Original Articles

In Vivo Efficacies of Pradimicin Derivatives,
BMS-181184 and BMY-28864, and Benanomycin
A against Systemic *Candida albicans* and
Aspergillus fumigatus Infections in Neutropenic Mice

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[Received : 1, October 1992. Accepted : 15, June 1993]

Abstract

The *in vivo* efficacies of pradimicin derivatives, BMS-181184 and BMY-28864, and benanomycin A were evaluated comparatively in experimental systemic candidiasis and aspergillosis in cyclophosphamide-treated neutropenic mice. Compounds were given intravenously once daily for 5 consecutive days beginning immediately after the infection of 10LD₅₀ of either *Candida albicans* A9540 or *Aspergillus fumigatus* IAM 2034. BMS-181184 was most effective in reducing the mortality among mice infected with *C. albicans* A9540, giving a PD₅₀ value of 33 mg/kg/day, while BMY-28864 and benanomycin A gave PD₅₀ values of 50 and 71 mg/kg/day, respectively. Against *A. fumigatus* IAM 2034, BMS-181184 and BMY-28864 were equally effective, giving PD₅₀ values of 41 and 43 mg/kg/day, respectively, while benanomycin A was less effective, giving a PD₅₀ value of 81 mg/kg/day.

Key words : systemic candidiasis, systemic aspergillosis, *in vivo* efficacy, pradimicin derivatives (BMS-181184 and BMY-28864), benanomycin A.

Introduction

Since the discovery of pradimicin A, a potent antifungal agent, from the fermentation broth of *Actinomadura hibisca* P157-2 (ATCC 53557)¹⁻³⁾, synthesis efforts have continued aiming at developing water-soluble pradimicin derivatives with the desired combination of antifungal activity and animal tolerance. BMY-28864 is a derivative of pradimicin FA-2⁴⁾, the first water-soluble compound with marked improvement in animal tolerance and pharmacokinetic

properties over pradimicin A⁵⁻⁸⁾. BMS-181184 is a new pradimicin derivative that contains a hydroxy substituent at C-4' position instead of the *N,N*-dimethyl functionality in BMY-28864⁹⁾. BMS-181184 showed excellent solubility in aqueous media, potent activity *in vitro* against a wide variety of fungi, and demonstrated *in vivo* efficacy in experimental *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* infection models¹⁰⁾. We sought, therefore, to determine whether BMS-181184 is superior to BMY-28864 and benano-

micin A^{11,12)}, and compared *in vivo* efficacies of these three compounds against *C. albicans* and *A. fumigatus* infections in persistently neutropenic mice.

Materials and Methods

Antifungal agents

Figure 1 shows the chemical structures of the three compounds, BMS-181184, BMY-28864, and benanomicin A, used in this study. All were produced by scientists at Bristol-Myers Squibb Research Institute, Tokyo, Japan and details of their preparations were described elsewhere^{4,9,13)}. A 10-mg/ml solution of each compound was prepared in 10% dimethyl sulfoxide (DMSO) at pH7.5 and serial two-fold dilutions were made with distilled water and filter sterilized (Millex-GV; pore size, 0.22 μ m).

Fungi

C. albicans A9540 and *A. fumigatus* IAM 2034 were used in this study. Their susceptibilities to known antifungal agents and virulence have been reported¹⁴⁾. Stock cultures were maintained at -80°C in 0.067 M phosphate buffer (pH7.2) containing 20% glycerol. *C. albicans* A9540 for inoculation into mice was

prepared in yeast extract-peptone-glucose broth (YPG: yeast extract, 0.4%; polypeptone, 1.0%; glucose, 1.5%; K_2HPO_4 , 0.05%; MgSO_4 , 0.05% [pH7.0]) and incubated at 28°C for 18h with shaking. The broth culture was diluted with saline to a concentration of 2×10^5 candida blastospores per ml. *A. fumigatus* IAM 2034 was grown at 28°C on YPG-1.5% agar slants for 7 days, and conidia were washed from the agar surface with 0.067 M phosphate buffer containing 0.2% (vol/vol) Tween 80 and suspended in saline containing 0.2% Tween 80. In each case, the inoculum was prepared fresh prior to use, with confirmation of the inoculum size by quantitative culture.

Animals

Four-week-old (22 to 26 g) male ICR mice (Japan SLC Inc., Shizuoka, Japan) were used in all experiments.

Experimental infections in mice

Groups of 10 mice at each dose level were treated intraperitoneally with 100mg of cyclophosphamide (CY: Shionogi, Osaka, Japan) per kg of body weight 4 and 1 days prior to an intravenous infection of 5×10^4 CFU (10 LD₅₀) of *C. albicans* A9540 per mouse or 1×10^4 conidia (10LD₅₀) of *A. fumigatus* IAM 2034 per mouse via the lateral tail vein. Test compounds were intravenously administered once daily for 5 consecutive days beginning immediately after the infection. The animals were kept in a neutropenic state ($\sim 70\%$ reduction in peripheral blood leukocyte count) for the entire observation period by administration of 100mg of CY per kg of body weight every 3-4 days. The 50% protective dose (PD₅₀) was calculated by the method of Litchfield and Wilcoxon¹⁵⁾ from the survival rate recorded 40 days after the infection.

Surviving mice after 40 days were sacrificed; the liver, lungs and kidneys were removed, chopped and homogenized in saline. Serial ten-fold dilutions of the homogenates were plated (100 μ g) in duplicate on petri dishes containing YPG-1.5% agar. Following incubation at 28°C for 2 days, colonies were counted. The minimal accurately detectable number of viable

Fig. 1. Chemical structures of BMS-181184, BMY-28864, and benanomicin A

cells was 100 CFU.

MIC determinations

The MICs of the three compounds were determined by the serial two-fold agar dilution method on yeast morphology agar (YMA: Difco Laboratories, Detroit Mich., USA) buffered with 0.067 M phosphate, pH 7.0, as described previously¹⁴).

Serum levels and urinary recovery in mice

A group of 48 mice was used for each compound to determine the drug concentrations in serum. A single dose of each compound at 20 mg/kg of body weight was administered intravenously and blood was collected, via cardiac puncture, from 6 mice at 0.17, 0.5, 1, 2, 3, 5, 7, and 24 h after drug administration. The concentrations of each compound in serum were determined by HPLC using a column of Waters μ Bondasphere (5μ , C18-100A, 3.9×150 mm, Nihon Millipore Ltd). The detector wavelength was set at 290 nm. The lowest concentration detectable by HPLC was $0.5\mu\text{g/ml}$. For urinary analysis, 20 mg/kg of body weight of each compound was given to six mice, and urinary samples (0-24 h) were collected, mixed with one-third volume of 2:1 acetonitrile-DMSO and centrifuged (12,000 rpm, 3 min). The supernatants were then filtered ($0.22\mu\text{m}$) and analyzed by HPLC.

Results

In vitro antifungal activity of BMS-181184, BMY-28864, and benanomicin A and their pharmacokinetics in mice

The MICs against *C. albicans* A9540 and *A. fumigatus* IAM 2034 determined on yeast morphology agar were $6.3\mu\text{g/ml}$ for BMS-181184, BMY-28864, and benanomicin A. Serum levels and urinary recovery of each compound were determined after a single intravenous administration at 20 mg/kg of body weight. Analysis by HPLC showed that these three compounds were similar to one another in pharmacokinetic profiles. They remained at high levels up to 7 h postdose, and >77% of total dose was excreted intact in urine during the first 24-h. The elimination half-lives of BMS-181184,

BMY-28864, and benanomicin A were 1.5, 2.0, and 1.7 h, respectively. Based on these pharmacokinetic parameters, a treatment schedule of once a day for 5 consecutive days was selected for comparative evaluation.

Effect of BMS-181184, BMY-28864, and benanomicin A on a systemic candidiasis in mice

Table 1 summarizes the effects of 5-day treatment of these compounds on the survival of mice infected with *C. albicans* A9540. In this model, mice were challenged with 5×10^4 CFU of candida blastospores per mouse. Infection control animals ($n=20$) died 5 to 10 days postinfection with a mean survival of 8.0 days. BMS-181184 therapy at a daily dose of 50 mg/kg for 5 consecutive days resulted in the survival of 70% of the infected mice with a PD_{50} value of 33 mg/kg/day. BMY-28864 and benanomicin A at the same dosing schedule gave 50 and 40% survival with PD_{50} values of 50 and 71 mg/kg/day, respectively. Semiquantitative culture results of organ cultures indicated that extensive infection occurred in the liver, lung, and kidney tissues of all untreated controls with a mean survival of 8.0 days as described above. Although all the survivors treated with these compounds had negative cultures in the liver and lungs on day 40, BMS-181184 was more effective than BMY-28864 and benanomicin A in clearing candida titers in the kidneys.

Effect of BMS-181184, BMY-28864, and benanomicin A on a systemic aspergillosis in mice

Table 2 summarizes the effects of 5-day treatment of these compounds on the survival of mice infected with *A. fumigatus* IAM 2034. In this model, mice were challenged with 1×10^4 conidia per mouse. Infection control animals ($n=20$) died 4 to 12 days with a mean survival of 7.0 days. The survival rate of mice treated with BMS-181184 or BMY-28864 was significantly higher than that of benanomicin A. Both BMS-181184 and BMY-28864 at a daily dose of 50 mg/kg for 5 days gave 60% survival, while benanomicin A gave 60 and 20% survival

Table 1. Effect of 5 days of treatment on a systemic candida infection in CY-treated neutropenic mice

Treatment (dose, mg/kg/day)	No. of survivors/treated after 40 days	No. of animals with negative kidney cultures
BMS-181184		
50	7/10	6
25	1/10	1
12.5	0/10	—
PD ₅₀	33 (31~35)	
BMV-28864		
50	5/10	5
25	1/10	0
12.5	0/10	—
PD ₅₀	50 (44~56)	
Benanomicin A		
100	6/10	4
50	4/10	2
25	0/10	—
12.5	0/10	—
PD ₅₀	71 (60~83)	

Figures in parentheses denote 95% confidence limits.

Table 2. Effect of 5 days of treatment on a systemic aspergillus infection in CY-treated neutropenic mice

Treatment (dose, mg/kg/day)	No. of survivors/treated after 40 days	No. of animals with negative kidney cultures
BMS-181184		
100	8/10	6
50	6/10	3
25	2/10	0
12.5	0/10	—
PD ₅₀	41 (35~47)	
BMV-28864		
100	9/10	7
50	6/10	3
25	1/10	0
12.5	0/10	—
PD ₅₀	43 (38~50)	
Benanomicin A		
100	6/10	2
50	2/10	0
25	0/10	—
12.5	0/10	—
PD ₅₀	81 (70~94)	

Figures in parentheses denote 95% confidence limits.

at daily doses of 100 and 50mg/kg, respectively. Tissue burden studies indicated that all the survivors treated with these compounds had negative cultures in the liver and lungs, similar to the case of candida infection, but BMS-181184 and BMY-28864 were superior to benanomycin A in clearing aspergillus titers in the kidneys.

Discussion

Fungal infection continues to represent a significant complication in immunocompromised individuals. The most common fungal pathogens encountered in neutropenic patients are *Candida* and *Aspergillus* species¹⁶⁾. Despite an increasing awareness of invasive mycoses and a greater mycologic acumen on the part of the medical community¹⁷⁾, amphotericin B still remains the drug of choice for presumed fungal infection in patients at high risk and for established candida and aspergillus infections¹⁸⁾. The newer triazoles such as fluconazole, itraconazole, and saperconazole have been shown to have inhibitory activity against *A. fumigatus* and demonstrated *in vivo* efficacy in the experimental models of invasive aspergillosis¹⁹⁻²¹⁾. However, these triazoles are not generally considered to be fungicidal and more experimental and clinical data are needed to establish their clinical potential against aspergillosis. Therefore, a need exists for fungicidal, broad-spectrum, less-toxic antifungal agents to supplement the list of currently available antifungal drugs.

The results of the present study demonstrated that the pradimicin derivatives, BMS-181184 and BMY-28864, were effective against both *C. albicans* and *A. fumigatus* infections in persistently neutropenic mice. These compounds may provide an invaluable adjunct to the therapy of serious fungal infections in humans, especially in neutropenic individuals. The present study also indicates that BMS-181184 and BMY-28864 are superior to benanomycin A in the treatment of systemic candidiasis and aspergillosis in CY-treated neutropenic mice. The MIC results appear to indicate that

the correlation between *in vitro* and *in vivo* results is uncertain at best among these compounds. Obviously, true confirmation will require additional studies with larger numbers of animals.

Acknowledgements

We thank the staffs of the Fermentation and Natural Products Chemistry Groups for the supply of BMS-181184, BMY-28864, and benanomycin A. We also thank Shinji Masuyoshi, Akemi Ohta, and Mieko Shinoda for their technical assistance.

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