EFFICACY AND PHARMACOKINETICS-PHARMACODYNAMICS OF ATI-2307 IN A RABBIT MODEL OF CRYPTOCOCCAL MENINGOENCEPHALITIS

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INTRODUCTION

ATI-2307 is a new aryl amidine compound with broad anti-fungal efficacy in multiple animal models. The mechanism of action, similar to pentamidine, inhibits mitochondrial function. The receptor required for transport into cells is present in fungal cells but not in mammalian cells. We tested the efficacy of ATI-2307 in a rabbit model of cryptococcal meningitis (CM). We performed dose response, clearance kinetic, and pharmacokinetic studies. We found strong efficacy in cerebrospinal fluid (CSF), brain tissue, a rapid rate of fungal clearance from the CSF, and evidence for drug accumulation in the meninges.

METHODS

All experiments were performed in compliance with the Duke University IACUC. Male New Zealand White rabbits, 2.3 – 2.8 kg, were ordered from RSI (Winston-Salem, NC). One day prior to infection rabbits started immune suppression with hydrocortisone acetate (5 mg/kg, IM), and received daily injections throughout the study. CSF was collected on day 2 post infection to quantify the baseline fungal burden. Drug therapy began on day 2 post infection. ATI-2307 was administered SC. CSF was collected on days 4, 7, and 10 post infection, and then the rabbits were euthanized. A subset of rabbits were euthanized on day 4 post infection to quantify ATI-2307 24 hour kinetics in the CSF and brain tissue. Fungal burden and drug levels were quantified in the CSF and brain tissue, and only drug levels in the meninges. Drug levels were obtained by LCMS at a third-party laboratory (QPS, Newark, DE). A PK/PD model is currently being developed. Figure construction and statistical analyses were performed using R, version 4.2.1.

AUTHOR AFFILIATIONS

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FIGURE 1 – CSF & BRAIN TISSUE FUNGAL BURDEN

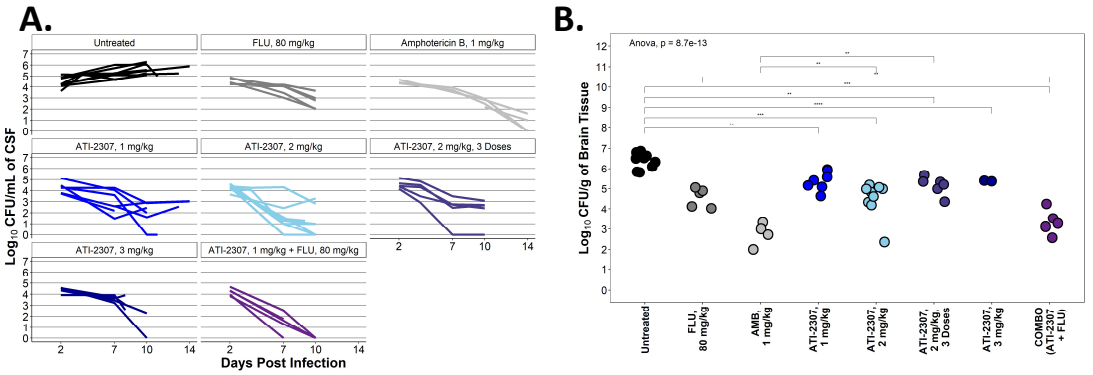

FIGURE 1. (A) Fungal burden in rabbit CSF. Each line represents data from a single rabbit. (B) Fungal burden in rabbit brain tissue. Each dot represents data from a single rabbit. Note, some experiments were carried out to 14 days post infection. Data are pooled from three independent experiments. Pairwise comparisons were made using T-tests with Holm's adjustment. Adjusted P value *P < 0.05, **P < 0.01, ***P < 0.001.

FIGURE 2 – CSF & MENINGES DRUG LEVELS

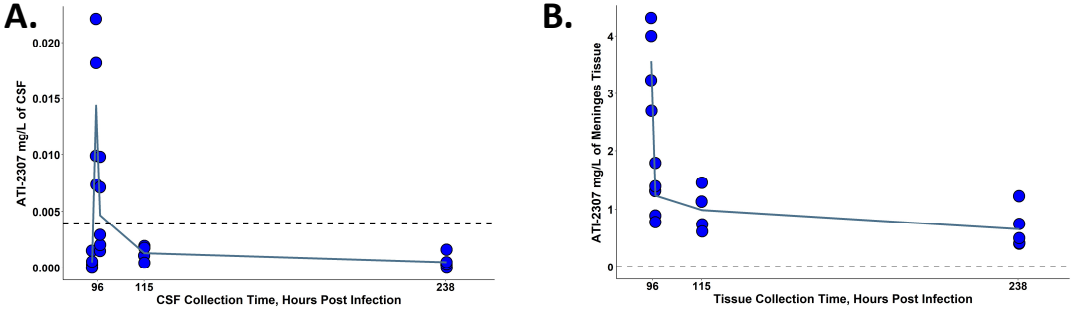

FIGURE 2. (A) ATI-2307 drug levels in CSF after three doses (2 mg/kg/day). Infection was time 0, and dose 1 was at approximately 48 hours post infection. Trough levels, approximately 93 hours, and time approximately 240 hours, were collected from the same rabbits, whereas all other points are from a single rabbit and CSF collection. The solid line is the mean value. (B) ATI-2307 levels in meninges tissue. Each point represents data from a single rabbit. The solid line is the mean value. The black dotted line in both plots is the MIC50 for H99 (0.004 µg/mL).

Summary Data: EFA and Tissue Drug Levels

Treatment	EFA: Change in Log_{10} CFU/mL/Day (95% CI)*	Concentration of ATI-2307, mg/L (95% CI)^		
		Meninges	Brainstem/ Cerebellum	Cerebrum
Untreated	0.13 (0.04 - 0.21)	ND	NA	NA
FLU, 80 mg/kg	-0.22 (-0.34 - -0.1)	ND	NA	NA
Amphotericin B	-0.32 (-0.43 - -0.21)	ND	NA	NA
ATI-2307, 1 mg/kg	-0.25 (-0.35 - -0.15)	ND	0.69 (0.56 – 0.83)	0.59 (0.51 – 0.67)
ATI-2307, 2 mg/kg	-0.43 (-0.52 - -0.34)	4.35 (3.99 – 3.72)	1.03 (0.85 – 1.21)	0.90 (0.78 – 1.01)
ATI-2307, 2 mg/kg, 3 Doses	-0.33 (-0.44 - -0.21)	0.66 (0.24 – 1.08)	0.26 (0.19 – 0.34)	0.26 (0.20 – 0.32)
ATI-2307, 3 mg/kg	-0.27 (-0.4 - -0.14)	ND	1.33 (-1.85 – 4.51)	1.14 (-1.77 – 4.05)
COMBO(ATI-2307, 1 mg/kg + FLU, 80 mg/kg)	-0.56 (-0.69 - -0.43)	ND	0.44 (0.28 – 0.60)	0.43 (0.28 – 0.59)

**EFA calculated using linear mixed effects model (R, lme4 1.1-30) Log10 CFU/mL ~ Day post Infection * Treatment + (0+Day|RabbitID), ^95% CI calculated using T distribution, ND = not done, tissue not collected, NA = Not applicable, these drug levels were not measured*

RESULTS

- ATI-2307 had antifungal activity which was equivalent to FLU and AMB in the CSF
- ATI-2307 had antifungal activity in the brain but was less than AMB
- ATI-2307 combined with FLU had antifungal activity which was superior to AMB in the CSF
- ATI-2307 combined with FLU had equivalent activity to AMB in the brain tissue
- Of the three doses tested, ATI-2307 at 2 mg/kg/day resulted in highest activity with fewest complications
- ATI-2307 given at 2 mg/kg for 3 doses and 9 doses had similar reductions in fungal burden in the CSF
- ATI-2307 concentrations were much higher in the meninges than in the brain tissue
- ATI-2307 levels in the meninges persisted at high levels 6 days after the last dose
- Effective fungicidal activity (EFA) of ATI-2307 at 2 mg/kg/day for only 3 doses or 2 mg/kg/day for 9 doses was equivalent to AMB

CONCLUSIONS

ATI-2307 has a CNS distribution pattern which is similar to amphotericin B, with high accumulation in the meninges which persist even after the dosing is completed. Also similar to amphotericin B, a short course of therapy reduced fungal burden. This makes it an attractive candidate for induction therapy. It's positive interaction with fluconazole and its novel target make it a good candidate for future clinical studies. With its prolonged antifungal activity, and an EFA which is similar to AMB, this drug might be used in a short therapeutic course of treatment and might not require long-term dosing.

FUNDING & DISCLOSURES

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