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Comparison between concentrations of amphotericin B in infected lung lesion and in uninfected lung tissue in a patient treated with liposomal amphotericin B (AmBisome)☆

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SUMMARY

Generally, the primary lesion of a mold infection is in the airway, an extravascular site. Therefore, the antifungal drug concentration at the actual tissue lesion of a mold infection is as important as in the blood compartment. Although our antifungal armamentarium has expanded recently, polyenes are still often needed in clinical practice because of their potent fungicidal activity and the rarity of resistance. Nevertheless, the distribution of amphotericin B (AmB) in infected lung tissue has not yet been evaluated. Using high-performance liquid chromatography analysis, we determined the concentrations of AmB in plasma and infected and uninfected tissues of resected lung simultaneously, in a patient with pulmonary aspergillosis treated with liposomal amphotericin B (L-AmB). The AmB concentration in the infected lesion of the lung was approximately 5.2 times higher than that in plasma and 3.7 times higher than in uninfected lung tissue. L-AmB accumulated in the infected lesion of the lung at a higher concentration. Although our data are from only one patient, they may be useful in helping to develop better strategies for the use of L-AmB against pulmonary fungal infections.

1. Introduction

The lung is the most common site of aspergillosis, a life-threatening disease with a dramatically increasing prevalence.[1] Recently, we have been able to formulate several antifungal strategies with new azoles and echinocandins, but polyenes remain important in clinical practice because of their potent fungicidal activity.[2] However, the use of conventional amphotericin B deoxycholate (CAB) is limited because it often causes renal failure, hypokalemia, or infusion-related reactions.

Liposomal amphotericin B (L-AmB, AmBisome®; Gilead Sciences, Sandimas, CA, USA) is a lipid formulation of amphotericin B (AmB): a preparation of small, unilamellar liposomes with an average size of 60–100 nm.[3] This drug exerts uniform fungicidal activity and its toxicity-related events are significantly reduced.[2] The improved adverse drug event profile and the contribution to survival advantages are superior to those of CAB.[2,4]

The primary portal of entry of *Aspergillus* is the airway, an extravascular site. Therefore, the antifungal drug concentration at this actual site of fungal infection is as important as that in the blood compartment. Smith et al. conjectured that more drug is delivered to the mouse lung when it is infected with *Aspergillus fumigatus*.[5] It was presumed that AmB would accumulate more in inflammatory lung tissue than at a normal lung site. However, to our knowledge, no human data have been reported regarding drug concentrations in infected and uninfected lung tissues in patients treated with known doses of L-AmB.

In this report we describe a patient with pulmonary aspergillosis who was treated with L-AmB. We were able to evaluate the concentrations of AmB in plasma, infected lung lesion, and uninfected lung site at the same time.

☆ This study was presented in part, in Japanese, with the title "A case of pulmonary aspergillosis associated with advanced esophageal cancer", at a poster session of the 83rd Annual Meeting of the Japanese Association for Infectious Diseases, held in Tokyo, April 23–24, 2009.

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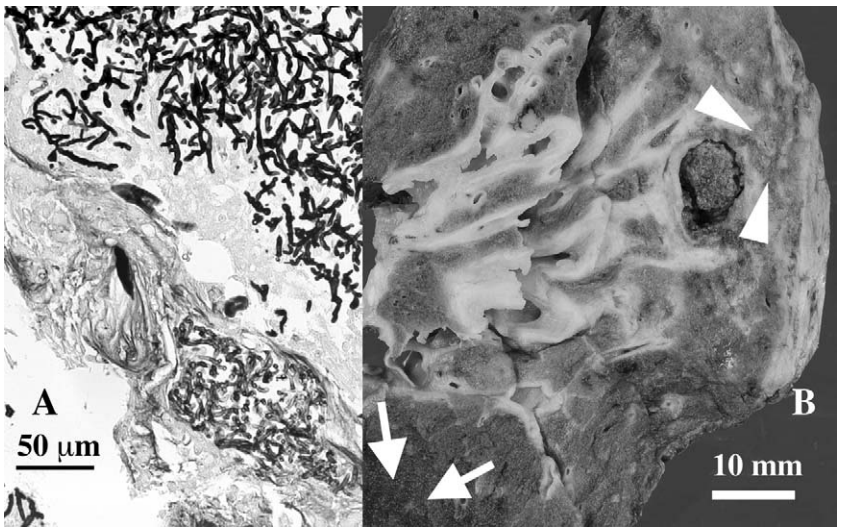

Fig. 1. The pulmonary aspergillosis lesion in the patient. (A) A trans-bronchial lung biopsy was performed from the right upper lobe. Thin separate hyphae showing dichotomous branching are seen; GMS stain (×400). (B) Two tissue samples were obtained from the resected lung. A sample was removed from the infected lesion (arrowheads), and another was cut from the uninfected area (arrows).

2. Case report

A 50-year-old male Japanese patient with advanced esophageal cancer was referred to our hospital for anticancer chemotherapy. He had multiple metastases, including lung and bone, and could not be treated with surgery. Full examinations including bronchoscopy were performed before chemotherapy, and a pulmonary fungal infection in the right upper lobe of the lung was revealed. Pathologically, brown-colored, thin separate hyphae showing dichotomous branching and crystals of calcium oxalate were observed. Using an in situ hybridization technique,[6] this fungus was revealed to be *Aspergillus* sp. From these findings, the causative pathogen was determined to be *A. niger* (Fig. 1A). Cultures of sputa and the biopsied specimen were negative. Renal and hepatic functions were not impaired. The patient expressed a strong desire to begin anticancer drugs; therefore, we started him on voriconazole (400 mg/day) concurrently with his cancer treatment.

After receiving the anticancer drugs, his body temperature rose sharply (39.5 °C), and there were increases in his white blood cell count (15.2×10^9/l) and C-reactive protein value (7.6 mg/dl). A chest computed tomography scan revealed deterioration of the aspergillosis. Based on these findings, the antifungal drug was changed to L-AmB (150 mg/day (2.8 mg/kg) for the first 4 days, subsequently 250 mg/day (4.7 mg/kg)), and remission was quickly achieved.

Twenty-two days after starting L-AmB, the patient again developed a fever (39.2 °C), and radiological findings indicated ingravescence of aspergillosis. Micafungin was added, but a drug rash forced its discontinuation. Flucytosine (5-FC; 10 g/day) was then added to the current antifungal therapy as a workaround. Fortunately, he soon became afebrile with this antifungal combination therapy.

In accordance with the patient's unwavering demand for continuance of his anticancer therapy, a right upper lobectomy was performed to remove the infectious focus. The total amount of AmB administered up until the surgery was 11 850 mg.

With written consent provided by the patient, we determined the concentrations of AmB in plasma and lung tissues. Blood and lung tissues were sampled during the operation. Blood samples had been taken just before L-AmB administration, immediately after administration was finished, and at the time of the right upper lobe resection. The duration of L-AmB infusion was 3 h. Plasma was separated by centrifugation. Two samples of lung tissue (approximately 1 g, wet weight) were obtained, one from an infected lesion and one from a distant, uninfected site that was assessed macroscopically (Fig. 1B). Later, we examined adjacent regions of each sample pathologically for confirmation. Plasma and tissue samples were frozen at −80 °C until the measurement of AmB concentration.

The concentrations of the drug were evaluated by a high-performance liquid chromatography (HPLC) method as previously described by Takemoto et al.,[7] with some modification. The internal standard solution was obtained by dissolving 1-amino-4-nitronaphthalene (Sigma-Aldrich, Tokyo, Japan) in methanol at 30 mg/l. The plasma sample (150 μl) was extracted by the addition of internal standard solution (150 μl) and methanol (500 μl). Methanol was added for protein precipitation. After vortexing for 1 min and centrifuging at 10 000 *g* for 10 min, 200 μl of supernatants was collected and evaporated. The residue was re-suspended in 100 μl of acetonitrile–2.5 mM EDTA (pH 5.0) 1:2 v/v and vortexed for 30 s. This sample, containing the total amount of AmB, was submitted to HPLC analysis (50 μl injected).

Lung tissue sample homogenization was carried out according to the method described by Vogelsinger et al.,[8] with some modification. Each lung tissue sample (0.15 g) supplemented with methanol (1 ml) was homogenized in a homogenizer (IKA T10 basic ULTRA-TURRAX, IKA Japan Co., Ltd, Yamatokoriyama, Japan) at 20 450 rpm. The tissue samples were used for preliminary and other studies as well, and therefore not all of them could be used repeatedly for evaluating the concentration. The homogenates were extracted by a method similar to the plasma extraction described above and submitted to HPLC analysis (50 μl injected).

The HPLC system consisted of LC-20AT solvent delivery pump systems and an SPD-10A$_{vp}$ detector (Shimadzu Co., Kyoto, Japan). The supernatants were separated on a CAPCELL PAK C_{18} SG120 reverse phase column (4.6 ∅ × 250 mm; Shiseido Co., Tokyo, Japan). The mobile phase consisted of acetonitrile–2.5 mM EDTA (pH 5.0) 1:2 v/v at a flow rate of 1.0 ml/min. Under these conditions, AmB and the internal standard were demonstrated at retention times of approximately 13 and 20 min, respectively, and were detected at 405 nm. The AmB concentration was determined from the ratio between the area of AmB and that of the internal standard. Standard curve samples were prepared with AmB