

# Comparison of the In Vitro and In Vivo Activity of the Bis-Triazole Derivative UK 49,858 with That of Amphotericin B against *Histoplasma capsulatum*

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**The antifungal activity of UK 49,858, a difluorophenyl bis-triazole derivative, was evaluated in vitro against seven strains of *Histoplasma capsulatum* and in vivo in AKR and C57BL/6 murine models of histoplasmosis. UK 49,858 had a lower toxicity for AKR and C57BL/6 mice than amphotericin B did. The therapeutic index of UK 49,858 was 4.3 for AKR mice and 7.1 for C57BL/6; with amphotericin B it was 2 for both mouse strains. Given orally, UK 49,858 compared favorably with amphotericin B given intraperitoneally in either AKR or C57BL/6 mice infected with *H. capsulatum*.**

The successful clinical trials of mono (N)-substituted imidazole compounds such as miconazole and ketoconazole against infections with yeasts and filamentous fungi (5) have led to the development of newer compounds in which the imidazole ring is replaced by the related 1,2,4-triazole ring (2). One such derivative, UK 49,858 [2-(2,4-difluorophenyl)-1-,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol], was synthesized by Pfizer Central Research, Sandwich, England (3). Like ketoconazole, it is active when given orally and well tolerated by healthy human volunteers; doses of 1.4 mg/kg for 7 consecutive days and up to 0.7 mg/kg for 28 consecutive days have not shown any toxicity (*Investigator's Reference Manual*, Pfizer Inc., Groton, Conn., 1984). Orally administered UK 49,858 also appears to possess activity superior to that of ketoconazole in mice with lethal *Candida albicans* (6) and *Aspergillus flavus* (P. F. Troke, R. J. Andrews, M. S. Marriott, and K. Richardson, IXth Int. Cong. Int. Soc. Human Anim. Mycol., abstr. R2-7, 1985) infections. Furthermore, UK 49,858 has the advantage of being equipotent by intravenous or oral administration (K. Richardson, M. S. Marriott, and P. F. Troke, IXth Int. Cong. Int. Soc. Human Anim. Mycol., abstr. R2-1, 1985).

In this study, we determined the acute lethal toxicity of UK 49,858 and amphotericin B for C57BL/6 and AKR mice and evaluated the efficacy of UK 49,858 compared with that of amphotericin B against *Histoplasma capsulatum* in broth susceptibility studies and in the treatment of disseminated histoplasmosis in two different strains of mice.

## MATERIALS AND METHODS

**Compounds.** UK 49,858 (lot no. R-9) was kindly provided by Anthony K. Knirsch, Pfizer Inc. Amphotericin B (Fungizone; control no. 3K 678, E. R. Squibb & Sons, Princeton, N.J.) was purchased. All compounds were suspended and diluted in 5% dextrose just before use.

**Animals.** Six- to eight-week-old female AKR (average weight, 21 g) and C57BL/6 (average weight, 18 g) mice were purchased from Charles River Mouse Farms, Wilmington, Mass. All mice were housed and held for 1 week prior to experimentation. They were fed Rodent Laboratory Chow

5001 (Ralston Purina Co., St. Louis, Mo.) and given water ad libitum.

**Organisms.** *H. capsulatum* 217B, 222B, 186B, and 186A were obtained from the American Type Culture Collection, Rockville, Md. *H. capsulatum* Downs was obtained from our permanent culture collection. *H. capsulatum* 186AS1, 186AS2, and 186AR were cloned from strain 186A and provided by William E. Goldman, Washington University School of Medicine, St. Louis, Mo. All strains were grown in the yeast phase morphology on 2% glucose-1% yeast extract broth incubated with constant agitation at 37°C. Cultures were passaged into fresh broth every 5 days according to procedures described previously (4). *Saccharomyces cerevisiae* (HLR), originally obtained from Hoffman-LaRoche, Inc., Nutley, N.J., was used as a drug control organism for our susceptibility studies.

The number of cells for inocula was quantitated by hemacytometer counts, and the numbers of viable units were determined by colony counts on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) supplemented with growth factor and cysteine as described in the procedure of Burt et al. (2).

**In vitro susceptibility studies.** Antifungal susceptibility tests were conducted in 2% glucose-1% yeast extract broth or yeast nitrogen base broth (Difco Laboratories), supplemented with 1% dextrose, using procedures previously described (7).

The MIC was the lowest concentration of drug that inhibited multiplication of the yeasts as determined by the absence of turbidity. Measurements were done when visible turbidity was noted in the control cultures (96 to 120 h).

**Drug toxicity studies.** Groups of 10 AKR and C57BL/6 mice were gavaged twice daily with 0.5-ml volumes of various concentrations of UK 49,858 dissolved in water for a total of 6 consecutive days. The concentrations of UK 49,858 used were serial twofold dilutions in water from 8.7 to 0.015 mg/ml. The range of daily doses for AKR mice was 414.3 to 0.7 mg/kg per day, and for C57BL/6 mice it was 483.3 to 0.9 mg/kg per day, for a total of 6 consecutive days. Deaths were recorded for up to 14 days after the final dose. Amphotericin B was administered by intraperitoneal (i.p.) injection on alternate days for a total of six injections according to procedures previously described (4). Control

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TABLE 1. Summary of the in vitro and in vivo comparisons of UK 49,858 and amphotericin B activities against *H. capsulatum* G217B

Drug	MIC ( $\mu\text{g/ml}$ )	Maximum tolerated dose (mg/kg per day for 6 days)		LD <sub>50</sub> , 95% confidence limit (mg/kg)		PD <sub>50</sub> , 95% confidence limit (mg/kg)				Therapeutic index <sup>a</sup>	
		AKR	C57BL/6	AKR	C57BL/6	AKR		C57BL/6		AKR	C57BL/6
						$5 \times 10^6$	$10^7$	$2.5 \times 10^6$	$5 \times 10^6$		
UK 49,858	15.6	414.3 <sup>b</sup>	483.3 <sup>b</sup>	>414.3 <sup>b</sup>	>483.3 <sup>b</sup>	6.2 $\pm$ 1.0	47.62 $\pm$ 12.6	9.2 $\pm$ 2.7	28.9 $\pm$ 8.6	4.3	7.1
Amphotericin B	0.2	14.5 <sup>c</sup>	13.9 <sup>c</sup>	23.8 $\pm$ 3.5 <sup>c</sup>	21.4 $\pm$ 3.4 <sup>c</sup>	1.8 $\pm$ 0.6	6.5 $\pm$ 1.6	1.4 $\pm$ 0.9	3.8 $\pm$ 1.5	2.0	2.0

<sup>a</sup> Maximally tolerated dose of drug/minimally effective dose to achieve 100% survival.

<sup>b</sup> Administered by gavage twice daily for 6 consecutive days.

<sup>c</sup> Administered by i.p. injection once daily on alternate days for a total of six injections.

animals were given identical volumes of 5% dextrose-water either orally or i.p. according to the schedules described above.

**Experimental therapy.** *H. capsulatum* G217B was used to establish our murine model of histoplasmosis because it is the most virulent of our strains (4). Groups of 10 mice each were injected via the tail vein with 0.2-ml suspensions of graded doses of viable *H. capsulatum* yeast cells ( $10^5$  to  $10^7$  organisms per mouse). The lowest inoculum of *H. capsulatum* G217B which killed 100% of the mice within 1 week after injection was determined (4). The experiments were repeated at least three times and the variability of the results was <20%. This lethal inoculum (100% lethal dose [LD<sub>100</sub>]) and twice this concentration (2LD<sub>100</sub>) of viable cells were used to infect mice for the drug efficacy tests. Treatment of mice with UK 49,858 given orally or amphotericin B given i.p. was begun 24 h after infection. Animals that received UK 49,858 were given graded doses by gavage once in the morning and once in the afternoon for a total of 6 consecutive days. The infected animals treated with amphotericin B were given the drug by i.p. injection beginning 24 h after infection and then on alternate days for a total of six doses. The animals were observed daily for 28 days after start of therapy and deaths were recorded. The 50% preventive dose (PD<sub>50</sub>) was estimated by the log dose probit response curve, which was constructed by fitting survival data to a logistic dose response model (1).

At termination of the experiment the surviving animals were necropsied and their spleens were removed. Each spleen was minced and cultured on Mycosel agar (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 25°C. The cultured specimens were examined daily for growth of *H. capsulatum* and kept for 4 weeks before discarding as negative for growth.

## RESULTS AND DISCUSSION

**In vitro susceptibility studies.** The MIC of UK 49,858 against the different strains of *H. capsulatum* ranged from 16 to 250  $\mu\text{g/ml}$ , compared with 0.12 to 0.47  $\mu\text{g/ml}$  for amphotericin B. The MIC of UK 49,858 against *S. cerevisiae* HLR was 125  $\mu\text{g/ml}$  versus 1.88  $\mu\text{g/ml}$  for amphotericin B. Therefore, on a weight basis, amphotericin B was 66.0 to 1,042 times more active than UK 49,858 against the isolates of *H. capsulatum* and *S. cerevisiae*. However, in vitro susceptibility testing with UK 49,858 is markedly influenced by the experimental conditions used. For example, peptone, Casamino Acids, pH, and agar itself modify or antagonize the activity of the triazole derivative (*Investigator's Reference Manual*, Pfizer, Inc., 1984). In fact, we observed that susceptibility studies with our control organism, *S. cerevisiae* HLR, and UK 49,858 could not be conducted in 2%

glucose-1% yeast extract broth since the MIC was >4 mg/ml. Therefore, despite the high in vitro MICs, we proceeded with the in vivo studies.

**Drug toxicity studies.** An exact lethal dose of UK 49,858 could not be determined in either strain of mice. No toxicity was evident at 414.3 mg/kg per day given orally by gavage in equally divided doses twice a day for 6 consecutive days to AKR mice and 483.3 mg/kg per day given in the same manner for 6 consecutive days to C57BL/6 mice. Higher daily doses could not be given by gavage because of the limits of drug solubility and fluid volume tolerated by the mice.

The LD<sub>50</sub> values of amphotericin B for AKR and C57BL/6 mice were 23.8  $\pm$  3.5 and 21.4  $\pm$  3.4 mg/kg per day, respectively. The maximum dose of amphotericin B which could be given i.p. without any deaths of AKR mice was 15.5 mg/kg per day; the equivalent dose in C57BL/6 mice was 13.9 mg/kg per day.

**Experimental therapy.** Untreated AKR mice injected with  $5 \times 10^6$  viable cells (LD<sub>100</sub>) of *H. capsulatum* G217B were all dead by 7 days after infection. With this inoculum the PD<sub>50</sub> was 6.2  $\pm$  1.0 mg of UK 49,858 and 1.8  $\pm$  0.6 mg of amphotericin B per kg. When an inoculum of 2LD<sub>100</sub> was used as the infecting dose, the PD<sub>50</sub> was 47.6  $\pm$  12.6 of UK 49,858 and 6.5  $\pm$  1.6 mg of amphotericin B per kg. To achieve a similar LD<sub>100</sub> for C57BL/6 mice, an inoculum of  $2.5 \times 10^6$  viable cells of *H. capsulatum* G217B was used. With this inoculum the PD<sub>50</sub> was 9.2  $\pm$  2.7 mg of UK 49,858 and 1.4  $\pm$  0.9 mg of amphotericin B per kg per day. When 2LD<sub>100</sub> was used as inoculum, the PD<sub>50</sub> was 28.9  $\pm$  8.6 mg of UK 49,858 and 3.8  $\pm$  1.5 mg of amphotericin B per kg per day.

The surviving AKR and C57BL/6 mice that received UK 49,858 or amphotericin B were not cured of their infections with viable *H. capsulatum* organisms cultured from spleens removed at necropsy from the survivors 4 weeks post-treatment. Exact quantitation of the spleens was difficult because of clumping and variations in spleen weights, but there did not appear to be differences between the two groups.

Therefore, despite its poor in vitro activity, UK 49,858 is an effective agent in vivo. This discrepancy between the in vitro and in vivo activity of this drug indicates the need for better and more relevant in vitro susceptibility testing.

Amphotericin B was 3.1 to 7.5 times more effective than UK 49,858 against this model of *H. capsulatum* infection in the two strains of mice. The difference between the 2 drugs depended on the strain of mouse and the infecting dose (Table 1). When the maximally tolerated doses of the drugs were divided by the minimally effective doses to achieve 100% survival of the mice, the therapeutic/toxic ratios of

each drug could be obtained. These values for UK 49,858 were 4.3 in AKR mice and 7.1 in C57BL/6; for amphotericin B they were 2 in both AKR and C57BL/6 mice. Therefore, it appears that UK 49,858 may be the more attractive agent in this infection, particularly because it achieved its therapeutic effects given orally.

UK 49,858 has been shown to have a broader spectrum of activity than ketoconazole (6). For example, the doses of UK 49,858 that prevented death of 50% of mice given a lethal dose of *Aspergillus flavus* were 4- to 20-fold lower than ketoconazole doses in this model of infection (Troke et al. IXth Int. Cong. Int. Soc. Human Anim. Mycol., abstr. R2-7, 1985). In addition, UK 49,858 has been reported to be more active against murine candidiasis than ketoconazole (6), it can be administered both orally and intravenously, and it is excreted unchanged in the urine (S. Jevons and M. H. Tarbit, IXth Int. Cong. Int. Soc. Human Anim. Mycol., abstr. R2-3, 1985). The latter property theoretically should make it useful in the treatment of fungal urinary tract infections. If its *in vivo* activity against other systemic fungal pathogens compares as favorably with amphotericin B as it does in the present study, UK 49,858 represents an exciting addition to antifungal therapy.

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