

# Efficacy of SPK-843, a Novel Polyene Antifungal in a Murine Model of Systemic Cryptococcosis

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

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Word count; the abstract: 51 words, the text: 976 words

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Antimicrobial Agents and Chemotherapy, 52 (5), pp. 1871-1872, 2008.

## **ABSTRACT**

SPK-843, a new polyene antifungal, possessed efficacy against a murine systemic infection caused by *Cryptococcus neoformans*. The administration of 4.0 mg/kg SPK-843 revealed better survival prolongation and fungal reduction than those observed with the administration of 0.7 mg/kg amphotericin B and 80 mg/kg fluconazole ( $p < 0.001$ ) without histopathological renal changes.

*Cryptococcus neoformans* is an important causative fungus of morbidity and mortality in immunocompromised patients. The central nervous system (CNS) infection of *C. neoformans* is a major clinical concern among those patients. Amphotericin B (AMB), lipid-associated formulations of amphotericin B, and fluconazole (FLC) are current standard antifungals used against cryptococcal CNS infection; however, the toxicity of AMB and the fungi-static characteristics of FLC sometimes limit their usage and clinical efficacy. Consequently, effective antifungal agents with fungicidal activity and low 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*. SPK-843 is reported to have in vitro inhibitory activity equal to or better than AMB against *Candida* spp., *C. neoformans*, and *Aspergillus* spp. (1, 5, 8). And the pharmacokinetics of SPK-843 was well analyzed to possess a suitable profile for its therapeutic effect (2, 3). In this work, we evaluated in a murine systemic cryptococcosis the treatment efficacy of SPK-843 compared to AMB and FLC.

AMB (Fungizone®, Bristol-Myers Squibb K.K., Tokyo, Japan) and FLC (Pfizer Inc., New York) were dissolved in a 5% glucose solution, and SPK-843 (Kaken Pharmaceutical Co., Tokyo, Japan) was dissolved in a 10% lipid emulsion (Terumo, Tokyo, Japan). The minimum inhibitory concentrations (MICs) of antifungals were determined by the microdilution method, according to the Clinical Laboratory Standards Institute, M27-A. In the cryptococcosis model Eight-week-old Balb/c female mice (Charles River Inc., Yokohama, Japan) were inoculated via the tail vein with a lethal dose of  $6$  to  $8 \times 10^5$  cells of *C. neoformans* (YC-11)(4). The mice were treated once daily intravenously with the drugs or the vehicles (n=10) daily for 5 days after the fungal

inoculation. Each experiment was repeated 2 times to confirm the reproducibility of results. The maximum tolerated dose of each drug was included as the highest dose for the mouse strain. For the analysis of fungal burden in the brain and histopathological changes in brain, kidneys and liver, the animals were sacrificed 7 days after inoculation. The guidelines of the Nagasaki University Laboratory Animal Center for Biomedical Research for animal experimentation were followed. Tests for differences in survival distributions were based on generalized log rank test from survival rates calculated by the Kaplan-Meier method. The mean numbers of CFU per brain from the mycological study were compared by Scheffe's multiple-comparison test. A  $p$ -value of less than 0.05 was considered statistically significant.

The MICs of SPK-843, AMB, and FLC for *C. neoformans* YC-11 were 0.125, 0.25, and 2.0  $\mu\text{g/ml}$ , respectively, suggesting the test strain has normal susceptibility to drugs. In the murine infection model, all the control mice died within 9 days after infection and were pathologically confirmed to have died with systemic cryptococcosis. *C. neoformans* was pathologically detected in the brain, lungs, spleen, and kidneys. CFU number from the brain was  $10^7$  to  $10^8$  cells per organ. The administration of all tested drugs significantly prolonged the survival of infected mice ( $p < 0.005$ ). The efficacies of SPK-843 at 1.0 mg/kg and AMB at 0.5 mg/kg were significantly better than those of FLC at 80 mg/kg. The administration of 4.0 mg/kg SPK-843 revealed better survival prolongation than the administration of 0.7 mg/kg AMB ( $p < 0.001$ ).

Clearance of infection from the brain is the key issue of cryptococcal infection. Table 1 indicates the amount of yeast cells in the brain 7 days after inoculation. AMB, FLC, and SPK-843 at 0.5, 20, and 1.0 mg/kg, respectively, inhibited the cryptococcal cell growth in brain tissue ( $p < 0.001$ ). The cell counts in the brain of 4.0 mg/kg SPK-843

treated mice were significantly lower than those of 0.7 mg/kg AMB or 80 mg/kg FLC treated mice ( $p < 0.001$ ).

In histopathology analysis, no brain abscesses were observed in mice receiving 4.0 mg/kg of SPK-843, while animals receiving 5% glucose, 80 mg/kg of FLC, 0.7 mg/kg of AMB, or 1.0 and 2.0 mg/kg of SPK-843 presented multiple cryptococcal brain abscesses. In the kidneys of mice sacrificed 7 days after infection tubular cell necrosis was observed in mice sacrificed after 5 days of treatment with AMB 0.7 mg/kg, suggesting kidney damage. However, no significant histopathological lesions were found in mice treated with FLC or in those receiving SPK-843 treatment at 4.0 mg/kg. Further, no histopathological changes were seen in livers treated with SPK-843 at 4.0 mg/kg or AMB at 0.7 mg/kg (data not shown).

The focus of this experiment is whether or not, high doses of SPK-843 are tolerable, as the acute or renal toxicity of conventional AMB sometimes limits its usage. The cytotoxicity on cell cultures revealed that SPK-843 was less toxic than AMB (7). In use of intravenous administration of AMB for mice conventional strains, such as ICR or DBA2/N, the antifungal efficacy of 2.0 mg/kg is less than 1.0 mg/kg due to acute toxicities (data not shown). The Balb/c mice were more sensitive to high doses of AMB and experienced acute toxicities (6). In our experiments, two mice died after slow administration of 0.7 mg/kg of AMB for 5 days and were not counted in our survival study. Consequently, approximately 0.7 mg/kg/day of AMB was estimated to be a maximal dose to avoid acute toxicities for Balb/c mice. No acute toxicities of SPK-843 were observed even through rapid intravenous administration of 4.0 mg/kg.

In summary, the same dose of SPK-843 was less effective than AMB in this experimental model, but the higher dose of SPK-843 was more effective than the lower

dose of AMB, without histopathological renal changes. Therefore, it may be possible that SPK-843 can be used for more frequent or longer-term therapy than can AMB. Further study is needed to determine the effect and safety of long-term administration of SPK-843. In conclusion, our results provide evidence that SPK-843 is a promising new alternative to AMB in the treatment of cryptococcosis.

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## FIGURE LEGENDS

### Table 1:

Amount of *Cryptococcus neoformans* recovered from the brains of mice with systemic cryptococcosis after 7 days of treatment with SPK-843, AMB, or FLC. Ten mice were used in each group. \*,  $p < 0.001$ , compared with results for 5% dextrose. \*\*,  $p < 0.0001$ , compared with results for 80 mg/kg of FLC and 0.7 mg/kg of AMB, by generalized T-test. No statistically significant difference was seen in 2.0 mg/kg SPK-843 compared to 0.7 mg/kg AMB or 2.0 mg/kg SPK-843 compared to 80 mg/kg FLC.

### Figure 1.

Survival rate of mice with experimental systemic cryptococcosis treated with an intravenous injection of 5% dextrose ( $\times$ ); 10% lipid emulsion (\*); dose, 20 mg ( $\triangle$ :open triangle), 40 mg ( $\blacktriangle$ :closed triangle), 80 mg ( $\diamond$ : open diamond) of FLC per kg of body weight; dose, 1.0 mg ( $\circ$ : open circle), 2.0 mg ( $\bullet$ :closed circle), 4.0 mg ( $\blacklozenge$ :closed diamond) of SPK-843 per kg of body weight; and dose, 0.5 mg ( $\square$ :open square), 0.7 mg ( $\blacksquare$ : closed square) of AMB per kg of body weight. Ten mice were used in each group. \* and \*\*,  $p < 0.005$ , compared with results for 5% dextrose and 80 mg/kg of FLC, respectively. \*\*\*,  $p < 0.001$ , compared with results for 0.7 mg/kg of AMB, by generalized log-rank test.

Table. 1

**Fungal burdens in brain of mice infected with *C. neoformans* (Log10 CFU/brain )**

	Log10 CFU/brain $\pm$ SD
5% glucose	7.70 $\pm$ 0.08
10% Lipid	7.74 $\pm$ 0.10
1.0mg/kg SPK-843	7.42 $\pm$ 0.11 *
2.0mg/kg SPK-843	6.44 $\pm$ 0.41 *
4.0mg/kg SPK-843	3.45 $\pm$ 0.48 * *
0.5mg/kg AMPH-B	6.77 $\pm$ 0.30 *
0.7mg/kg AMPH-B	6.36 $\pm$ 0.20 *
20mg/kg FLCZ	7.03 $\pm$ 0.10 *
40mg/kg FLCZ	6.71 $\pm$ 0.14 *
80mg/kg FLCZ	6.34 $\pm$ 0.22 *

Figure. 1

### Survival rate of mice with experimental systemic cryptococcosis

