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In Vivo Characterization of A-192411: A Novel Fungicidal Lipopeptide (II)

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Abstract—The ability of the novel antifungal cyclic hexalipopetide A-192411 to treat fungal infections in rodents is presented. Efficacy was demonstrated against *Candida albicans* as both prolonged survival of systemically infected mice and clearance of viable yeasts from kidneys. The efficacy of A-192411, administered intraperitoneally, was equivalent to amphotercin B at a 4-fold lower dose by weight in the systemic candidiasis models in mice, while the efficacy of A-192441 administered intravenously was equivalent to amphotercin B by weight in the *Candida pyelonephritis* model in rats. A-192411 also slightly prolonged the survival of immunocompromised mice infected systemically with *Aspergillus fumigatus*.

Life-threatening human mycoses arising from increased numbers of immunocompromised patients at risk for acquiring fungal infections due to HIV infection, chemotherapy-induced neutropenia, organ transplantation, hemodialysis and use of broad-spectrum antibiotics and glucocorticosteroids are a serious medical concern.[1] In the preceding paper, we reported on the discovery and SAR as well as on the synthesis, biochemical and pharmacokinetic characterization of a novel cyclic lipopeptide: A-192411 (Fig. 1); the in vitro antifungal activity of A-192411 was reported previously.[2] We report herein on the treatment of *Candida albicans* and *Aspergillus fumigatus* infections with A-192411 in rodent models.

The in vitro spectrum of A-192411 includes the most clinically important fungal pathogens, with potencies similar to those of amphotericin B.[2] The minimum inhibitory concentrations (MICs) for A-192411 against 41 strains of *C. albicans* range from 0.25 to 1.0 μg/mL with 90% of the strains inhibited by A-192411 at 1.0 μg/mL, and it is equally active against fluconazole-susceptible and resistant strains. Like amphotericin B, exposure of *C. albicans* to A-192411 at four-times the MIC reduced the number of viable cells by >99% within 6 h; in contrast, fluconazole was unable to reduce cell viability. Moreover, A-192411 was highly active in vitro against 80 isolates representing non-*albicans Candida* species. The activity against *C. tropicalis* was equivalent to that of *C. albicans* while *C. glabrata*, *C. krusei,* and *C. lusitaniae* and by *C. kefyr* and *C. parapsilosis* are 2- to 4-fold less susceptible than *C. albicans*. A-192411, demonstrating MICs of 4 μg/mL, is more active in vitro against *Cryptococcus neoformans* than the other echinocandins caspofungin (MK-0991), LY-303366, and FK-463; however, the potential for clinical utility for treatment of cryptococcal menigitis will rely on the penetration of effective drug concentrations across the blood–brain barrier.

A-192411 was evaluated in vivo against acute systemic infection caused by *C. albicans* CAF2. In this model, death of untreated animals occurred within 24 h of infection.[3] Intraperitoneal treatment with a single, 20 mg/kg dose of A-192411 resulted 100% survival (Fig. 2) which was confirmed with two additional trials. With a single dose of 1.25 mg/kg, A-192411, the time to death of treated mice was prolonged compared with untreated controls, although no mice survived to day 10. All 10 mice treated with A-192411 at 20 mg/kg had no detectable yeast in kidney homogenates at the termination of the trial. Amphotericin B, administered 5 and 0.31 mg/kg, resulted

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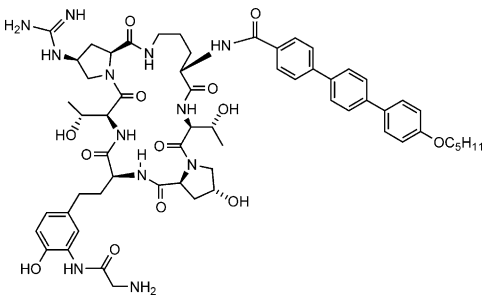

Figure 1.

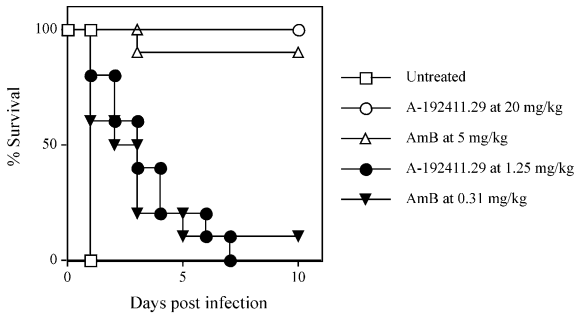

Figure 2. Survival in the acute candidiasis mouse model.[3]

in survival patterns similar to A-192411 yet did not result in the same level of curative efficacy at the 5 mg/kg dose as seen for A-192411 at 20 mg/kg.

A-192411 was also evaluated against *C. albicans* CCH442 in a chronic systemic infection model, in which 100% mortality in the untreated control group occurred about day 8 after infection.[4] A 10-day dosing regimen of A-192411 administered intraperitoneally at 20 mg/kg/day was equivalent to amphotericin B administered at 5 mg/kg/day. The treatments resulted in 100% survival and 80% cure for A-192411 and 100% survival and 100% cure for amphotericin B (Fig. 3). Intraperitoneal administration of fluconazole at 20 mg/kg/day prolonged the survival of infected mice, although none survived to day 35. As found in the acute systemic infection model, A-192411 administered at 1.25 mg/kg/day delayed the death of infected animals, but no mice survived to day 35. Amphotericin B administered at 0.31 mg/kg/day resulted in 40% survival of infected mice.

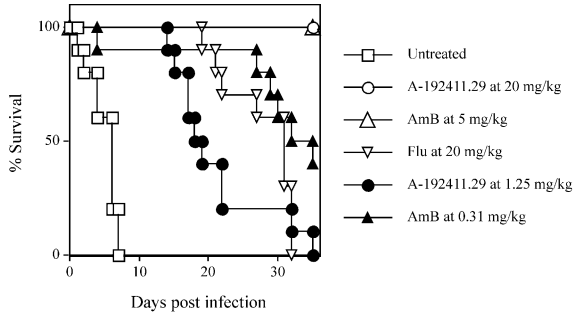

Figure 3. Survival in the chronic candidiasis mouse model.

A-192411 was also evaluated against *C. albicans* CCH442 in a rat model of experimental pyelonephritis.[5] Intravenous therapy with A-192411 and amphotericin B were equally effective. The calculated ED_{50}s to reduce the number of viable yeasts in the kidney by 3-$\log_{10}$ were 1.1 and 0.9 mg/kg/day for A-192411 and amphotericin B, respectively. Oral fluconazole required a 6.5 mg/kg/day dose to achieve efficacy equivalent to A-192411 and amphotericin B.

A-192411 was evaluated in a model of systemic infection caused by *A. fumigatus* 13073 in immunocompromised mice.[6] In untreated mice, the mean survival time (MST) is 7.9 days post inoculation. A 5 day course of therapy with A-192411 at 20 mg/kg administered intraperitoneally resulted in a 1.7-fold increase in the MST to 13.3 days as compared to untreated controls and 30% survival at day 21. Therapy with amphotericin B was more effective on a dose basis. For amphotericin B, 5 mg/kg/day administered intraperitoneally produced a 2.1-fold increase in the mean survival time to 16.8 days, with a 20% survival rate (Figs. 4 and 5).

In summary, A-192411 has potent in vitro antifungal activity against *Candida* species, including *C. albicans*, that is comparable to amphotericin B.[2] The activity seen in vitro correlated with effective treatment of *C. albicans* infections in rodent models. Efficacy was demonstrated as both prolonged survival of systemically infected mice and clearance of viable yeasts from

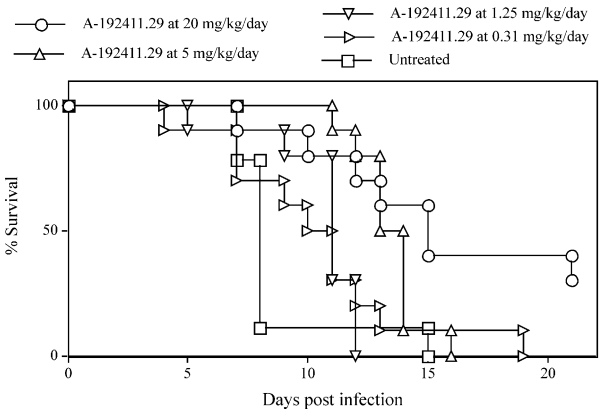

Figure 4. Survival in the murine systemic aspergillosis model.

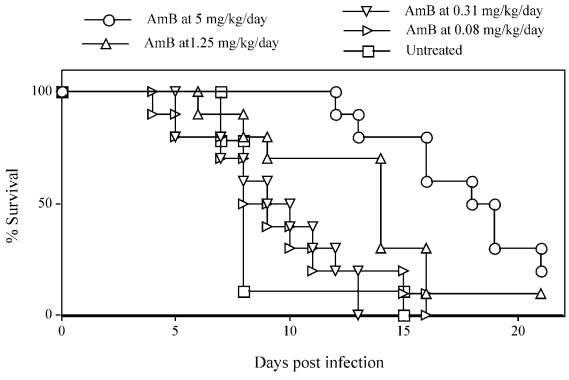

Figure 5. Survival in the murine systemic aspergillosis model.

kidneys. The efficacy of A-192441 administered intraperitoneally was equivalent to amphotercin B at a 4-fold lower dose by weight in the systemic candidiasis models in mice, while the efficacy of A-192441 administered intravenously was equivalent to amphotercin B by weight in the *Candida* pyelonephritis model in rats. A-192411 slightly prolonged the survival of immunocompromised mice infected with *A. fumigatus*, despite the fact that activity against *A. fumigatus* was not detected in vitro.[2]

References and Notes

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3. Mice were inoculated intravenously via the lateral tail vein with an undiluted sample (1.6×10^7 cfu/mouse) of a 24-h culture of *C. albicans* CAF2 grown in Sabauroud broth. Compounds were administered by intraperitoneal injection as a single dose at 1 h post infection using 10 mice per treatment group. Mice were monitored daily for survival for 11 days. Survival results are reported as the percentage of mice still alive on day 11. Cure of infection was determined by plating kidney homogenates from surviving mice on Sabouraud agar; cure results are reported as the percentage of mice with no viable yeast recovered from the homogenates. The MICs for *C. albicans* CAF2 were 0.25 and 0.5 μg/mL for A-192411 and amphotericin B, respectively.

4. Mice were inoculated intravenously via the lateral tail vein with a 1:10 dilution (9.0×10^5 cfu/mouse) of a 24-h culture of *C. albicans* CCH442 grown in Sabauroud broth. Compounds were administered by intraperitoneal injection, beginning at 5 h post infection, then continuing once daily on days 1–9. Mice were monitored for mortality until day 35 post infection. Survival results are reported as the percentage of mice still alive on day 35. Cure of infection was determined by plating kidney homogenates from surviving mice on Sabouraud agar; cure results are reported as the percentage of mice with no viable yeast recovered from the homogenates. The MICs for *C. albicans* CCH442 were 0.5 and 1 μg/mL for A-192411 and amphotericin B, respectively.

5. Rats were inoculated intravenously via the lateral tail vein with a 1:20 dilution (1.6×10^6 cfu/rat) of a 24-h culture of C. *albicans* CCH442 grown in Sabauroud broth. A-192411 and amphotericin B were administered by intravenous injection and fluconazole was administered by oral gavage beginning at 5 h post inoculation, then continuing once daily on days 1 and 2 post infection. On day 3 post infection, kidney homogenates were plated on Sabauroud agar. The dose required to reduce the number of viable yeasts in the kidney by 3 $\log_{10}$ for 50% of the rats in comparison with untreated control animals was calculated by linear regression analysis. The MICs for *C. albicans* CCH442 were 0.5, 1, and 0.19 μg/mL for A-192411, amphotericin B, and fluconazole, respectively.

6. C3H/HeN mice were immunosuppressed by providing dexamethasone in their drinking water at 4.6 mg/L beginning 4 days prior to infection and continuing throughout the trial. Mice were inoculated by intravenous injection with *A. fumigatus* 13073 conidia at 1×10^4 cells/mouse. Therapy by intraperitoneal administration was initiated within 15–30 min after infection, then continued once daily on days 1–4. Mice were monitored for mortality until day 21 post infection. Survival results are reported as the percentage of mice still alive on day 21. Mean survival time was calculated as the mean time to death in days, excluding surviving mice from this calculation. The MICs for A. fumigatus 13074 were >100 and 0.78 μg/mL for A-192411 and amphotericin B, respectively.