

ANTI-MALARIAL ACTIVITY OF CEM-101, A FLUOROKETOLIDE ANTIMICROBIAL, IN BOTH BLOOD STAGE AND PRESUMPTIVE CAUSAL PROPHYLACTIC MOUSE MODELS

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ABSTRACT

Background: CEM-101 is a new broad spectrum macrolide that has completed Phase 1 trials that inhibits protein synthesis through binding to bacterial ribosomal RNA. A comparator drug, azithromycin, causes a delayed death effect *in vitro*. *P. falciparum* blood stage assays and demonstrates antimalarial activity against liver stage parasites. CEM-101 was recently shown to be active *in vitro* against *P. falciparum* in extended incubation assays which measure the potency of inhibitors that demonstrate delayed death effects. CEM-101 is also active against blood stages in *P. berghei*-Infected mice.

Method: Dose-response for CEM-101 was characterized in both blood stage treatment and causal prophylactic *P. berghei*-infected mice models using 3 day PO or SC dosing. Efficacy was measured by number of mice with delayed parasitemia and mice that were malaria free at day 31. Antimalarial liver stage activity was assessed in mice infected with Luciferase expressing *P. berghei* parasites using an *in vivo* imaging system.

Results: For blood stage infections, the minimum curative SC dose was 40 mg/kg/d X 3 days, while 80 mg/kg/d X 3 was the minimum active dose for PO route. In the *P* berghei causal prophylactic mouse model, CEM-101 was curative at 40 mg/kg/d X 3 days with SC or PO dosing. No systemic toxicity was observed with SC or PO dosing as high as 160 mg/kg/d X 3 days. No demonstrable antimalarial activity against liver stage parasites was observed by in vivo imaging analysis of fuiderase-expressing *P* berghei with PO dosing at 40 mg/kg/d X 3 days. While drug activity against liver stage parasites could not be measured by *in vivo* imaging, no blood stage infection was detected in mice dosed as low as 40 mg/kg/d X 3 days and the minimum active dose was 20 mg/kg/d X 3 days.

Conclusion: CEM-101 shows 100% prophylactic activity in causal mouse malaria models with PO dosing at 40 mg/kg/d X 3 days and 3/5 mice remain parasite free at 20 mg/kg/d X 3 days. The *in vivo* imagining analysis of liver stage parasites suggests that CEM-101 does not affect parasite growth at 40 mg/kg/d X 3 days. Based on *in vitro* blood stage drug assays and the mechanism of inhibition of this class of compounds, the lack of demonstrable liver stage activity suggest that CEM-101, like azithromycin, demonstrate a delayed death effect; that is, developing liver stage mercociles are effectively non-viable blood stage parasites.

BACKGROUND

CEM-101 (solithromycin)



·New macrolide antibiotic of the fluoroketolide subclass under development by Cempra Pharmaceuticals, Inc

-Acts to inhibit protein synthesis by binding to domain V (like other macrolides) and domain II (like ketolides) with additional interactions with the peptide tunnel of the 23S component of the 50S ribosomal subunil supports the concept that it may be active against *Plasmodum sp*, through targeting apicoplast replication²

·Potent broad spectrum antibacterial drug active against macrolide and lincosamide resistant bacteria

 Comparators, azithromycin and doxycycline, causes delayed death in vitro against P. falciparum and P. berghei liver stage parasites by blocking developing apicoplast during exo-erythocytic schizogony³

 Recently shown to be active against in vitro against P. falciparum in extended incubation assays and against blood stage in P. berghei-infected mice⁴

Clinical Pharmacology¹

hERG channel blocker; normal dog telemetry study
In vitro CYP 3A4 substrate and inhibitor
Absolute oral BA 11-16% rodents; 10-17% monkeys
Non-linear kinetics in all species tested
Phase I single escatating dose and multiple dose studies

•Non-linear kinetics with C_{out} and AUC increases non-proportional at doses 50-400 mg and approx. linear from 400-1200mg -T_{ima} also increased from 1.5 h to 1sh a dose increased -Terminal elimination half-life varied from 2.2 h to 7.9h -Multiple dose skuly showed onch-linear increases C_{out} and AUC for lower doses and accumulation ratios of 2-3

•5-6 mg/kg for 7 days in humans equivalent exposure to 100 mg/kg/d for 7 days in monkeys

METHODS

P. berghei-infected Mice for Blood Stage Activity and for Causal Prophylaxis

•Test Facility: Vet Med, AFRIMS Thailand

•Test Systems: Plasmodium berghei-Anopheles dirus Sporozoites-ICR Mice for Screening Excerythrocytic and Blood Stage Antimalarial Drugs

•Animals: 45 female mice, 9 groups of 5 mice

 Day 0 Inoculate for Causal Prophylaxis Screen: 0.1ml IV, 100,000 P. berghei ANKA sporozoites from mosquitoes fed on donor mice, PBS 5% BSA

-Day 0 Inoculate for Blood Stage Activity: 0.1ml, 1 x 10⁸ *P. berghei* P line infected red blood cells -Blood smears q3 day through Day 31 post-inoculation followed by necropsy

Group #	Drug	Dose (mg/kg/day)	#Days	Route	Days Dosing	Vehicle
Vehicle	None	0	3	SC and PO	-1,0,1 (CP) 3,4,5 (BS)	HECT
Test Group	25					

Luciferase expressing P. berghei parasites in vivo imaging system⁵

•Test Facility: WRAIR, Experimental Therapeutics

Test System: Luciferase expressing Plasmodium berghei-Anopheles stephensi Sporozoites-C57BL/6 Male Mice
Day 0 Inoculate: 0.1ml IV, 5 x 10⁴ luciferase expressing P. berghei sporozoites

•Three day oral dosing schedule

 In vivo luciferase activity measured by IVIS at 24, 48, and 72 hours post inoculation to assess liver and blood stage infection

Luciferin 150mg/kg IP administered 3 minutes prior to isoflorane anesthesia and imaging
Positive and negative controls used for IVIS calibration each experiment

•Flow cytometry and microscopy used to assess for blood stage infection out to 30d

RESULTS

P. berghei-infected Mice for Blood Stage Activity

P. berghei-infected Mice for Causal Prophylaxis

DRUG	Dose mg/k g	No.	Route	Vehicle	Study days of dosing	No. Cured	No. Suppr essed
Vehicle	0	0	PO	0	3, 4, 5	0	0
	40	5	PO	5	3, 4, 5	0	5
	80	5	PO	5	3, 4, 5	3	2
	120	5	PO	5	3, 4, 5	3	2
CEM 101	160	5	PO	5	3, 4, 5	5	•
	40	5	SC	5	3, 4, 5	5	
	80	5	SC	5	3, 4, 5	5	
	120	5	SC	5	3, 4, 5	5	•
	160	5	SC	5	3, 4, 5	5	•
Vehicle	0	0	SC	0	3, 4, 5	0	0

DRUG	Dose mg/k g	No	Route	Vehicle	Study days of dosing	No. Protecte d	No. delay onset
Vehicle	0	0	PO	0	-1, 0,1	0	0
	40	5	PO	5	-1, 0,1	5	-
	80	5	PO	5	-1, 0,1	5	-
	120	5	PO	5	-1, 0,1	5	-
CEM 101	160	5	PO	5	-1, 0,1	5	
	40	5	SC	5	-1, 0,1	5	
	80	5	SC	5	-1, 0,1	5	-
	120	5	SC	5	-1, 0,1	5	-
	160	5	SC	5	-1, 0,1	5	-
Vehicle	0	0	SC	0	-1, 0,1	0	0

CEM 101 vs. Azithomycin Dose-Response against P. berghei-infected Mice

DRUG*	CEI	M 101	Azithro		CEM 101		Azithro		
Model		Blood	Stage		Causal Prophylactic				
Route _{days}	SC3	PO3	SC3	PO3	SC3	PO3	SC3	PO3	
MCD	< 40	>40<80	10	20	< 40	< 40	~20	~64	
ED25**	< 40	>40<80	10	<20	< 40	< 40	~20	>40<64	
ED _{50**}	< 40	>40<80	<20	20	< 40	< 40	~40	83	
ED _{99**}	< 40	>80 <160	>20<80	80	< 40	< 40	~160	160	

*Doses mg/kg per day for three day dosing

ND= no data ED 25.00- E Stimated dose required to prevent malaria in 25-90% test animals MCD=Minimal curative dose seen in actual testing

RESULTS

IVIS for P. berghei-infected Mice for CEM 101, doxycycline, and azithromycin







CONCLUSIONS

•CEM 101 is more active than azithromycin by both the SC and the PO route in preventing malaria in mice but is not definitively causal

CEM 101 has blood stage activity against mice malaria

·Azithromycin is more active than CEM 101 by the oral route against blood stages of mouse malaria

 Absolute bioavailability between CEM 101 and azithromycin (15% vs 46%) in rodents may explain the differences in oral activity

 Further dose-ranging in both blood and causal prophylaxis models will be necessary to elucidate clear dose-response relationships

•CEM 101 was well tolerated systemically in the mice with self-limited skin ulcers seen in the SC injection sites

•The *in vivo* imagining analysis of liver stage parasites suggests that CEM-101 does not demonstrate liver stage activity at 40 mg/kg/d X 3 days despite preventing malaria in 5/5 mice

•This lack of demonstrable activity is most likely explained by insufficient dosing and short liver stage period

•These results suggest that CEM-101, like azithromycin and doxycycline ,demonstrate a delayed death effect; that is, developing liver stage merozoites are effectively non-viable blood stage parasites

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