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

Hiroshi Kakeya,1* Yoshitsugu Miyazaki,2 Hisato Senda,3 Tsutomu Kobayashi,1 Masafumi Seki,1 Koichi Izumikawa,1 Kazunori Yanagihara,1 Yoshihiro Yamamoto,1 Takayoshi Tashiro,1 and Shigeru Kohno1

Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; Department of Bioactive Molecules, National Institute of Infectious Disease, Tokyo, Japan; and Kaken Pharmaceutical Co., Ltd., Kyoto, Japan

Received 24 October 2007/Returned for modification 20 November 2007/Accepted 16 February 2008

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

Pulmonary aspergillosis in immunocompromised patients is a major clinical concern. Amphotericin B (AMB; Fungizone) was the most commonly used drug, but it has severe side effects (9). Azoles and echinocandins (7) 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 (8, 12). 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 watersoluble diaascorbate 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 that of AMB against Candida spp., Cryptococcus neoformans, and Aspergillus spp. (6). The pharmacokinetics of SPK-843 was analyzed, and the drug was found to possess a suitable profile for its therapeutic effect (1, 2). In this study, we evaluated the efficacy of SPK-843 compared to those of AMB, liposomal AMB (L-AMB) (AmBisome), and micafungin in experimental pulmonary aspergilloses.

AMB deoxycholate (Bristol-Myers Squibb K.K., Tokyo, Japan), L-AMB (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 MICs of the antifungal agents against challenge strains were determined by the microdilution method according to Clinical Laboratory Standards Institute (CLSI) M38-A (4). In the MIC measure-

* Corresponding author. Mailing address: Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Phone: 81-95-819-7273. Fax: 81-95-849-7285. E-mail: kakeya@nagasaki-u.ac.jp.

† Published ahead of print on 25 February 2008.

FIG. 1. Effect of SPK-843 (a), AMB (b), and L-AMB (c) 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).
ment, SPK-843 and MCFG were dissolved in water, and AMB (Sigma-Aldrich K.K., Tokyo, Japan) was dissolved in dimethyl sulfoxide.

In experimental aspergilloses, Aspergillus fumigatus MF-13 (MIC for SPK-843, 0.5 μg/ml; MIC for AMB, 0.25 μg/ml; MIC for MCFG, 0.0156 μg/ml), which was obtained from the Nagasaki University Hospital, and A. niger TIMM 2814 (MIC for SPK-843, 0.0625 μg/ml; MIC for AMB, 0.25 μg/ml) and A. flavus TIMM 0057 (MIC for SPK-843, 0.25 μg/ml; MIC for AMB, 0.5 μg/ml), which were obtained from Teikyo University, were used for infection (11). 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 (3, 5). Animals were given drinking water containing 250 mg/800 ml tetracycline (Nacalai Tesque Inc., Kyoto, Japan) throughout the experiment to prevent bacterial infection (10). Three days before infection, mice were subjected to immunosuppression by subcutaneous injection of 50 to 60 mg/kg of body weight of triamcinolone acetonide (Bristol-Myers K.K., Tokyo, Japan) (3). 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 10-ml conidial suspension at concentrations of $5 \times 10^8$ to $9 \times 10^8$ cells/ml by a glass nebulizer at 1-kg/cm² pressure for 30 min. 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 testing. Each experiment was repeated twice to confirm the reproducibility of results. To investigate renal toxicity, non-infected immunosuppressed mice 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 stain. The experimental procedures followed the ethical rules from Kaken Pharmaceutical Co. and Nagasaki University Laboratory Animal Center.

Two replicated experiments for A. fumigatus, one comparing SPK-843 to AMB and L-AMB (Fig. 1) and another comparing SPK-843 to MCFG (Fig. 2), were performed under the same conditions. 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 (Fig. 1). Compared with the administration of MCFG (Fig. 2), the administration of SPK-843 at 0.25 to 1 mg/kg resulted in survival prolongation comparable to that seen for 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 (Fig. 3) and A. niger infection (Fig. 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

![FIG. 2. Effect of SPK-843 (a) and MCFG (b) on survival curves of mice with pulmonary infection caused by Aspergillus fumigatus (**, $P < 0.01$; compared to vehicle controls; log rank test).](http://aac.asm.org/)

![FIG. 3. Effect of SPK-843 (a), AMB (b), and L-AMB (c) 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).](http://aac.asm.org/)
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 aspergilloses, 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 at 1.0 mg/kg, tubular cell necrosis and cast formation were observed, suggesting kidney damage. No significant histopathological lesions, however, were found for 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 in vitro activity comparable to or better than that of AMB against three *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 of higher than 1.0 mg/kg exhibited a tendency toward better survival prolongation than AMB, without renal toxicities. The data obtained in the present study are encouraging for further studies of SPK-843.

**REFERENCES**