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Short communication

Activity of pradimicin BMS-181184 against *Aspergillus* spp

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Abstract

The pradimicins are a new class of antifungal agents with activity against the majority of human fungal pathogens. In this study, the in vitro activity of pradimicin BMS-181184 was investigated against a range of the most common species of *Aspergillus*. The results were compared with itraconazole and amphotericin B. BMS-181184 was found to be active against most *Aspergillus* spp., but at higher concentrations than itraconazole and amphotericin B. © 1999 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

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1. Introduction

During recent years the frequency of serious fungal infection has increased dramatically. Increasing numbers of patients requiring immunosuppressants and/or cytotoxic chemotherapy, transplantation, intensive care medicine and the advent of acquired immune deficiency syndrome (AIDS) have contributed to the rise in systemic fungal infections seen worldwide [1].

The increase in the incidence of invasive aspergillosis and other fungal infections has generated a greater need for effective antifungal agents [2]. Invasive aspergillosis is usually treated with amphotericin B whose effectiveness is limited (0–50% response rate) and often causes severe side effects [3]. Lipid-associated amphotericin B preparations have ameliorated toxicity, particularly renal and infusion related toxicity, but data on efficacy in invasive aspergillosis are limited. The only other efficacious antifungal agent for aspergillosis is itraconazole [4] but responses to this drug are variable due to poor drug absorption in certain patient groups [5].

BMS-181184 (BMS) is a fermentation-derived antifungal antibiotic belonging to a new class of drugs called the pradimicins [6]. It has a broad range of activity against human fungal pathogens including *Candida* spp. [7,8] and *Cryptococcus neoformans* [9].

The pradimicin class of antifungal compounds has a unique mechanism of action related to calcium binding in the cell wall and, therefore, cross-resistance with amphotericin B and azole antifungals is unlikely. In a neutropenic rabbit model of invasive aspergillosis, BMS-181184 was as active as amphotericin B 1 mg/kg [10] although its pharmacokinetics were not favourable [11]. In this study we tested the in vitro activity of BMS-181184 against a range of *Aspergillus* spp. and compared it directly with amphotericin B and itraconazole.

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2. Materials and methods

A total of 54 clinical *Aspergillus* isolates consisting of 37 *A. fumigatus*, seven *A. terreus*, and six each of *A. flavus* and *A. niger* were used in this study. The isolates had been collected over many years from both the US and the UK. For susceptibility tests, all isolates were grown on Sabouraud dextrose agar (Lab M, Bury, UK) at 30°C for 3–4 days.

BMS-181184 (Bristol Myers Squibb, Wallingford, CT, US) was prepared by dissolving the BMS free acid in sterile water in a glass bijou bottle. The appropriate amount of drug was added to 0.5 ml of sterile water and a total volume of 0.2 ml of 0.2 M NaOH. To completely dissolve the drug the solution was sonicated for 30 min. The final concentration was 6400 mg/l. Amphotericin B with desoxycholate (Squibb, Middlesex, UK) was suspended in sterile water to a final concentration of 1600 mg/l. Itraconazole (Janssen Research Foundation, Beerse, Belgium) was dissolved in 50% acetone and 50% 0.2M HCl to produce a final concentration of 3200 mg/l. All drugs were stored in glass vials in the dark at −20°C.

MICs were performed using a broth macrodilution method. RPMI-1640 (Sigma, Poole, UK) supplemented with 2% glucose was used for BMS-181184 and itraconazole and yeast nitrogen base (YNB) (Difco, UK) with 0.5% glucose buffered to pH 7.0 with morpholinopropanesulfonic acid was used for amphotericin B as described previously [12]. Two-fold dilutions of 1 ml of drug at each concentration were made in 5-ml sterile plastic tubes. The final range of drug concentrations (in mg/l) was 0.125–64 for BMS, 0.03–16 for itraconazole and 0.125–32 for amphotericin B.

The inoculum was prepared by wetting a sterile loop into PBS (Oxoid, Basingstoke, UK) with Tween 80 (concentration of 0.05%) (Fisons, Loughborough, UK) and transferring a loopful of *Aspergillus* conidia into sterile PBS Tween. The resulting suspension was counted using a haemocytometer and diluted to a concentration of 2×10^3 ml in appropriate medium. Inoculum (1 ml) was then delivered to every tube in the drug dilution range. The final inoculum contained 1×10^3 spores/ml. A negative control was also included to ensure sterility of the medium. The tubes were incubated with loose caps at 37°C on a gyratory shaker at 30° to the horizontal for a period of 40–42 h. The MIC was read by comparing all tubes against the positive control. The concentration of drug in the first tube that showed no growth was designated the MIC. To check the reproducibility of the study, eight of the isolates were retested in triplicate.

Minimum fungicidal concentrations (MFCs) were also performed. 100 μl were transferred from every tube with no growth on to the surface of horse blood agar. The liquid was allowed to soak in so as to avoid carry over of drug. The plates were then streaked and incubated at 37°C for 48 h. The MFC was defined as the lowest drug concentration to yield no growth or less than three colonies. This represents greater than 98% killing of the original inoculum.

3. Results and discussion

The range of MICs, geometric means, and MIC_{50} and MIC_{90} results are summarised in Table 1. BMS had considerably higher MICs when compared with currently used antifungal agents itraconazole and ampho-

Table 1
In-vitro activity (mg/l) of BMS-181184 (BMS) against *Aspergillus* spp. and comparison with itraconazole (ITZ) and amphotericin B (AMB)

Species	Number of isolates	Antifungal agent	MIC range	Geometric mean	MIC_{50}	MIC_{90}	MFC range	MFC geometric mean*
A. fumigatus	35	BMS	4–16	8	8	8	32–>64	86.1
		ITZ	0.125–16	0.37	0.25	0.5	0.125–32	10.55
		AMB	0.5–2	0.87	1	2	0.5–4	1.08
A. terreus	7	BMS	8–16	13.1	16	16	8–>64	52.5
		ITZ	0.06–0.125	0.11	0.125	0.125	0.125–0.5	0.25
		AMB	0.5–2	1	2	1	0.5–8	1.811
A. flavus	6	BMS	8–16	12.7	16	16	64–>64	101.6
		ITZ	0.06–0.125	0.09	0.125	0.125	0.06–0.25	0.14
		AMB	1–4	1.78	2	2	1–32	2.52
A. niger	6	BMS	8–16	8.9	8	8	16–>64	32.0
		ITZ	0.06–1	0.24	0.25	0.5	0.06–1	0.35
		AMB	0.5–2	0.9	1	1	0.5–16	2.0
Total	54	BMS	4–16	9.1	8	16	8–>64	73.7
		ITZ	0.06–16	0.26	0.25	0.5	0.125–32	0.41
		AMB	0.5–4	0.96	1	1	0.5–32	1.36

* In calculation of geometric mean MFCs, a value of >64 is assumed to be 8.128 mg/l.

tericin B. The MICs for BMS ranged from 4–16 mg/l and the geometric mean was 9.1 mg/l for all of the isolates tested in the study. There was little difference in MIC results between species. *A. fumigatus* had the lowest GM value of 8 mg/l and the highest GM MIC was observed in *A. terreus* at 13.1 mg/l. BMS-181184 was fungicidal against 42% of the isolates in the drug range tested (with a range of 8–64 mg/l and a geometric mean of 73.7 mg/l).

In comparison, itraconazole was considerably more active with MICs ranging from 0.06 to 16 mg/l and with a GM value of 0.26 mg/l. *A. terreus* appeared to be the most susceptible species to itraconazole with a GM value of 0.09 mg/l. Three of the *A. fumigatus* isolates tested against itraconazole were resistant (MICs of >16 mg/l). There was no cross-resistance observed between BMS and itraconazole as the BMS MICs were all 8 mg/l for the resistant isolates. The MICs observed for amphotericin B ranged from 0.5 to 4 mg/l with an overall GM MIC of 0.96 mg/l for all isolates tested. *A. fumigatus* appeared to be the most susceptible species to AB with a GM of 0.87 mg/l. MFC values showed 94 and 100% of isolates were killed by the concentrations tested of itraconazole and amphotericin B, respectively.

BMS-181184 produced clear and defined end-points using this method despite the deep red colour of the compound. However, determination of MICs using a colorimetric end-point such as Alamar Blue [13] might be problematic. The reproducibility study showed that for all drugs eight of eight isolates gave MIC results identical to or within one tube difference of the MIC on re-testing.

In conclusion, pradimicin BMS-181184 shows moderate activity against a range of *Aspergillus* spp. It has less in vitro activity than itraconazole and amphotericin B, although no cross-resistance was observed between BMS-181184 and itraconazole. However pharmacodynamice considerations could alter the apparent differences between the compounds in vivo. Unfortunately, the clinical development of BMS-181184 has been discontinued because of elevated liver function tests in human volunteers receiving the compound. However clearly further studies on this new class of antifungal compounds are warranted as activity, albeit modest, has been demonstrated in this study.

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