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Pharmacokinetics of Aerosol Amphotericin B in Rats

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The distributions of amphotericin B (AmB) in tissue were compared after intraperitoneal or aerosol administration. Rats were sacrificed 24 h after receiving single or repeated daily doses; AmB concentrations in tissues were determined by high-performance liquid chromatography. After intraperitoneal doses of 4 mg/kg of body weight per day for 7 days, mean concentrations of AmB were 122.7, 55.2, and 4.31 μg/g in the spleen, liver, and lung, respectively. After aerosol doses (aero-AmB) of 1.6 mg/kg per day, the mean concentrations of AmB in the lung were 2.79 μg/g after a single dose and 9.88 μg/g after four doses, while the drug was undetectable (<0.1 μg/g) in serum, spleen, liver, kidney, and brain. The half-life of elimination of AmB from the lungs was 4.8 days according to serial sacrifices done after a single dose of 3.2 mg of aero-AmB per kg. Treatment with 60 mg of aero-AmB per kg was well tolerated and produced no histopathologic changes in the lungs. The aerosol route was much more efficient than the systemic route in delivering AmB to the lungs, and it limited the accumulation of AmB in other organs. Because AmB is eliminated slowly, infrequent dosing schedules can be used. These pharmacokinetic characteristics and its proven effectiveness in an animal model make aero-AmB a highly promising new method for the prevention of pulmonary aspergillosis. Aero-AmB should also be considered for use as an adjunct to intravenous AmB for treatment of fungal pneumonias.

Intravenous amphotericin B (AmB) remains the standard treatment for most serious fungal infections (1, 4, 6–8) and is the only proven treatment for pulmonary aspergillosis. Unfortunately, the drug is toxic, and treatment failures are common. There is no proven chemoprophylaxis for pulmonary aspergillosis. The toxicity of intravenous AmB has limited its prophylactic use for this indication.

We recently showed that aerosol AmB (aero-AmB) was effective in the prevention and treatment of pulmonary aspergillosis in an experimental model (9). A better understanding of the pharmacokinetics and toxicity of aero-AmB is needed in order to design appropriate regimens for clinical trials.

The distributions of AmB in tissue in rats were compared after aerosol or systemic administration. The drug was extracted from tissues, and the extract was assayed by high-performance liquid chromatography as previously described (2, 3). Accumulation of the drug in the lungs and other organs was determined after multiple doses of aero-AmB; elimination of the drug was studied by measuring AmB in the lungs at different intervals after a single dose. High doses of aero-AmB were also administered to determine whether they produced mortality, morbidity, or histopathologic changes.

MATERIALS AND METHODS

Animals. Animal research procedures were approved by the Institutional Animal Care and Use Committee of Memorial Sloan-Kettering Cancer Center. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing 125 to 150 g were used for all experiments. Ethrane anesthesia was used when intraperitoneal doses were administered. At sacrifice, anesthetized animals were bled by cardiac puncture and were euthanized with carbon dioxide gas.

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Administration of AmB. The AmB preparation for intravenous infusion (Fungizone; Squibb & Sons, Princeton, N.J.) was used. Solutions of the drug were prepared in sterile, pyrogen-free water. For intraperitoneal administration, the dose injected was 0.5 to 1.0 ml.

Rats were treated with aero-AmB as previously described (9). Rats in groups of four or five were placed in a glass chamber while it was swept with a stream of aero-AmB. The aerosol was produced by air flowing at 8 liters/min through a jet nebulizer (Cadema Medical Products, Middletown, N.Y.). The drug solution was aerosolized at a rate of 0.3 ml/min, and particles were generated with a mean mass aerodynamic diameter of 1.0 μm.

Determination of AmB concentrations in tissues. Methods for tissue extraction and high-performance liquid chromatography assay of AmB were based on a previous report (2). In each experiment the following tissues were obtained: serum, lung, spleen, brain, and left kidney. Weighed tissue samples were homogenized (model PT 10/35; Brinkmann Instruments Co., Westbury, N.Y.) in distilled water (3 ml/g of tissue). A 1.0-ml portion of the homogenate was combined with 1.5 ml of 95% ethanol. After vortex mixing, this mixture was allowed to stand for 1 h and then was centrifuged (1,200 × *g*, 15 min), and the supernatant was saved for high-performance liquid chromatography analysis.

A modular high-performance liquid chromatography system, consisting of a SP-8800 pump, SP-8775 autosampler with a 20-μl sampler loop, SP-8450 UV–visible-spectrum detector at 407 nm, and WlNner chromatography data station (Spectra Physics, San Jose, Calif.), was used. The analyses were performed with a Reliance Zorbax C8 column (4 by 80 mm) with a Zorbax C8 guard cartridge (4 by 12.5 mm; Du Pont Co., Wilmington, Del.). The mobile phase was 40% acetonitrile in 25 mM ammonium phosphate buffer (pH 3.0); the flow rate was 1.0 ml/min. The retention time for AmB was 2.8 to 2.9 min.

All samples were assayed in duplicate. Concentrations of AmB in tissues were determined from a linear regression

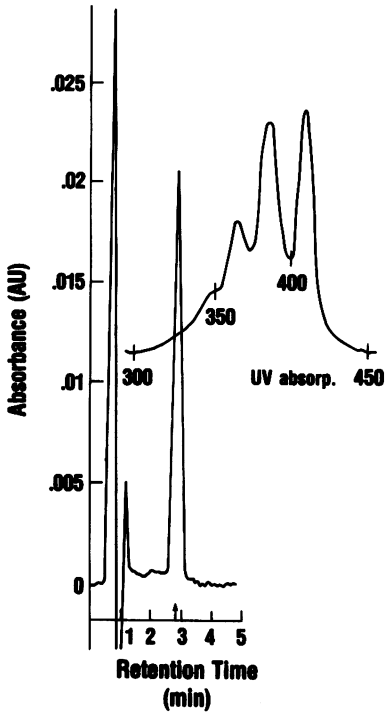

FIG. 1. High-performance liquid chromatogram of extract of lung. The AmB peak had a retention time of 2.85 min. Detector, UV at 407 nm. The insert shows the UV absorption spectrum for the peak at 2.85 min. AU, Absorption units.

analysis of the peak areas obtained with standard solutions of AmB in water.

RESULTS

Figure 1 shows a typical high-performance liquid chromatogram of a lung homogenate extract; the lung sample was obtained from a rat that received aero-AmB. The AmB peak at 2.85 min was well resolved; there were no interfering peaks in lung, liver, spleen, or serum samples from untreated animals. The peak at 2.85 min had the UV absorption spectrum expected for the polyene moiety of AmB; absorption maxima were at 366, 388, and 407 nm. The minimum detectable concentrations of AmB were 0.10 μg/g in tissue and 0.025 μg/ml in serum.

The distribution of AmB in tissue was determined in rats that were treated with daily intraperitoneal doses for 7 days. Animals were sacrificed 24 h after the last dose. Table 1

TABLE 1. AmB levels in tissues of rats after intraperitoneal administration of 4 mg/kg per day for 7 days

Tissue	Concn (μg/g) in tissue[a]
Lung	4.34 ± 0.73
Spleen	122.68 ± 81.27
Liver	55.24 ± 56.23
Kidney	5.03 ± 1.26
Serum	0.10 ± 0.03
Brain	<0.1

[a] Mean ± standard deviation; $n = 5$ per group. All animals were sacrificed 24 h after the last dose.

TABLE 2. AmB levels in lungs of rats after daily doses of 1.6 mg of aero-AmB per kg

No. of doses	Concn (μg/g) in lung[a]
1	2.79 ± 0.43
2	4.18 ± 1.26
4	9.88 ± 1.26

[a] Mean ± standard deviation; $n = 4$ per group. All animals were sacrificed 24 h after the last dose, and AmB was undetectable in all other organs.

shows the AmB concentrations in different organs. The highest concentrations of AmB were found in the spleen and liver; concentrations in the lung and kidney were nearly 30-fold lower than in the spleen. Low concentrations were detected in serum; the drug was undetectable in the brain. Approximately 16% of the total dose was recovered from the liver, and 1% of the total dose was recovered from the lungs.

The distribution of AmB in tissue was determined in rats that were treated with one, two, or four doses of aero-AmB at 1.6 mg/kg of body weight per day; doses were given on consecutive days. Animals were sacrificed 24 h after the last dose. The inhaled dose was calculated as the product of the minute volume (respiration rate × lung volume), AmB concentration in the chamber, and exposure time. For example, rats treated with an inhaled dose of 1.6 mg/kg were exposed for 15 min in a chamber that contained about 0.2 μg of AmB per ml; assuming a minute volume of 70 ml/min, the inhaled dose was (70 ml/min) × (0.2 μg/ml) × 15 min, i.e., 210 μg. Since the mean weight of the rats was 130 g, the approximate inhaled dose was 1.6 mg/kg.

Table 2 shows the concentrations of AmB that were measured in the lungs of rats treated with aero-AmB. There was a stepwise increase in concentrations with multiple doses. After four doses, the concentration of AmB in the lung was nearly 10 μg/g. The drug was undetectable in spleen, liver, kidney, serum, and brain after aerosol administration.

To study the kinetics of elimination of AmB from the lungs, concentrations of the drug in tissue were determined in rats that were sacrificed at different intervals after a single dose of aero-AmB. Rats received 3.2 mg of aero-AmB per kg, and groups were sacrificed 1, 4, and 16 days after treatment. Figure 2 shows a semi-log plot of concentrations of AmB in the lung versus time. The half-life of elimination from the lungs was 4.8 days. The drug was undetectable in spleen, liver, kidney, brain, and serum at all intervals.

The accumulation of AmB in tissue after long-term aerosol treatment was also studied. The dose of aero-AmB was 0.8 mg/kg; it was administered four times during week 1 and twice weekly for 8 additional weeks. A total of 20 doses were administered over 9 weeks. No mortality or morbidity was observed during the trial. Groups of animals were sacrificed 24 h after 10, 16, and 20 doses; another group was sacrificed 7 days after completion of the 20-dose regimen. Concentrations of AmB in the lung are shown in Fig. 3. The mean concentration of AmB rose to a peak of 21.1 μg/g at the end of the treatment period.

The steady accumulation of AmB with this regimen suggests that the drug was eliminated more slowly after chronic administration than after a single dose. Alternatively, as rats became habituated to this treatment regimen, their respiratory rates appeared to slow and there was a reduced tendency to huddle. These or other changes may have caused increased retention with successive doses.

To study the acute toxicity of aero-AmB, we administered a single dose of 60 mg/kg over 6 h and then sacrificed groups

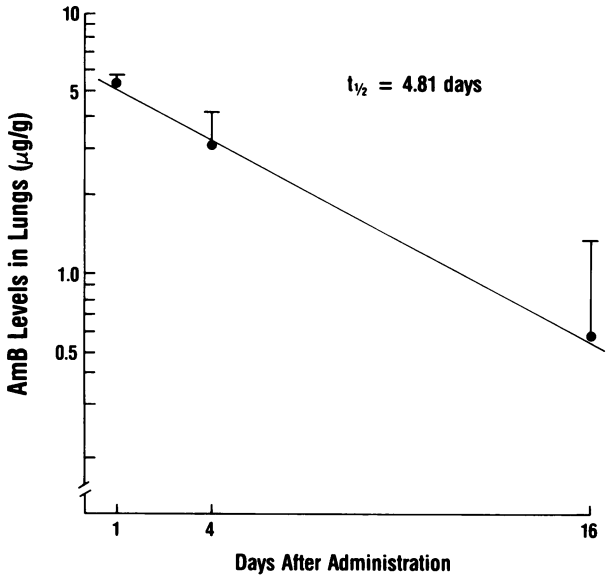

FIG. 2. Pulmonary concentrations of AmB at different intervals after a single dose of 3.2 mg of aero-AmB per kg. At each interval, four animals were sacrificed. $t_{1/2}$, Half-life.

of animals after 1 and 24 h. The mean concentration of AmB in the lung at 1 h was 52.1 ± 8.33 μg/g. This dose was well tolerated; animals did not appear toxic during or after treatment; at necropsy, there was no gross or microscopic evidence of toxicity.

DISCUSSION

In a previous study we showed that aero-AmB was highly effective in both treatment and prophylaxis of pulmonary aspergillosis in a rat model (9). In the model, severely immunocompromised animals were challenged with a large inoculum of aspergillus spores, which produced a pulmonary infection that progressed rapidly and was usually fatal within 7 days.

In patients receiving induction chemotherapy for leukemia or lymphoma, the immunosuppression is usually less severe and the exposure to aspergillus is lower by several orders of magnitude (10). These patients need protection against a less

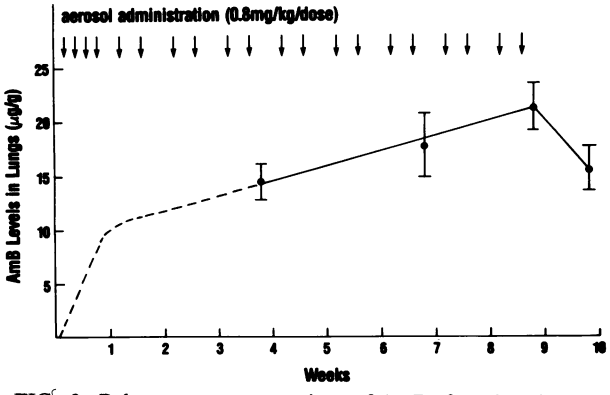

FIG. 3. Pulmonary concentrations of AmB after chronic aerosol administration of 0.8 mg/kg per dose. At each interval, four animals were sacrificed. – – –, Theoretical projection.

overwhelming challenge; however, it may be necessary to maintain a high level of protection through the 1 to 3 months of myelosuppressive chemotherapy (5).

The purpose of these studies was to determine the pharmacokinetic characteristics of aero-AmB in order to provide a rational basis for the design of safe and practical regimens that might provide protection against pulmonary aspergillosis for a prolonged period.

Aerosol administration efficiently delivered AmB to the target organ, the lung, while limiting accumulation of the drug in other organs. The drug was eliminated slowly from the lungs, and relatively stable levels were maintained with twice-weekly doses. Because there was no detectable accumulation in other organs, the aerosol route is unlikely to cause toxicity at extrapulmonary sites.

If the pharmacokinetic characteristics of aero-AmB in humans are similar to those found in rats, it should be possible to maintain protective levels of the drug in the lungs with low twice-weekly or weekly doses. At the outset, more frequent or loading doses should be administered in order to establish protective levels as quickly as possible. The dose administered should be high enough to ensure that protection is maintained, even if one or two doses must be delayed because of intercurrent illness.

More studies are needed to determine the physiologic and histopathologic effects of chronic doses of aero-AmB in animals. The mechanisms and kinetics of elimination of the drug from the lungs also need further study, since clearance appeared to slow with chronic dosing. Finally, it is important to determine whether prophylactic regimens of aero-AmB are likely to select for resistant strains of fungi.

Clinical studies should be undertaken to determine the safety of three or more doses or schedules, and the best-tolerated of these should be tested in properly controlled trials. Prophylactic regimens of aero-AmB will establish concentrations of the drug in the lung that are no higher than those that are established with systemic therapy (2). Safety studies with humans should focus on pulmonary function tests, as the lung is the only organ that is likely to receive significant exposure to the drug.

AmB is the most effective agent currently available for treatment of serious fungal infections. This new route of administration, i.e., aero-AmB, promises to extend the use of this important agent to prophylaxis.

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