Efficacy of SPK-843, a Novel Polyene Antifungal, in Comparison with Amphotericin B, Liposomal Amphotericin B, and Micafungin against Murine Pulmonary Aspergillosis

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Efficacy of SPK-843, a Novel Polyene Antifungal in Murine Pulmonary Aspergillosis in Comparison with Amphotericin B, AmBisome® and Micafungin

Running title: EFFICACY OF SPK-843 IN MURINE ASPERGILLOSIS MODEL

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ABSTRACT

SPK-843, a new polyene antifungal, exhibited dose dependent efficacy on murine pulmonary aspergillosis models. Doses of SPK-843 higher than 1.0 mg/kg exhibited no renal toxicities and a tendency toward better survival prolongation than the estimated maximum tolerated dose of amphotericin B (1.0 mg/kg) and AmBisome® at 8.0 mg/kg.
BODY TEXT

Pulmonary aspergillosis in immunocompromised patients is a major clinical concern. Amphotericin B (AMB) was the most commonly used drug, but it has severe side effects (6). Azoles and echinocandins (4) have fewer side effects than those of AMB, but they frequently fail in therapy because these agents are not entirely satisfactory alternatives due to limitations in spectrum (5, 9). Consequently, more effective antifungal agents with broad spectrum of action and reduced toxicity are required.

SPK-843 is a new polyene antifungal, which is a water-soluble diascorbate salt from SPA-S-752, an amide derivative of Partricin A produced by a mutant strain of *Streptomyces aureofaciens*. Clinical trials to clarify the therapeutic efficacy of SPK-843 for deep-seated mycoses are now being performed. SPK-843 is reported to possess in vitro inhibitory activity comparable to or better than AMB against *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus* spp. (3). The pharmacokinetics of SPK-843 was analyzed and found to possess a suitable profile for its therapeutic effect (1, 2). In this study, we evaluated the efficacy of SPK-843 in experimental pulmonary aspergilloses as compared to AMB, AmBisome®, and micafungin.

Amphotericin B deoxycholate (AMB, Fungizone®, Bristol-Myers Squibb K.K., Tokyo, Japan), liposomal amphotericin B (L-AMB, AmBisome®, Fujisawa Healthcare, Inc., Deerfield, IL), sodium micafungin (MCFG, Funguard®, Fujisawa Pharmaceutical Co., Osaka, Japan), and SPK-843 (Kaken Pharmaceutical Co., Tokyo, Japan) were used in this study. The minimum inhibitory concentrations (MICs) of the antifungal agents against challenge strains were determined by the microdilution method according to the Clinical Laboratory Standards Institute (CLSI) M38-A. In the MIC measurement,
SPK-843 and MCFG were dissolved in water, and amphotericin B (Sigma-Aldrich K.K., Tokyo, Japan) was dissolved in dimethyl sulfoxide.

In experimental aspergilloses, *Aspergillus fumigatus* MF-13 (MIC: SPK-843 0.5µg/ml; AMB 0.25µg/ml; MCFG 0.0156µg/ml) which was obtained from the Nagasaki University Hospital, *A. niger* TIMM 2814 (MIC: SPK-843 0.0625 µg/ml; AMB 0.25 µg/ml) and *A. flavus* TIMM 0057 (MIC: SPK-843 0.25 µg/ml; AMB 0.5 µg/ml) which were obtained from Teikyo University, were used for infection (8). The strains were subcultured on potato dextrose agar (Nissui Pharmaceutical Co., Tokyo, Japan) at 30°C for 6 or 7 days, and the conidia were harvested with sterile saline containing 0.05% Tween 80, and diluted with sterile saline for inhalation. Six-week-old male DBA/2N mice (Charles River Inc., Yokohama, Japan) were used (10, 11). Animals were given drinking water containing 250 mg/800 ml tetracycline (Nacalai Tesque Inc., Kyoto, Japan) throughout the experiment to prevent bacterial infection (7). Three days before infection, mice were subjected to immunosuppression by subcutaneous injection of 50 to 60 mg/kg of triamcinolone acetonide (Bristol-Myers K.K., Tokyo, Japan) (11). About 60 to 80 mice at a time were confined in an aerosol apparatus (Ikemoto Scientific Technology Co., Ltd., Tokyo, Japan) and inhaled with a 10ml conidial suspension at a concentrations of 5×10⁸ to 9×10⁸ cells/ml by a glass nebulizer at 1 kg/cm² pressure for 30 minutes. Infected mice received intravenous treatments of the drugs or the vehicles (n=10) once daily for 5 days, starting on the next day after inhalation. AMB, L-AMB, and MCFG were dissolved in 5% glucose, and SPK-843 was dissolved in 10% lipid emulsion (10% Intralipid, Terumo Co., Tokyo, Japan) according to clinical preparations. The surviving mice were monitored and analyzed by log-rank test. Each experiment was repeated twice to confirm the reproducibility of results. To investigate renal toxicity,
non-infected immunosuppressed mice were received intravenous treatments of SPK-843 or AMB, once daily for 5 days, and sacrificed 2 days after the last administration. Renal histopathological damages were compared in kidneys stained with periodic acid-Schiff. The experimental procedures followed the ethical rules from Kaken Pharmaceutical Co. and Nagasaki University Laboratory Animal Center.

Two replicated experiments for *A. fumigatus*, one is comparing SPK-843 to AMB and L-AMB (Figure 1) and another is comparing SPK-843 to MCFG (Figure 2) were performed under the same condition. In the *A. fumigatus* infection, all the vehicle-treated mice died within 4 days after the infection and the pathological examination confirmed that they died with invasive aspergillosis. The administration of SPK-843 and AMB at doses of 0.5 mg/kg or higher, and those of L-AMB at doses of 4.0 mg/kg or higher significantly prolonged the survival of infected mice compared to the vehicle-treated mice (Figure 1). When compared with MCFG (Figure 2), the administration of SPK-843 at 0.25 to 1 mg/kg resulted in survival prolongation comparable to MCFG at 2.0 to 4.0 mg/kg, and the efficacy of SPK-843 at 2.0 mg/kg is comparable to that of MCFG at 8.0 mg/kg. In *A. flavus* infection (Figure 3) and *A. niger* infection (Figure 4), SPK-843 had dose-dependent efficacy on survival prolongation at doses of 1.0 mg/kg or higher for *A. flavus* and 0.25 mg/kg or higher for *A. niger*. The high dose of SPK-843 (4.0 mg/kg for *A. flavus*, 2.0 mg/kg for *A. niger*) exhibited better efficacy than AMB (1.0 mg/kg) and L-AMB (8.0 mg/kg).

In all tested aspergiloses, AMB at 2.0 mg/kg was less effective at prolonging survival than at 1.0 mg/kg, suggesting some toxicity. Survival prolongations at 8.0 mg/kg of L-AMB were no more than those at 4.0 mg/kg. In the kidneys of mice treated with AMB 1.0 mg/kg, tubular cell necrosis and cast formation were observed, suggesting kidney
damage. No significant histopathological lesions, however, were found in daily SPK-843 treatment of 1.0 mg/kg or 4.0 mg/kg. The dose-dependent renal toxicity of AMB is well known. SPK-843 is likely to be less toxic against kidneys than is AMB.

In these experiments, at doses higher than 1.0 mg/kg, SPK-843 exhibited dose-dependent efficacy with a tendency toward better efficacy than 1.0 mg/kg of AMB or 8.0 mg/kg of L-AMB. SPK-843 exhibited comparable or better in vitro activity than that of AMB against 3 *Aspergillus* species used for the infection models, reflecting comparable efficacy at relatively low doses against the aspergilloses. SPK-843 at 1.0 mg/kg or less was as effective as AMB at the same dose, but without renal toxicities. Doses of SPK-843 higher than 1.0 mg/kg exhibited a tendency toward better survival prolongation than AMB, and without renal toxicities. The data obtained in the present study are encouraging for further studies of SPK-843.
REFERENCES


FIGURE LEGENDS

Figure 1.
Effect of (a) SPK-843, (b) AMB, and (c) L-AMB on survival curves of mice with pulmonary infection caused by *Aspergillus fumigatus* (*: p < 0.05, **: p < 0.01, compared to vehicle controls, log-rank test).

Figure 2.
Effect of (a) SPK-843 and (b) MCFG on survival curves of mice with pulmonary infection caused by *Aspergillus fumigatus* (**: p < 0.01, compared to vehicle controls, log-rank test).

Figure 3.
Effect of (a) SPK-843, (b) AMB, and (c) L-AMB on survival curves of mice with pulmonary infection caused by *Aspergillus flavus* (*: p < 0.05, **: p < 0.01, compared to vehicle control, log-rank test).

Figure 4.
Effect of (a) SPK-843, (b) AMB, and (c) L-AMB on survival curves of mice with pulmonary infection caused by *Aspergillus niger* (*: p < 0.05, **: p < 0.01, compared to vehicle control, log-rank test).
Figure 1

(a) SPK-843

(b) AMB

(c) L-AMB
Figure 2

(a) SPK-843

(b) MCFG
Figure 3