Activity of aminocandin (IP960; HMR3270) compared with amphotericin B, itraconazole, caspofungin and micafungin in neutropenic murine models of disseminated infection caused by itraconazole-susceptible and -resistant strains of *Aspergillus fumigatus*

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ABSTRACT

Aminocandin (IP960; HMR3270; NXL201) is a new echinocandin with broad-spectrum in vitro activity against *Aspergillus* and *Candida* spp. We compared the activity of aminocandin with that of amphotericin B (AmB), itraconazole (ITC) and caspofungin (CAS) in murine models of disseminated aspergillosis against three strains of *A. fumigatus*, two of which were fully susceptible (AF293 and A1163) and one was resistant to ITC (AF91). Mice were rendered temporarily neutropenic or persistently neutropenic with cyclophosphamide and were infected intravenously 3 days later. Temporarily neutropenic mice were treated with either intraperitoneal (i.p.) AmB (5 mg/kg/dose), oral (p.o.) ITC (25 mg/kg/dose), intravenous (i.v.) aminocandin (0.25–10 mg/kg/dose), i.p. aminocandin (1 mg/kg/dose) or solvent control for 9 days. Mice were euthanised 11 days post infection and the kidneys and liver were removed for quantitative culture. Following infection with AF293, only aminocandin 5 mg/kg i.v. yielded 100% survival. Aminocandin 1 mg/kg i.v., AmB 5 mg/kg i.p. or ITC 25 mg/kg p.o. were equivalent ($P > 0.05$). Aminocandin 5 mg/kg was superior to aminocandin 0.25 mg/kg ($P < 0.0001$) as well as all controls ($P < 0.0001$) in reducing mortality. Following infection with AF91, only aminocandin at 5 mg/kg and 1 mg/kg i.v. yielded 100% survival, which was superior to ITC, aminocandin 0.25 mg/kg and controls (all $P < 0.0001$). In the persistently neutropenic model with A1163, aminocandin, CAS and micafungin (2–10 mg/kg) were all effective at prolonging survival, with some impact on reducing culture burdens, even with alternate-day dosing (4 mg/kg). The only fungicidal regimen was aminocandin 5 mg/kg, which sterilised 40% and 50% of mice following infection with AF293 and AF91, respectively. Aminocandin at doses of ≥ 1 mg/kg is highly effective in reducing mortality and organ burden in disseminated infection caused by ITC-susceptible and -resistant *A. fumigatus*.

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1. Introduction

Invasive aspergillosis (IA) causes ca. 30% of fungal infections in patients with cancer and is a major threat to life in patients receiving cytotoxic chemotherapy resulting in an immunocompromised state [1,2]. IA is now a more common cause of death than candidiasis in the USA [3]. Amphotericin B (AmB) and itraconazole (ITC) are only moderately effective in IA, with persistently high mortality rates [1], although the introduction of voriconazole (VCZ) has resulted in significant improvements in outcome [4–7]. Posaconazole (PCZ) is also active against *Aspergillus* spp. and is licensed in some

countries for salvage therapy of IA, but it is only available orally [8].

The echinocandins have recently been introduced. They act by inhibiting the synthesis of 1,3-β-D-glucan, an essential component of the fungal cell wall—a novel mode of action. In vitro studies have demonstrated that existing echinocandins are inhibitory, but not fungicidal, against clinical isolates of *Aspergillus* spp. [9] and this activity is also seen in animal models of disseminated aspergillosis [10,11].

Aminocandin (IP960; HMR3270; NXL201) is a new water-soluble antifungal drug of the echinocandin class [12,13] that has demonstrated excellent in vitro and in vivo activity both against *Candida* [14] and *Aspergillus* [15]. In this study, we tested aminocandin in temporarily and persistently immunocompromised mouse models of disseminated aspergillosis against three *Aspergillus* strains, two of which were susceptible in vitro to both AmB and ITC (AF293 and A1163) and one was susceptible to AmB

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but resistant to ITC (AF91). Aminocandin was compared with AmB and ITC and separately with caspofungin (CAS) and micafungin (MFG) in other murine models.

2. Method

2.1. Test strains

Three strains of *Aspergillus fumigatus* [AF293 (NCPF 7367), AF91 (NCPF 7100) and A1163 (FGSC A1163)] were used in this study. Susceptibilities were determined by a modified European Committee on Antimicrobial Susceptibility Testing (EUCAST) method [16] with lower inocula of 5×10^4 colony-forming units (CFU)/mL (AmB and ITC) or 0.5–2.5×10^3 CFU/mL (aminocandin, CAS and MFG). A no-growth endpoint was used for ITC and AmB, but for aminocandin, CAS and MFG interpretation of the minimum inhibitory concentration (MIC) was modified to the lowest concentration at which a prominent decrease in turbidity occurred (the minimum effective concentration) [17]. *Aspergillus fumigatus* AF293 was susceptible to AmB and ITC (MICs of 1.0 mg/L and 0.5 mg/L, respectively) and echinocandins (MICs of 0.25 mg/L for aminocandin and CAS and 0.125 mg/L for MFG). AF91 was isolated from a patient with acquired immune deficiency syndrome (AIDS) treated with ITC and was susceptible to AmB (0.5 mg/L), aminocandin (0.125 mg/L), CAS (0.25 mg/L) and MFG (<0.015 mg/L) but was resistant to ITC (MIC > 8.0 mg/L) and in murine models. A1163 is a derivative of *A. fumigatus* CEA17 and is susceptible to AmB, ITC, aminocandin and CAS (0.5, 0.25, 0.06 and 0.06 mg/L, respectively).

2.2. In vivo studies

2.2.1. Animals

Male CD1 mice (4–5 weeks old; 18–20 g) (Charles River UK Ltd., Margate, UK) were virus-free and were allowed free access to food and water. Mice were randomised into groups of 10. Each cage was inspected at least four times daily.

2.2.2. Immunosuppression

Mice were immunosuppressed with a single dose (temporary neutropenia, AF239 and AF91) or multiple doses (persistent neutropenia, A1163) of 200 mg/kg intravenous (i.v.) cyclophosphamide (Sigma-Aldrich) every 3 days starting on Day – 3. This yields either a temporary profound neutropenia (until 2 days post infection) or persistent neutropenia throughout the experiment starting on Day 0 [18].

2.2.3. Infection of mice

Aspergillus conidia were harvested in phosphate-buffered saline (PBS) plus 0.05% Tween 80 (Sigma). The count was determined by serial dilution on Sabouraud dextrose agar (SDA).

Prior to each experiment, inoculum finding studies [90% lethal dose (LD$_{90}$)] for each isolate were performed, resulting in LD$_{90}$ values of 1.2×10^6 CFU/mouse for AF293, 6.0×10^5 CFU/mouse for AF91 and 4.0×10^5 CFU/mouse for A1163. Mice were infected with the LD$_{90}$ dose on Day 0 (3 days post immunosuppression) via the lateral tail vein. Post-infection viability counts were performed to ensure the correct inoculum had been given. All experiments were performed once.

2.2.4. Antifungal therapy

Amphotericin B deoxycholate (Fungizone®; E.R. Squibb, Hounslow, UK) was dissolved in 5% glucose (Baxter Healthcare, Norfolk, UK) to a stock concentration of 5.0 mg/mL. The stock solution was stored at 4 °C for up to 7 days before use and was further diluted with 5% glucose for use. AmB (5 mg/kg) was administered intraperitoneally on Days 1, 2, 4 and 7.

ITC (Janssen-Cilag Ltd.) in 2-hydroxypropyl-β-cyclodextrin (HPBC) (25 mg/kg) [19] was administered by gavage three times daily on Days 1 and 2 then twice daily on Days 3–9.

Aminocandin powder (Aventis, Romainville, France) 13.88 mg (equivalent to 12.5 mg of active drug) was dissolved in 1 mL of sterile water. The stock was further diluted in 5% glucose as required and was stored for up to 48 h at 4 °C before use. Aminocandin was administered via the i.v. (10, 5, 1 and 0.25 mg/kg) or intraperitoneal (i.p.) (1 mg/kg) routes.

Control mice were infected but received no active treatment. Groups received either 5% glucose i.v. or i.p. as appropriate or HPBC solution by gavage.

For direct comparison with other echinocandins, MFG (Mycamine™; Astellas Pharma, Staines, UK) was reconstituted in 5% glucose and was administered intravenously, and CAS (Cancidas®; Merck, Whitehouse, NJ) was reconstituted in 0.9% saline and administered intravenously. Treatments were aminocandin, MFG or CAS 10, 4 or 2 mg/kg once daily, 4 mg/kg every 2 days or solvents as appropriate (persistently neutropenic).

Each treatment or control group consisted of 10 mice. Treatment was initiated 24 h post infection (AF293 and AF91) or 4 h post infection (A1163) and continued for 7 days.

2.2.5. Experimental endpoints

Mice were examined at least four times daily. Any infected animals with severely reduced mobility, unable to reach the drinker or otherwise in substantial distress were humanely terminated. On Day 11 (azole and polyene experiment) or Day 15 (echinocandin comparison experiment), all surviving mice were humanely terminated. The kidneys and liver were removed and homogenised in PBS, then colony counts determined using serial 10-fold dilutions plated on the surface of SDA supplemented with 0.5% (w/v) chloramphenicol. Single colonies were accorded a negative result because of the possibility of airborne contamination. This method detected *A. fumigatus* at >30 CFU/organ.

2.3. Pharmacokinetics of itraconazole

Blood samples were collected from a separate group of immunosuppressed mice treated with 25 mg/kg itraconazole (three times a day on Days 1 and 2, then twice a day) by cardiac puncture 2 h post dose on Day 5 to determine adequate absorption and pharmacokinetics of ITC. Serum samples were stored at −20 °C until analysis. ITC was measured by bioassay [20].

2.4. Statistical analysis

Mortality data were analysed using Peto's log-rank test in which *P*-values reflect the χ^2 for equivalence of death rates. Culture data were analysed using the Kruskal–Wallis pairwise comparisons test (Conover–Inman). All data analysis was performed using the computer package StatsDirect (Ashwell, UK).

3. Results

3.1. In vivo results

The mortality curves (Figs. 1–3) demonstrate that all isolates caused lethal infections in mice (80–100% mortality in vehicle controls). To aid clarity, control groups (glucose i.v. and i.p. and HPBC oral) were combined in the figures (the mortality rates/times were similar and statistically indistinguishable). No toxicity was noted after treatment with aminocandin in any model.

Following infection with AF293, aminocandin 5 mg/kg i.v. was the only treatment with 100% survival. Survival after treatment with aminocandin 1 mg/kg i.v., AmB 5 mg/kg i.p. or oral ITC
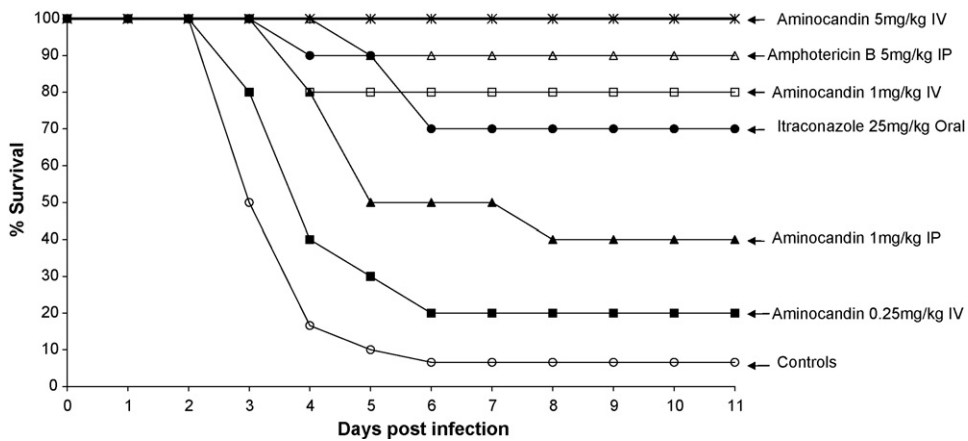
Fig. 1. Cumulative mortality versus time in a murine model against *Aspergillus fumigatus* AF293 infection. ✳, aminocandin 5 mg/kg i.v.; □, aminocandin 1 mg/kg i.v.; ■, aminocandin 0.25 mg/kg i.v.; ▲, aminocandin 1 mg/kg i.p.; △, amphotericin B (AmB) 5 mg/kg i.p.; ●, itraconazole (ITC) 25 mg/kg oral; ○, controls. Treatment with 5 mg/kg or 1 mg/kg aminocandin i.v. was superior to 0.25 mg/kg aminocandin (*P* = 0.0082); treatment with 5 mg/kg or 1 mg/kg aminocandin i.v., AmB and ITC was superior to controls (*P* < 0.008). i.v., intravenous; i.p., intraperitoneal.
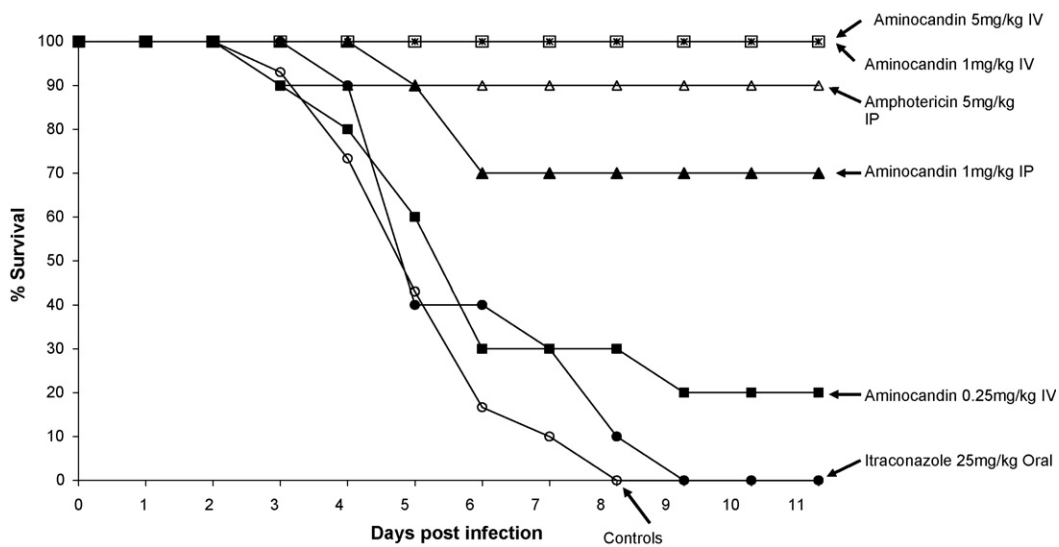

Fig. 2. Cumulative mortality versus time in a murine model against *Aspergillus fumigatus* AF91 infection. ✳, aminocandin 5 mg/kg i.v.; □, aminocandin 1 mg/kg i.v.; ■, aminocandin 0.25 mg/kg i.v.; ▲, aminocandin 1 mg/kg i.p.; △, amphotericin B (AmB) 5 mg/kg i.p.; ●, itraconazole (ITC) 25 mg/kg oral; ○, controls. Treatment with 5 mg/kg or 1 mg/kg aminocandin i.v. was superior to 0.25 mg/kg aminocandin (*P* = 0.0003); treatment with 5 mg/kg or 1 mg/kg aminocandin i.v. and AmB was superior to ITC and controls (*P* < 0.0001). i.v., intravenous; i.p., intraperitoneal.
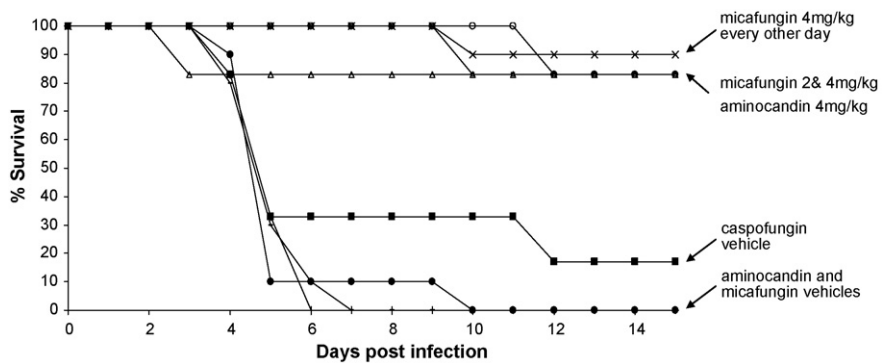

Fig. 3. Cumulative mortality versus time in a murine model against *Aspergillus fumigatus* A1163 infection. ✳, micafungin 4 mg/kg EOD i.v.; △, aminocandin 4 mg/kg i.v.; □, micafungin (MFG) 4 mg/kg; ○, MFG 2 mg/kg; ■, caspofungin (CAS) vehicle daily; ●, aminocandin and MFG vehicle EOD; +, CAS vehicle EOD; −, aminocandin and MFG vehicle daily. All other treatments had 100% survival (aminocandin 10 mg/kg/day, 2 mg/kg/day and 4 mg/kg EOD, caspofungin 10, 4 and 2 mg/kg/day and 4 mg/kg EOD and micafungin 10 mg/kg/day). EOD, every other day; i.v., intravenous.

Table 1
Geometric mean organ counts following infection with *Aspergillus fumigatus* AF293 and AF91.

Treatment group	A. fumigatus AF293			A. fumigatus AF91		
	% with no growth	Count[a]		% with no growth	Counts[a]	
		Liver	Kidney		Liver	Kidney
Aminocandin 5 mg/kg i.v.	40[*]	1.62[**]	1.08	50[*]	1.86[†]	2.16[†]
Aminocandin 1 mg/kg i.v.	0	3.58[††]	3.13[††]	0	2.4[†]	3.83[†]
Aminocandin 0.25 mg/kg i.v.	0	5.08	4.18	10	4.27	4.77
Aminocandin 1 mg/kg i.p.	0	5.14	4.23	0	2.92	4.36
Amphotericin B 5 mg/kg i.p.	0	2.93	2.51	10	1.56	3.58
Itraconazole 25 mg/kg	0	4.55	3.65	N/D	N/D	N/D
Vehicle[b]	0	5.13	4.3	N/D	N/D	N/D

CFU, colony-forming units; i.v., intravenous; i.p., intraperitoneal; N/D, no data.

[a] All counts expressed as the geometric mean $\log_{10}$ CFU/g of tissue in mice culled at the end of the study.

[b] Vehicle groups combined (glucose i.v., glucose i.p. and oral 2-hydroxypropyl-β-cyclodextrin).

[*] $P < 0.04$, Fishers exact test.

[**] Superior to aminocandin 1 mg/kg i.p. ($P < 0.013$), aminocandin 0.25 mg/kg i.v., itraconazole and vehicle ($P < 0.0001$).

[†] Superior to aminocandin 0.25 mg/kg ($P = 0.0002$, aminocandin 5 mg/kg i.v.; and $P = 0.0011$, aminocandin 1 mg/kg i.v.).

[††] Superior to aminocandin 0.25 mg/kg i.v. and aminocandin 1 mg/kg i.p. ($P < 0.02$).

25 mg/kg was numerically inferior to treatment with aminocandin 5 mg/kg but were statistically equivalent ($P > 0.05$). Treatment with aminocandin 5 mg/kg was superior to aminocandin 0.25 mg/kg ($P = 0.0003$), aminocandin 1 mg/kg i.p. ($P = 0.0041$) and all controls ($P < 0.0003$) in reducing mortality. Aminocandin 1 mg/kg i.v. was superior to the same dose administered intraperitoneally. Treatment with aminocandin demonstrated a dose-dependent response, with 0.25 mg/kg i.v. being much less effective than 5 mg/kg/day or 1 mg/kg/day (Fig. 1).

Organ counts following infection with AF293 (Table 1) show that the only partially fungicidal regimen was aminocandin 5 mg/kg, which organ burden reduced to below detectable levels in 40% of mice ($P < 0.04$, Fishers exact test). Treatment with aminocandin 5 mg/kg was superior in reducing organ burden both in the liver and kidneys compared with all treatments except AmB ($P < 0.045$). Organ burden was lower after treatment with either aminocandin 5 mg/kg or 1 mg/kg compared with aminocandin 0.25 mg/kg or controls ($P < 0.002$). Treatment with aminocandin delivered intravenously was superior to the same dose administered intraperitoneally at lowering organ burden (liver, $P = 0.029$; kidney, $P = 0.006$).

Following infection with AF91, treatment with aminocandin at 5 mg/kg or 1 mg/kg i.v. yielded 100% survival; this was superior to ITC, aminocandin 0.25 mg/kg and controls (all $P < 0.0003$). Treatment with AmB 5 mg/kg resulted in 90% survival and treatment with aminocandin 1 mg/kg i.p. resulted in 70% survival

(not statistically different from aminocandin 5 mg/kg or 1 mg/kg i.v.). In this model, aminocandin again demonstrated a dose-dependent response. Treatment with ITC 25 mg/kg did not prolong survival compared with the control therapies (Fig. 2).

Treatment of infection with AF91 with aminocandin 5 mg/kg resulted in a reduction in organ burden to below detectable levels in 50% of the mice that were infected (Table 1). Treatment with either aminocandin 5 mg/kg or 1 mg/kg i.v. was superior in reducing organ burden compared with aminocandin 0.25 mg/kg ($P < 0.007$).

Following infection with A1163, mortality in untreated mice was high in this persistently neutropenic model, with death occurring 6–10 days post infection. All three echinocandins were highly effective at improving survival with regimens as low as 2 mg/kg/day. Alternate-day therapy was as effective as once-daily treatment ($P > 0.05$) for MFG and aminocandin (Fig. 3).

The burdens of A1163 in vehicle-treated mice 4 days post infection were high, with the liver, spleen and kidneys the main target organs. Treatment with echinocandins (≥ 4 mg/kg/day) reduced the burden in all organs but was particularly effective at reducing the burden in the kidneys (Table 2). There was a 1–1.5 log reduction at 4 days in most treatment groups compared with vehicle-treated mice. Aminocandin 4–10 mg/kg sterilised 4 (36%) of 11 mice compared with 2 (18%) of 11 mice treated with MFG and 6 (50%) of 12 mice treated with CAS at the same doses ($P > 0.05$, Fishers exact test).

Table 2
Geometric mean organ counts following infection with *Aspergillus fumigatus* A1163 at 100 h and at the end of the study.

Treatment	Tissue burden 100 h post infection ($\pm$ S.D.)[a]		Tissue burden 11 days post infection in survivors ($\pm$ S.D.)[a]	
	Liver	Kidney	Liver	Kidney
Aminocandin/MFG vehicle daily	3.92 ± 3.94	4.11 ± 3.56	N/D	N/D
CAS vehicle daily	4.24 ± 4.24	4.19 ± 4.28	0	3.15
10 mg/kg aminocandin daily	3.06 ± 2.81	2.68 ± 2.59[*]	0	3.21 ± 3.03
4 mg/kg aminocandin daily	2.96 ± 2.72	2.49 ± 2.30[*]	1.85 ± 2.03	2.69 ± 3.07
2 mg/kg aminocandin daily	2.57 ± 1.91	2.31 ± 3.34[*]	1.72 ± 1.63	2.49 ± 2.43
10 mg/kg MFG daily	2.51 ± 2.20	2.64 ± 2.32[*]	0	2.61 ± 2.73
4 mg/kg MFG daily	2.87 ± 2.61	2.78 ± 2.83[*]	1.24 ± 1.58	2.94 ± 2.97
2 mg/kg MFG daily	3.11 ± 3.12	2.38 ± 2.08[*]	1.85 ± 2.0	2.75 ± 2.97
10 mg/kg CAS daily	3.03 ± 2.57	2.66 ± 2.38	0.77 ± 1.14	2.88 ± 2.93
4 mg/kg CAS daily	3.20 ± 3.10	2.48 ± 0.01	0.67 ± 1.92	2.95 ± 3.34
2 mg/kg CAS daily	3.22 ± 2.71	3.10 ± 3.20	1.88 ± 1.61	3.32 ± 1.61
4 mg/kg aminocandin EOD	2.77 ± 2.70	2.47 ± 4.14[*]	2.54 ± 2.71	3.84 ± 2.71
4 mg/kg MFG EOD	2.91 ± 2.73	2.81 ± 4.45[*]	1.0 ± 2.31	1.90 ± 2.32
4 mg/kg CAS EOD	2.65 ± 2.67	2.21 ± 2.02[*]	1.51 ± 1.79	1.51 ± 1.79

S.D., standard deviation; CFU, colony-forming units; MFG, micafungin; CAS, caspofungin; N/D, no data; EOD, every other day.

[a] All counts expressed as $\log_{10}$ CFU/g.

[*] Superior to vehicle ($P < 0.02$).

ITC plasma concentrations were a mean of 1.7 mg/L in samples collected 2 h post dose on Day 5 of treatment, confirming the expected drug exposure.

4. Discussion

As expected from previous work, AF293 was susceptible to both AmB and ITC, whereas AF91 was susceptible to AmB and resistant to ITC in vivo [19,21]. This study further demonstrated that the echinocandin aminocandin administered at therapeutic levels ($\geq$1 mg/kg i.v.) to temporarily or persistently neutropenic mice was highly active against all isolates in terms of reducing mortality (and reducing organ burden below detectable levels at $\geq$4 mg/kg). As reported previously, we have again demonstrated no cross-resistance of echinocandins with azoles [22–24]. Given the emerging resistance in *Aspergillus* to AmB, ITC and other azoles [18,25] as well as few alternative therapies for diseases caused by *Aspergillus*, the continued development of new antifungal drugs is essential.

Aminocandin demonstrated a dose-dependent response in all models (with 1–10 mg/kg being more effective than 0.25 mg/kg) in terms of prolonged survival and reduction of organ burden in the liver and kidneys. This activity was superior to that seen in the same models with MFG [18] as well as being superior to PCZ (SCH 56592) [26,27]. A criticism of this study is that we did not compare the activity of aminocandin with VCZ, the leading treatment for IA. This is because VCZ undergoes autoinduction of metabolism in mice [27] with poor results, despite its good activity in other species and humans with IA.

Doses of aminocandin of $\geq$1 mg/kg were superior to a dose of 0.25 mg/kg (all administered intravenously). It is interesting to note that aminocandin 1 mg/kg administered intravenously was superior to the same dose administered intraperitoneally both at improving survival and in reducing organ burden. Unfortunately, we were unable to measure aminocandin plasma concentrations to determine whether the reason for the apparent reduced effect was a function of a reduced maximum serum concentration ($C_{\max}$) or reduced area under the concentration–time curve (AUC) or some other pharmacodynamic parameter. Andes et al. [13] reported that after a singe i.p. injection of aminocandin 1 mg/kg the serum drug level remained above 0.5 μg/mL for 48 h and that in a murine candidiasis model in vivo concentration-dependent killing was observed; i.v. administration of aminocandin is likely to increase the $C_{\max}$ and therefore increase the peak/MIC ratio, improving efficacy. Aminocandin did not demonstrate any toxicity in neutropenic mice even at the highest doses administered.

It has previously been reported that the echinocandins have little fungicidal activity against *A. fumigatus* in vivo and in vitro [28]. Staining using fluorescent indicators of cell viability and death after treatment with caspofungin acetate illustrated that the tips and branch points of young germlings were killed preferentially by the drug but parts of the hyphal structure remained viable [29] and that echinocandins fail to clear organs of *Aspergillus* following disseminated infection [29,30]. It has also been noted that high counts of *Aspergillus* are recovered (sometimes higher than untreated controls) in organ burden studies, possibly due to microcolonies within intact organs being fragmented during homogenisation procedures [29]. This problem has been partly addressed in a recent study using quantitative polymerase chain reaction (qPCR) as an endpoint of disseminated infection, but this was flawed in design as treatment was given immediately after infection and only kidneys were examined [30]. The study therefore addressed the activity of echinocandins in the very early stages of infection during germination of conidia. O'Sullivan et al. [31] describe a fluorescence resonance energy transfer (FRET)-qPCR that was more sensitive than culture in detecting *Aspergillus* in bronchoalveolar lavage,

but overall the pattern of detection was similar to quantitative culture.

The studies of cell viability [and MIC/minimum fungicidal concentration (MFC)] following echinocandin treatment have all been performed in air, but recent data indicate that increased inhibition occurs if susceptibility tests take place in hypoxic conditions similar to those encountered in tissue [32]. In hypoxic conditions ($\leq$1% oxygen) almost complete inhibition of hyphal growth occurs after contact with echinocandins. It is therefore possible that the lack of fungicidal activity and pattern of inhibition in cell viability assays may be influenced by culture conditions.

It is particularly noteworthy that treatment with aminocandin 5 mg/kg was able to totally clear *Aspergillus* from the organs of 45% of the temporarily neutropenic mice in this study (AF293 and AF91) and 29% of persistently neutropenic mice (A1163). It therefore seems likely that the drug is interacting with the recovering immune system in temporarily neutropenic mice to enhance clearance of fungi, as recently demonstrated with isavuconazole [33]. The immunomodulatory effects of glucan have recently been noted [34].

CAS was the first drug of the echinocandin class to be approved for the treatment of IA in patients refractory to or intolerant of conventional therapy. It has demonstrated excellent safety and tolerability as well as useful efficacy in completed studies [35], although relapse rates may be higher than with azole therapy, probably reflecting either shorter periods of therapy or fungistatic activity only or combinations of both. MFG also has clinical activity in IA [36]. In this direct comparative study, aminocandin was essentially equivalent to CAS and MFG in terms of survival and organ sterilisation. Furthermore, alternate-day dosing with all three echinocandins was as effective as daily dosing with the same cumulative dose. This raises the possibility of alternate-day dosing in patients requiring prolonged i.v. therapy.

These data are consistent with the remarkable efficacy of aminocandin compared with other licensed echinocandins in the treatment of murine IA and warrant further investigation of this new member of the class.

Ethical approval: All animal experiments included in this study were part of ongoing studies performed under UK Home Office project licence PPL40/1523 entitled 'Invasive Fungal Infections' and had received ethical clearance from the University of Manchester Ethics Review Panel.

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