigatus* from an immunocompromised patient with IPA was used. Minimum inhibitory concentration (MIC) and minimal fungicidal concentration of amphotericin B for this strain are 0.4 and 0.8 mg L^{-1}, respectively (Leenders et al 1996). This strain was stored under oil on SDA. At least once every two months, the strain was passed through a rat to maintain its virulence. For inoculation, conidia were harvested and suspended in sterile phosphate buffered saline, as previously described (Leenders et al 1996).

Immunosuppression and supportive care

Leukopenia was induced by intraperitoneal administration of 90 mg kg^{-1} cyclophosphamide at five days before fungal inoculation followed by additional dosages of 60 mg kg^{-1} every four days throughout the study. This treatment resulted in a persistent leukopenia ($< 0.5 \times 10^9$ L^{-1}) from the time of *A. fumigatus* inoculation up to the end of the study (Leenders et al 1996). To prevent bacterial superinfection, strict hygienic care was applied, and animals received ciprofloxacin (660 mg L^{-1}) and polymyxin E (100 mg L^{-1}) in their drinking water during the whole experiment. Furthermore, intramuscular administration of amoxicillin (40 mg kg^{-1}/day) was added to this regimen. Shortly before and after inoculation, gentamicin (6 mg kg^{-1}) was administered intramuscularly.

Experimental lung infection

Infection of the lung was established according to the method described by Bakker-Woudenberg et al (1982). Briefly, under general anaesthesia the left main bronchus was intubated. A canula was passed through the tube and the left lobe of the lung was inoculated with 0.02 mL of the conidial suspension containing 1.5×10^5 conidia. This resulted in a left-sided pneumonia.

Nebulization procedure

AMB-DOC, L-AMB, ABLC and ABCD were reconstituted according to the manufacturer's instructions and further diluted in 5% glucose. L-AMB, ABLC and ABCD were diluted with glucose up to an AMB concentration in the nebulizer reservoir of 4 mg mL^{-1}. AMB-DOC was diluted up to a nebulizer reservoir concentration of 2 mg mL^{-1}. Previous work in our laboratory describes the rationale for these concentrations (Ruijgrok et al 2000). In short: for AMB-DOC, 2 mg mL^{-1} is the maximum dose which is tolerated by rats, whereas the dose of the lipid formulations is limited to 4 mg mL^{-1} because of technical limitations to nebulization. The aerosol procedure was as previously described

(Ruijgrok et al 2000): rats were constrained in cone-ended plastic tubes and placed in a nose-only inhalation apparatus (CH Technologies USA Inc., Westwood, NJ, USA). Aerosols were generated by a Collison six-jet nebulizer system (Model CN, BGI Inc., Waltham, MA, USA). The nebulizer operated at 20 L min^{-1} air flow. Animals were exposed to aerosol treatment for one or more periods of 60 min.

Droplet size measurements

The droplet size distribution of aerosols was measured using a laser velocity particle sizer (Aerosol Particle Sizer 3320 A, TSI, Inc., St Paul, MN, USA). Aerosols were generated with compressed air at a flow rate of 10 L min^{-1}. Distribution of the number of generated aerosol particles was directly measured with this technique. For extrapolation of mass distribution from the number distribution, specific gravity was set at that of the solvent (1 g/cm^3) and relative humidity at 60%. From the mass distribution, the mass median diameter and the percentage of particles $< 5\,\mu$m were calculated.

Deposition of amphotericin B in lungs after nebulization of AMB-DOC, L- AMB, ABLC, or ABCD

Deposition experiments were performed in healthy rats. Directly after and at six weeks after nebulization of AMB-DOC, L-AMB, ABLC or ABCD, rats were killed and blood was sampled via a cardiac punction. Right and left lung lobes were removed and weighed. Lungs were not washed. Lungs were homogenized in 5 mL glucose 5%. Amphotericin B was extracted from blood and homogenate with ethanol in a 2:3 (v/v) ratio. The extracts were centrifuged for 5 min at 13 000 *g* and concentrations of amphotericin B in the supernatants were determined by HPLC with an UV detector operating at 382 nm. The mobile phase consisted of 0.1 M sodium acetate solution (pH 7.2) containing 0.2% (v/v) triethylamine and 60% acetonitrile and was pumped through a guard pre-column (Chromguard, 10×3 mm; Chrompack, Middelburg, The Netherlands) followed by a reverse-phase ODS 2 C18 column (100×3 mm i.d.; particle size 5 μm; Chrompack, Middelburg, The Netherlands) at a flow rate of 0.5 mL min^{-1}. The recovery was 70% for blood and 75% for lung tissue. The lower limit of quantification of this assay was 0.2 mg mL^{-1}.

Effect of AMB-DOC, L-AMB, ABLC and ABCD on survival of rats

Groups of 15 infected rats each were treated with a single dose of nebulized AMB-DOC, L-AMB, ABLC or ABCD at different times after or before fungal inoculation. Treatment given after fungal inoculation started at 16 h from inoculation. Histopathological examination (periodic acid–Schiff stain) confirmed that mycelial outgrowth began at 16 h. Prophylactic treatment was given at one week, two weeks, or six weeks before fungal inoculation. The endpoint was survival of treated animals as compared with controls. Controls received nebulized glucose 5%. Animals were checked twice daily and mortality was recorded for 12 days after fungal inoculation. Surviving animals were killed after day 12. To check for bacterial superinfections, the left lung, right lung and liver were dissected post mortem and homogenized in 20 mL phosphate-buffered saline for 45 s at 20 000 rev min^{-1} in a VirTis homogenizer (The VirTis Co. Inc., Gardiner, NY). Volumes of 0.2 mL and 2 mL and the remainder of each homogenate were spread onto or poured into SDA plates. Plates were incubated for 24 h at 37°C followed by 24 h at 25°C. Animals with bacterial infections were left out of the analysis.

Influence of amphotericin B products on surfactant function

Freeze-dried natural surfactant prepared from bovine lavages was a gift of the Department of Anaesthesiology of the Erasmus University Medical Center Rotterdam. It consisted of approximately 90–95% phospholipids, 1% hydrophobic proteins (surfactant protein B & C) and 1% free fatty acids. This surfactant is highly surface active at low concentrations. Influence of different amphotericin B formulations on surfactant function was determined by means of a modified Wilhelmy balance system (E. Biegler GmbH, Mauerbach, Austria). This system records surface tensions of an air–liquid film over several cycles of mechanical compression and expansion of this film. The lower the surface tension at minimal surface area, the higher the surface activity of the applied film.

The trough of the Wilhelmy balance was filled with warm saline (37°C) and calibrated. After calibration, 100 μL of surfactant (1 mg mL^{-1} total lipids) alone or in the presence of amphotericin B formulations was applied onto the saline hypophase and allowed to spread for 2 min. The concentration of amphotericin B or desoxycholate can be found in Table 3. The surface area was compressed and expanded with a cycling speed of 1 cycle/3 min and an area reduction from 100% to 20%. Minimal surface tension (γ_{min}) was measured after 3 cycles at 20% surface area, and is expressed as mN m^{-1}. Inhibition of surfactant function of natural surfactant by amphotericin B formulations resulted in increased surface tension at minimal surface area.

Statistical analysis

Survival curves were generated by the method of Kaplan and Meier. Statistical evaluation of differences in the survival curves was performed by the log rank test. This test examines the length of survival as well as the percentage of survival. Differences in mean minimal surface tension (γ_{min}) of surfactant after addition of several amphotericin B formulations were calculated using a two-sided *t*-test.

Results

Aerosol droplet size

Aerosol droplet sizes of all four formulations defined as the mass median diameter and the percentage of particles $< 5\,\mu$m

Table 1 Mass median diameter and % of aerosol particles < 5 μm as determined by laser diffraction analysis

Formulation	Mass median diameter (μm)	Geometric standard deviation (μm)	% particles < 5 μm
AMB-DOC	1.38	2.26	83.2
L-AMB	2.43	1.97	86.6
ABLC	0.90	2.02	91.2
ABCD	2.29	2.32	86.7

are given in Table 1. For all the formulations, more than 80% of the particles were below 5 μm mass diameter.

Deposition of amphotericin B in lungs after nebulization of AMB-DOC, L-AMB, ABLC, or ABCD

The pulmonary concentrations of amphotericin B in uninfected lungs for aerosolized AMB-DOC (nebulizer reservoir concn 2 mg mL^{-1}) and aerosolized L-AMB, ABLC and ABCD (nebulizer reservoir concn 4 mg mL^{-1}) are given in Table 2. The concentrations were determined directly after and at six weeks after a single aerosol dose of one of the four formulations. For all formulations, substantial amphotericin B concentrations were deposited in the lungs directly after nebulization. There was no difference in the deposited amphotericin B between the four formulations. With all four products, amphotericin B was still detected in lungs at six weeks after nebulization, which indicated a very slow clearance of amphotericin B from the lungs. The amount of amphotericin B in blood was below the limit of detection in all samples.

Table 2 Deposition of amphotericin B in lungs (μg g^{-1}) directly after (0 h) and six weeks after aerosol administration (60 min) of four different amphotericin B formulations

Formulation	Amphotericin B concn (mg mL^{-1}) in nebulizer	Amphotericin B in lungs (μg g^{-1}) at 0 h	Amphotericin B in lungs (μg g^{-1}) at six weeks
AMB-DOC	2	26.9 (8.5)[a]	11.4 (1.3)
L-AMB	4	46.7 (15.5)	11.1 (2.9)
ABLC	4	24.9 (11.4)	7.7 (0.4)
ABCD	4	30.9 (5.1)	14.8 (2.5)

[a]Values are mean (s.d.), n = 3.

Effect of nebulized AMB-DOC, L-AMB, ABLC and ABCD on survival of rats

The effect of aerosol treatment on survival is shown in Figure 1. In this model of IPA, control rats died between

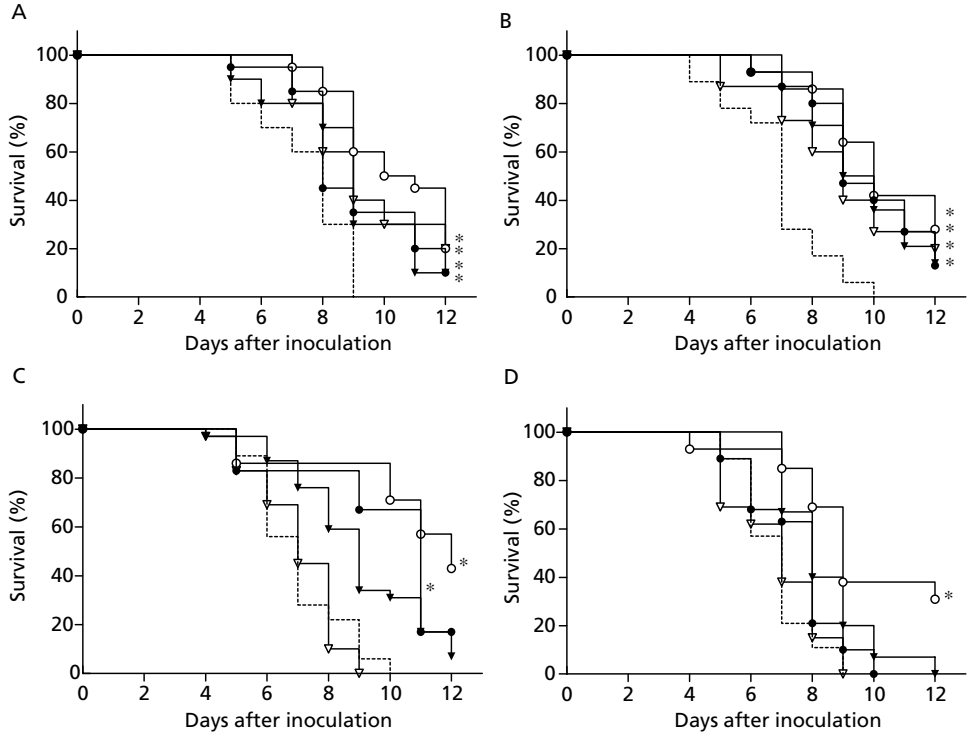

Figure 1 Effect of a single dose of aerosolized AMB-DOC (●), L-AMB (○), ABLC (△) and ABCD (▲) on survival of persistently granulocytopenic rats with pulmonary aspergillosis (Kaplan–Meier plot). Treatment was started at (A) 16 h after or (B) one week, (C) two weeks or (D) six weeks before fungal inoculation. Nebulizer reservoir amphotericin B concentrations were 4 mg mL^{-1}, except for AMB-DOC, where the nebulizer reservoir concentration was 2 mg mL^{-1}. Each group of 15 animals was exposed for 60 min. Control animals received nebulized 5% glucose (---). *P < 0.05 compared with controls.

day 4 and day 9 after fungal inoculation. Empiric treatment started at 16 h after fungal inoculation, the time at which hyphal outgrowth began. This treatment resulted in a significantly prolonged survival as compared with controls for all four regimens ($P < 0.05$) (Figure 1A), with no difference between the four drug formulations. Prophylactic treatment was started at one week, two weeks or six weeks before fungal inoculation. Treatment started at one week before inoculation resulted in a significantly prolonged survival as compared with controls for all four regimens ($P < 0.05$) (Figure 1B), again with no difference between the four amphotericin B formulations. Treatment started at two weeks before fungal inoculation resulted in a significantly prolonged survival for AMB-DOC and L-AMB ($P < 0.05$), whereas ABLC showed borderline significance ($P = 0.06$). Treatment with ABCD did not result in a significantly prolonged survival ($P = 0.77$). Only for L-AMB did treatment at six weeks before inoculation result in a significantly prolonged survival as compared with controls ($P < 0.05$).

Influence of amphotericin B products on surfactant function

Table 3 shows the mean minimal surface tensions (γ_{min}) of surfactant alone or in combination with amphotericin B alone, desoxycholate, AMB-DOC, L-AMB, ABLC or ABCD. The natural surfactant was highly surface active at the examined concentrations (γ_{min} 1.97 ± 1.23). Minimal surface tensions after mixing of surfactant with amphotericin B alone, L-AMB, ABLC or ABCD yielded similar low values. Addition of AMB-DOC to natural surfactant resulted in significantly increased minimal surface tensions, indicating a loss of surface activity of the mixtures. An increase in minimal surface tension of natural surfactant was also seen with addition of desoxycholate alone.

Table 3 Mean minimal surface tension (γ_{min}) of surfactant ($1\,mg\,mL^{-1}$) together with saline, amphotericin B, desoxycholate, AMB-DOC, L-AMB, ABLC and ABCD

	Concn ($mg\,mL^{-1}$)		Minimal surface tension ($mN\,m^{-1}$)[a]
	Amphotericin B	Desoxycholate	
Saline	–	–	1.97 ± 1.23
Amphotericin B	2		2.70 ± 0.53
Desoxycholate		2.1	23.13 ± 1.16[c]
AMB-DOC[b]	2	1.6	47.53 ± 0.89[c]
L-AMB	2		1.42 ± 0.83
ABLC	2		1.45 ± 0.39
ABCD	2		3.33 ± 0.39

[a]Each value represents the mean ± s.d. of three individual experiments. [b]In AMB-DOC, 54% (g g^{-1}) was amphotericin B, 46% was desoxycholate. Therefore, $2\,mg\,mL^{-1}$ amphotericin B correlated with $1.6\,mg\,mL^{-1}$ desoxycholate. [c]$P \leq 0.05$ as compared with saline.

Discussion

IPA is a serious opportunistic disease in patients with immunosuppression. A difficult diagnosis, which is often confirmed late in the course of the infection, hampers an adequate treatment of IPA. To treat IPA effectively, multiple high doses of intravenous AMB-DOC are needed, but these can usually not be given due to nephrotoxicity of amphotericin B. Using aerosol administration of amphotericin B formulations, the drug is directly deposited at the intended site of action, which could be highly attractive, both in terms of avoidance of toxicity and in terms of efficacy. In this study, we have examined the value of aerosol administration of four commercially available amphotericin B formulations in a model of IPA in persistently granulocytopenic rats.

The amount of deposition of an aerosol in the respiratory tract is mainly determined by the particle size distribution of the aerosol droplets. Large particles with a diameter of $> 5\,\mu m$ are deposited in the upper airways and will not be distributed to the peripheral regions of the lungs. Particles with a diameter of $< 0.5\,\mu m$ are exhaled (Hickey 1996). Inhaled *A. fumigatus* spores, with an average particle size of 1–2 μm, are deposited in the peripheral (alveolar) region of the lungs and invasive disease develops from thereon. It is suggested that the optimal range of particle sizes for peripheral deposition for amphotericin B aerosols is between 1 and 5 μm. We have shown before that AMB-DOC and L-AMB aerosols are characterized by an adequate particle size (Ruijgrok et al 2000). Although the four tested amphotericin B formulations have distinct different physicochemical properties, all formulations were efficiently nebulized by the nebulizer used in this study. Although the mass median diameter was different for the four formulations, this was probably not relevant for their peripheral deposition. The median particle size of all four formulations was between 1 and 5 μm, and $> 80\%$ of generated particles were below 5 μm. A substantial peripheral deposition of amphotericin B could therefore be expected for all formulations.

In this study, we have shown that for all four formulations, the pulmonary concentrations of amphotericin B directly after nebulization were substantial and exceeded the MIC for *A. fumigatus*, which was 0.4–0.8 $mg\,L^{-1}$ for non-lipid associated amphotericin B. The deposition data of the different formulations directly after nebulization were not in discrepancy with the differences in aerosol particle sizes. The pulmonary deposition seemed not to differ significantly between the different products. Furthermore, we have shown that amphotericin B could still be detected in pulmonary tissue at six weeks after nebulization of any of the four formulations. Amphotericin B was not detected in blood at any time. This indicated that aerosol administration led to very low systemic exposure of amphotericin B. The substantial deposition as well as the extensive pulmonary retention and low systemic exposure of amphotericin B rendered the aerosol delivery of any one of the four products very suitable for empiric as well as prophylactic treatment of pulmonary aspergillosis.

Few clinical data are available on the nebulization of amphotericin B formulations in patients with pulmonary fungal infections (Hertenstein et al 1994; Erjavec et al 1997; Schwartz et al 1999). Until now, the exact value of this approach still had to be determined. In a model of aspergillosis in mice and rats with corticosteroid-induced immunosuppression, prophylaxis or treatment of IPA with nebulized AMB-DOC or L-AMB was evaluated (Schmitt et al 1988; Allen et al 1994). In those studies, the nebulized formulations were effective in decreasing the number of viable *A. fumigatus* counts in the lungs and in increasing survival of animals. L-AMB was more effective than AMB-DOC (Allen et al 1994). In rats with corticosteroid-induced immunosuppression, prophylactically nebulized ABLC resulted in improved survival of IPA (Cicogna et al 1997). These studies were all focused on the prophylaxis of IPA, and temporary or mild immunosuppression was applied in the experimental model. In the clinical situation however, patients with severe IPA are generally persistently granulocytopenic. An important feature of the experimental set-up of this study was therefore the persistently immunosuppressed state. In this model, we have described previously the therapeutic efficacy of nebulized AMB-DOC and L-AMB, with the start of treatment at 30 h after fungal inoculation (Ruijgrok et al 2001). In that study, we showed that nebulized AMB-DOC as well as nebulized L-AMB significantly prolonged survival in rats with established IPA. In this study, the start of treatment was at 16 h after and at different times before inoculation. Histopathological examination confirmed that at 16 h after fungal inoculation hyphal outgrowth began. We have seen that aerosol treatment started at 16 h with any one of the four formulations resulted in a significantly prolonged survival as compared with control rats. However, we showed before that aerosol administration of amphotericin B formulations did not prevent dissemination of the infection (Ruijgrok et al 2001). We concluded therefore, that aerosol administration of amphotericin B would be of limited value as treatment of IPA.

The deposition data showed that long-term protection could be expected from the nebulized amphotericin B products, since at six weeks after a single dose of nebulized AMB-DOC, L-AMB, ABLC or ABCD, amphotericin B was still present in lung tissue. The measured amphotericin B concentrations exceeded the MIC value of *A. fumigatus* strain for amphotericin B. Whether this amphotericin B was indeed biologically active remains to be proven by the efficacy data. We showed prophylactic efficacy for all four formulations when aerosols were given at one week before fungal inoculation. The efficacy seemed not to differ between the four products. When aerosol treatment was given at two weeks before fungal inoculation, we saw that nebulized AMB-DOC and L-AMB still showed prophylactic efficacy, whereas nebulized ABCD did not and ABLC did not conclusively. When given at six weeks before only nebulized L-AMB showed prophylactic efficacy. The differences between the four formulations in the efficacy experiments were not reflected by the aerosol particle size or the deposition data. We can therefore only speculate about the reason for the observed discrepancy between the chemical presence and the biological activity of amphotericin B. It is known that amphotericin B tightly binds to cell membranes and sterol receptors which may render it inactive over time; however, we found the amphotericin B to be extractable with organic solvents. It is tempting to suggest that the liposomal formulation acted as a slow release pool, which resulted in long-term efficacy as was shown in the survival data.

The toxicity of aerosolized amphotericin B formulations was evaluated in an in-vitro experiment in which their influence on surfactant function was determined. It appeared that AMB-DOC, and not the lipid formulations, had a detrimental effect on surfactant. This was due to the detrimental effects of desoxycholate on surfactant function, since this agent alone showed high influence on surface activity of natural surfactant as opposed to amphotericin B without desoxycholate. The detrimental effect of deoxycholate was probably caused by its deterging capacities. The lipid formulations showed no detrimental effects, which was as expected because the lipid formulations consisted in a large part of phospholipids, which were the surface active components of pulmonary surfactant. Therefore, it was assumed that the lipid formulations would be safer for the surfactant function of the respiratory tract and that administration of aerosols of AMB-DOC was more likely to lead to local toxic effects.

Conclusions

We have described the nebulization of four commercially available amphotericin B formulations. All formulations were nebulized efficiently in terms of aerosol particle size and deposition in the lungs. Nebulization of all four formulations did not lead to systemic amphotericin B levels and therefore side effects which are frequently related to intravenous administration of amphotericin B formulations were expected to be minimal. Local toxicity on the lungs was expected to be minimal with the lipid formulations. All nebulized formulations showed promising efficacy when administered prophylactically. However, L-AMB was superior as it was effective for a relatively longer period compared with the other formulations. This work provides support for more effective strategies to prevent the threat of *Aspergillus* infections during periods of prolonged immunosuppression. The positive results have instigated a clinical trial with nebulized L-AMB in our institution.

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