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

Susan Fracisco1, Montip Gettayacarin2, Anchalee Tungtaeng2, Michael Kozar1, Michael O’Neil1, Carl Craft3, Prabha Fernandes3
1 WRAIR Experimental Therapeutics, Silver Spring, MD, United States, 2 Armed Forces Research Institute of the Medical Sciences, Bangkok, Thailand, 3 Cempra Pharmaceuticals, Inc, Chapel Hill, NC, United States

ABSTRACT

Background: CEM-101 is a new broad spectrum macrolide that has completed Phase 1 trials that inhibit protein synthesis by 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 vivo 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. Efficiency was measured by number of mice with delayed parasitaemia 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 X 3 days, while 80 mg/kg 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 X 3 days with SC or PO dosing. No systemic toxicity was observed with SC or PO dosing as high as 160 mg/kg X 3 days. No demonstrable antimalarial activity against liver stage parasites was observed by in vivo imaging analysis of luciferase-expressing P. berghei with PO dosing at 40 mg/kg 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 X 3 days and the minimum active dose was 20 mg/kg X 5 days.

Conclusion: CEM-101 shows 100% prophylactic activity in causal mouse malaria models with PO dosing at 40 mg/kg X 3 days and 80 mg/kg X 3 days remain parasite free at 20 mg/kg X 3 days. The in vivo imaging analysis of liver stage parasites suggests that CEM-101 does not affect parasites growth at 40 mg/kg X 3 days. Based on these data, drug activity assays and the measurement of inhibitory activity of CEM-101 in vitro, the demonstrable liver stage activity suggest that CEM-101, like azithromycin, demonstra a delayed death effect, that is, developing liver stage merozoites are effectively non-viable blood stage parasites.

BACKGROUND

CEM-101 (solithromycin)1

- 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 subunit, supporting the concept that it may be active against Plasmodium ap. through targeting apicoplast replication2
- Potent broad spectrum antibacterial drug active against macrolide and tetracycline resistant bacteria
- Comparisons, azithromycin and doxycycline, causes delayed death in vivo against P. falciparum and P. berghei liver stage parasites by blocking developing apicoplast during exo-erythocytic schizogony3
- Recently shown to be active in vivo against P. falciparum in extended incubation assays and against blood stage in P. berghei-infected mice
- Clinical Pharmacology4

- T31/4 channel blocker; normal dog telemetry study in vivo; CYP 3A4 substrate and inhibitor
- Single and multiple dose PK studies in dogs, rats and monkeys
- Non-linear kinetics in all species tested
- Phase I single escalating dose and multiple dose studies

- Non-linear kinetics with Cmax increased non-proportionally at doses ≥50 mg/kg and approx. dose from 60-120 mg
- Cmax also increased from 7.6 to 36 mg/kg as dose increased
- Cmax/clofibrate halved dose varied from 2.6 to 1.4
- AUC increased from 316 to 1170 mg/min with food
- AUC increased from 624 to 1320 mg/min for liver dose and accumulation ratios of 2-3
- 8-9 mg/kg for 7 days humans equivalent exposure to 100 mg/kg for 7 days in monkeys

METHODS

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

- Test Facility: Vet Med, ARFRMS Thailand
- Test Systems: Plasmodium berghei-etoschistosoma IR mouse for Screening Erythrocytic and Blood Stage Antimalarial Drugs
- Animals: 40 female mice, 5 groups of 5 mice
- Day 0 inoculate for Causal Prophylaxis: 0.1 ml IV, 100,000 P. berghei ANKA sporozoites from mosquitoes fed on donor mice; PBS 5x, RSA
- Day 0 inoculate for Blood Stage Activity: 0.1 ml, 1 x 10^6 P. berghei infected red blood cells
- Blood smears p3 day through Day 31 post-inoculation followed by necropsy

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 did not show in vivo efficacy
- 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-limit skin ulcers seen in the SC injection of azithromycin
- The in vivo imaging analysis of liver stage parasites suggests that CEM-101 does not demonstrate liver stage activity at 40 mg/kg X 3 days despite preventing malaria in 55 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

REFERENCES


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