Nebulized Liposomal Amphotericin B Prophylaxis for *Aspergillus* Infection in Lung Transplantation: Pharmacokinetics and Safety

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Background: The main problem with using nebulized liposomal amphotericin (n-LAB) as prophylaxis for *Aspergillus* infection after lung transplantation is the lack of knowledge of its pharmacokinetics and its possible adverse effects. The aim of this study was to measure post-inhalation amphotericin B concentration in the respiratory tract and serum of lung transplant patients and assess the effects of n-LAB on respiratory function.

Methods: Thirty-two consecutive bronchoscopies were performed on 27 lung transplant patients at two hospitals. Amphotericin B concentration in the first and third aliquot of bronchoalveolar lavage material was measured in steady state. The first aliquot approximates most closely the true amphotericin B concentrations in the proximal airway, whereas the third aliquot provides an optimum sample from the distal airway.

Results: At 2 days, mean amphotericin B concentrations were 11.1 μg/ml (95% confidence interval [CI]: 16.5 to 5.7 μg/ml) and 9.0 μg/ml (95% CI: 14.3 to 3.8 μg/ml) in the first and third aliquot, respectively. Thereafter, concentrations declined progressively. At 14 days, concentrations were 3.0 μg/ml (95% CI: 4.4 to 1.5 μg/ml) in the first aliquot and 4.1 μg/ml (95% CI: 6.1 to 2.1 μg/ml) in the third aliquot (p = not statistically significant). Traces of amphotericin B (0.1 μg/ml) were found in serum samples from only 1 of 27 patients. Mean value of forced expiratory volume in the first second (FEV_1) was similar before and after n-LAB.

Conclusions: Amphotericin B concentrations after n-LAB remained high for 14 days, at adequate concentrations for prophylaxis of *Aspergillus* infection. No significant systemic absorption of amphotericin B was detected and no effect was observed on respiratory function. This promising prophylactic regimen warrants assessment in future clinical studies. J Heart Lung Transplant 2009;28:170–5. Copyright © 2009 by the International Society for Heart and Lung Transplantation.

Intrapulmonary and extrapulmonary infections[1,2] have been described for several species of fungi[3–5]; however, lung infection caused by *Aspergillus* spp is the most common. Despite the continuing advances in treatment for fungal conditions,[6,7] infection by *Aspergillus* spp is still associated with high morbidity and mortality in lung transplant recipients,[8–10] which indicates the need for prophylactic strategies in these patients. The use of prophylaxis has led to a considerable decrease in the incidence of *Aspergillus* infection in this population. According to a survey by Dummer et al,[11] 76% to 80% of centers in the USA currently prescribe some type of anti-fungal prophylaxis for transplant patients. The agent used was inhaled amphotericin at nearly two thirds of centers. Several studies performed with inhaled conventional amphotericin B (with deoxycholate) in lung transplant patients have reported on efficacy and safety data,[12–14] as well as pharmacokinetics and distribution in the respiratory tract.[15] In a previous report from our group, the overall incidence of *Aspergillus* spp infection in the lung transplant population was 33%. Prophylaxis with inhaled conventional amphotericin reduced the overall incidence of *Aspergillus* infection to 14.4%.[12]

Inhaled conventional amphotericin has the advantage that distribution is limited to the respiratory tract and there is no systemic absorption. Moreover, it is well tolerated and does not interact with immunosuppressive drugs. The main drawbacks include poor adherence to treatment, because frequent administration is required,[12–15] and the potential for contamination of

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Submitted July 25, 2008; revised October 27, 2008; accepted November 6, 2008.

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 1053-2498/09/$–see front matter. doi:10.1016/j.healun.2008.11.004

the nebulization system.[16] Other formulations of inhaled amphotericin B (lipid complex and liposomal amphotericin) have been proposed for prophylactic purposes, with promising results.[17–20] In some experimental models, nebulized liposomal amphotericin B (n-LAB) has been shown to be more effective[21] and long-lasting[22] than the conventional formulation.

In a previous pilot study of lung transplant recipients, we observed high concentrations of amphotericin B in the respiratory tract at 7 days after a single 25-mg dose of n-LAB.[23] The aim of the present study was to measure post-inhalation amphotericin B concentration in the respiratory tract and serum of lung transplant patients, and also to assess the effects of n-LAB on respiratory function.

METHODS

Patients and Study Design

Because of an insufficient supply of amphotericin B deoxycholate on the Spanish market, nebulized amphotericin B deoxycholate was switched to n-LAB as prophylaxis for *Aspergillus* infection in all lung transplant patients at our hospital, starting in June 2003. The dose of n-LAB administered was based on the results of our previous pilot study.[23] Patients received 25 mg (6 ml) 3 times per week up to Day 60 post-transplantation, 25 mg once per week between Days 60 and 180, and 25 mg once every 2 weeks thereafter for life.

From June 2003 to July 2004, a cross-sectional study was performed. Twenty-seven consecutive patients from two hospitals were enrolled, including 21 men and 6 women, with a mean age of 46.8 years (range: 18 to 66 years). Five patients received single-lung transplants and 22 sequential had double-lung transplants. The surgical procedure was similar for all patients. Underlying diseases were idiopathic pulmonary fibrosis in 10 patients, chronic obstructive pulmonary disease (COPD) in 9, cystic fibrosis in 2, Langerhan's cell histiocytosis in 3, lymphangioleiomyomatosis in 1, bronchiolitis obliterans in 1 and hemosiderosis in 1. Other characteristics of the patients are shown in Table 1.

Thirty-two bronchoscopies were performed on the 27 patients, 10 at 2 days after nebulization of 25 mg n-LAB, 10 at 7 days and 12 at 14 days. Amphotericin B concentration in bronchoalveolar lavage material was measured in steady state after several doses of n-LAB. Moreover, in all patients, serum amphotericin B concentration was measured at the time bronchoscopy was performed. In patients undergoing more than one bronchoscopy, the interval between the procedures was at least 1 month. The indications for bronchoscopy were always established on clinical criteria or surveillance criteria (Table 1). Patients with fever, pneumonia, hypoxemia, hemodynamic instability or mechanical ventilation were excluded.

Table 1. Patient Characteristics and Bronchoscopies Performed

Number of bronchoscopies	32
Bronchoscopy/patient	Mean: 1.18 (range: 1–3)
Time from transplant to bronchoscopy	Mean: 333 days (range: 44–2,168)
Bronchoscopies in patients with acute rejection	11 of 32 (34.3%)
Bronchoscopies in patients with infectious bronchitis	14 to 32 (43.7%)
Bronchoscopies in patients with bronchiolitis obliterans	11 to 32 (34.3%)
Bronchoscopies in patients with significant bronchial stenosis	12 to 32 (37.5%)

Nebulized Liposomal Amphotericin B Preparation and Administration

Fifty milligrams of liposomal amphotericin B for injection (Ambisome; Gilead Sciences SL, Madrid, Spain) was dissolved in 12 ml of sterile water. The solution remained stable for at least 7 days at 2°C to 8°C. The technique consisted of amphotericin B nebulization by a jet nebulizer (Ventstream or Sidestream, Respironics, Murrysville, PA) with a CR60 compressor (air pressure: 27.2 psi; flow: 7.3 liters/min), equipped with a disposable bacterial exhale filter. This system produces aerosol droplets having a median mass diameter of 3 μm and a respirable fraction (percent output contained in particles <5 μm) of 80% of particles. Patients were instructed by a trained staff nurse to inhale through a mouthpiece and exhale through the nose. The procedure took 10 to 15 minutes. To avoid contamination, the nebulizer was washed and brushed with soap and water after each administration; once rinsed, it was submerged in 1% sodium hypochlorite solution (Milton).

Bronchoscopic Procedure and Sample Collection

Bronchoscopy was performed through the nose in most cases. Prior to sample collection, 10 ml of lidocaine 2% was administered as local anesthetic and immediately aspirated. Bronchoalveolar lavage (BAL) samples were obtained. The tip of the bronchoscope was wedged into a sub-division of a segmental bronchus, preferably in the right middle lobe or lingula. BAL was then performed by instillation of a preliminary aliquot of 20 ml of sterile isotonic saline solution, which was excluded from the analysis, and three separate 50-ml aliquots of saline. The first and third aliquots were used for the amphotericin B assays. The first aliquot approximates most closely the true amphotericin B concentrations in the proximal airway, whereas the third aliquot provides an optimum sample from the distal airway.[24] The instilled fluid was then re-aspirated by gentle manual suction. Dwell time of the instilled fluid in BAL

averaged 20 seconds. After BAL sampling, transbronchial lung biopsies were performed in 31 of 32 procedures.

Amphotericin B Assay and Calculation of Final Concentration

In a previous study, we reported on a reversed-phase high-performance liquid chromatography (HPLC) method for amphotericin B assay in respiratory samples.[25] As BAL is a diluted sample, the final drug concentration was calculated assuming that 1% of the recovered BAL corresponded to the volume of epithelial lining fluid.[26] Hence, the following formula was used: $C_{final} = (C_{BAL} \times V_{BAL})/(0.01 \times V_{BAL})$, where C_{final} is the final amphotericin concentration present in epithelial lining fluid, C_{BAL} is the concentration found in BAL analysis, and V_{BAL} is the volume recovered in BAL. The HPLC method used to assay amphotericin B in human serum was described in another study.[27]

Respiratory Function Assessment

Spirometry (Spirodoc; Abmedica SA, Barcelona, Spain) was performed before, and at 30 minutes and 2 hours after nebulization in 22 consecutive patients. Differences were considered significant when forced expiratory value in 1 second (FEV_1) decreased by >12% with respect to FEV_1 measured before n-LAB administration.

Statistical Analysis

The mean and 95% confidence interval (CI) of amphotericin B concentrations from the first and third aliquot of BAL were calculated at 2, 7 and 14 days post-nebulization. The Mann–Whitney *U*-test was used to compare mean amphotericin B concentration between the first and third aliquot, and FEV_1 value before and after n-LAB administration. $p < 0.05$ was considered significant.

The study protocol was approved by the Ethics Committee for Clinical Research of Hospital Universitari Vall d'Hebron and by the Spanish Ministry of Health and Consumer Affairs. Informed written consent was obtained from all patients taking part in the study.

RESULTS

Mean fluid volume recovered in the 32 samples was 26.1 ml (range 6 to 25 ml) and 34.3 ml (range: 15 to 50 ml) in the first and third BAL aliquot, respectively. Mean amphotericin B concentrations in the first and third aliquot at each time-point are shown in Figure 1. At 2 days, mean amphotericin B concentrations were 11.1 μg/ml (95% CI: 16.5 to 5.7 μg/ml) in the first aliquot and 9.0 μg/ml (95% CI: 14.3 to 3.8 μg/ml) in the third aliquot. Thereafter, concentrations decreased progressively. At 7 days, mean concentrations were 4.4 μg/ml (95% CI: 7.9 to 0.9 μg/ml) and 8.2 μg/ml (95% CI: 13.4 to 3.0 μg/ml) in the first and third aliquot, respectively. At 14 days, concentrations were 3.0 μg/ml (95% CI: 4.4 to 1.5 μg/ml) and 4.1 μg/ml (95% CI: 6.1 to 2.1 μg/ml) in the first and third aliquot. There were no significant differences in n-LAB concentrations between the two aliquots. None of 31 transbronchial biopsies performed yielded findings suggestive of lipid pneumonitis. In 1 of 27 patients, traces of amphotericin B were found in serum samples (0.1 μg/ml).

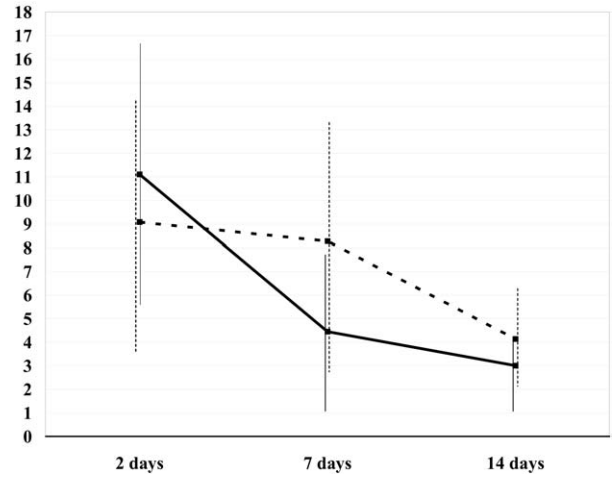

Figure 1. Mean amphotericin B concentrations and 95% confidence intervals (μg/ml) in the first (solid line) and third (dashed line) bronchoalveolar lavage aliquot. No significant differences were observed between the first aliquot, which is an indication of proximal airway concentrations, and the third aliquot, an indication of distal airway concentrations. Thus, amphotericin B levels were similar in the distal and proximal tree and remained high for at least 14 days.

To provide a more detailed assessment of the results, several sub-analyses were performed when the number of cases permitted. The findings are presented in Table 2. Higher levels of amphotericin B were seen in patients with single-lung transplants than in those with double-lung transplants (in the third aliquot at 2 days). In addition, higher levels of the drug were seen at 7 days in patients with bronchial stenosis than in those without stenosis, only in the first aliquot. However, at 14 days, drug levels were lower (in the third aliquot) in patients with bronchial stenosis. Higher concentrations of the drug were also seen in patients with infectious bronchitis with respect to the remaining recipients (in the first aliquot at 14 days).

None of the patients had ulcerative tracheobronchitis or pseudomembrane at the anastomosis. Four patients showed colonization by *Aspergillus* spp and 1 patient had *Scedosporium apiospermum* infection. All these patients presented high enough levels of amphotericin B to inhibit growth of *Aspergillus* spp (>2 μg/ml), with the exception of 2 patients showing levels of <1 μg/ml in the first aliquot.

Table 2. Amphotericin B Concentrations in Patients Grouped According to Several Factors

Factors studied	Time	*n*	First aliquot	Third aliquot
Single/double	2 d	4/6	14.8 (24.3–5.3) / 8.7 (14.9–2.5)	16.4 (25.3–7.5)[a] / 4.2 (6.3–2.1)[a]
Days post-transplant (<365/>365 days)	7 d	4/6	8.7 (15.2–2.3)[b] / 1.5 (3.0–0.1)[b]	9.0 (15.1–2.9) / 7.8 (15.5–0.1)
	14 d	6/6	3.93 (6.3–1.7) / 2.06 (3.4–0.6)	4.95 (8.1–1.9) / 3.3 (5.4–1.0)
Bronchial stenosis (no/yes)	7 d	5/5	1.1 (2.9–[–0.7])[a] / 7.8 (2.4–3.3)[a]	8.3 (17.6–[–0.9]) / 8.2 (13.1–3.3)
	14 d	5/7	4.9 (6.9–3.0)[b] / 1.6 (3.0–0.2)[b]	7.1 (10.2–3.9)[a] / 2.0 (2.7–1.3)[a]
Aspergillus colonization (no/yes)	7 d	7/3	3.4 (6.1–0.6) / 2.8 (5.8–[–0.2])	9.3 (16.2–2.3) / 8.1 (15.7–0.4)
BOS (no/yes)	7 d	5/5	1.48 (3.3–[–0.3])[b] / 7.4 (13.1–1.7)[b]	8.1 (17.5–[–1.2]) / 8.4 (13.9–3.71)
	14 d	6/6	3.2 (5.6–0.8) / 2.8 (4.5–1.1)	5.2 (8.3–2.1) / 3.1 (5.2–0.9)
Bronchial infection (no/yes)	2 d	7/3	10.2 (15.2–5.1) / 15.4 (28.8–[–2.1])	6.2 (10.7–1.7)[b] / 15.7 (27.9–3.0)[b]
	14 d	9/3	1.8 (2.8–0.7)[a] / 6.6 (8.4–4.8)[a]	3.0 (4.5–1.4)[b] / 7.6 (12.3–3.1)[b]

These sub-analyses are limited by the small sample size. Nonetheless, significant differences were observed depending on the type of transplant, and the presence of bronchial stenosis or bronchial infection. Amphotericin B values are expressed in micrograms per milliliter (95% confidence interval).

[a]Significant difference, $p < 0.05$.

[b]Statistical tendency, $p < 0.2$.

Mean FEV_1 value was 1.97 liters (range: 1.02 to 3.02 liters) before n-LAB, 1.96 liters (range: 1.01 to 3.05 liters) at 30 minutes post-nebulization and 2.07 liters (range: 1.01 to 3.06 liters) at 2 hours (p = not statistically significant [NS]). A significant FEV_1 decrease (14%) was observed in 1 of 22 patients, who, nonetheless, remained asymptomatic.

DISCUSSION

To our knowledge, this is the first study investigating amphotericin B concentrations in the respiratory tract of lung transplant patients receiving nebulized liposomal amphotericin B prophylaxis. We found that amphotericin B concentrations are high enough after nebulized inhalation to inhibit the growth of most *Aspergillus* species, with persistently elevated levels even 14 days after administration. Moreover, the safety of the drug proved to be excellent, with virtually no systemic absorption or respiratory function alterations.

In a study by Cuenca-Estrella et al, the geometric mean minimum inhibitory concentration (MIC) of amphotericin B for 697 different strains of *Aspergillus* spp was 0.41 μg/ml, and all the species isolated with the exception of *A terreus* presented MICs of ≤2 μg/ml.[28] The amphotericin B levels found in the present study were higher than these values even at 14 days; hence, it is feasible to administer n-LAB every 2 weeks, thereby improving adherence to treatment and convenience for the patient. It should be remembered, however, that the part of the respiratory tract studied ran from the segmental bronchus to the parenchyma, and concentrations at the suture site were not determined. Because this area is particularly susceptible to infection, and until more information becomes available, it seems reasonable to maintain a high frequency of n-LAB administration (every 2 or 3 days) until the suture has completely healed.

The sub-analyses yielded differing concentrations of amphotericin in the bronchial tree, depending on the type of transplant and the presence of bronchial infection or significant stenosis. The other factors investigated, such as the time post-transplantation or presence of bronchiolitis obliterans syndrome (BOS) or fungal colonization, showed no significant differences. Because of the limited sample size, however, we cannot rule out an influence of these factors on drug levels. In any case, in all the situations analyzed, amphotericin B levels were high enough to inhibit the majority of *Aspergillus* strains.

The highest concentrations of amphotericin B were found in patients undergoing single-lung transplantation. This may have been because the greatest part of n-LAB was directed toward the graft, which was better ventilated. We also found a higher concentration of the drug in patients with bronchial infection. Although the reason for this is difficult to explain, one possibility might be that amphotericin B is deposited in bronchial secretions that accumulate in the airways when there is an infection. The presence of a higher concentration of the drug in the first aliquot at 7 days in patients with stenosis is also difficult to interpret, and may be due to a problem with statistics. Nonetheless, in a previous study using technetium-labeled conventional amphotericin B, we found that the labeled drug accumulated in the area of stenosis.[15] This observation could explain the higher concentration of the drug at this level. At 14 days, however, the drug concentration was lower in patients with stenosis, a result that may be attributable to more restricted drug diffusion because of the obstruction.

The patients' tolerance to nebulization was optimal. There were no changes in the mean FEV_1 value before and at various time-points after treatment. Only 1 patient showed a significant FEV_1 decrease on spirom-

etry after n-LAB administration, but had no clinical symptoms. Palmer et al reported similar data with inhaled amphotericin B lipid complex. In their study, a significant pulmonary function decline was observed in <5% of 335 administrations of the drug.[17] In addition, we found no evidence of lipid deposits in any of the transbronchial biopsies performed in the present study. Along this line, it has been shown that n-LAB has little impact on the surfactant function when compared with the conventional amphotericin B formulation.[29]

Plasma concentrations of amphotericin B were insignificant at the doses used. Other investigators have reported comparable results. Lowry et al found no significant amphotericin B levels in lung transplant patients receiving n-LAB prophylaxis.[20] This characteristic averts the risk of nephrotoxicity and allows the drug to be administered over lengthy periods. In addition, the optimal safety profile allows dose increases in certain circumstances, such as in BOS or even single-lung transplantation. There are indications that the distribution of the drug in these cases may not be uniform, as has been observed with other types of inhaled amphotericin B. In a previous study with technetium-labeled nebulized conventional amphotericin B, we found that drug distribution was heterogeneous in the lung with BOS and in the native lung.[15] Corcoran et al also reported sub-optimal amphotericin B distribution in the native lung with the use of a radiolabeled lipid complex amphotericin B.[19]

The cost of prophylaxis with n-LAB is higher than with conventional nebulized amphotericin B, but lower than with other drugs. The cost of our current protocol (25 mg 3 times per week up to Day 60, 25 mg once per week up to Day 180) has been estimated at 2,997 euros/patient in the first 6 months. This figure is higher than that for the amphotericin B deoxycholate prophylaxis formerly used in our department; that is, 511 euros/patient for the same period (18 mg/day up to Day 120, and 6 mg/day thereafter). However, the cost is somewhat lower than that for itraconazole in solution (3,591 euros/patient with a dose of 400 mg/day) and much lower than voriconazole (14,105 euros/patient at a dose of 400 mg/day) for the same period. The cost of maintaining n-LAB prophylaxis after 6 months (25 mg every 2 weeks) is 140 euros/month in Spain.[30]

In conclusion, drug concentrations achieved in the respiratory tract of lung transplant patients with nebulized liposomal amphotericin B are high and remain high for at least 14 days. Moreover, n-LAB administration is safe and does not affect respiratory function, nor is there significant systemic absorption. These findings may be of help in attempts to optimize this type of prophylaxis. However, only a prospective clinical trial will determine whether these high amphotericin B concentrations in the respiratory tract can prevent *Aspergillus* infection in lung transplantation.

The authors thank Merche Catalan for her skillful work in the fiberbronchoscopy procedures, Celine Cavallo for English editing, and Rosa Llòria and Maite Valdeolivas for technical assistance.

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