Poster 269

Solithromycin (CEM-101) - A New Fluoroketolide with Antimalarial Activity

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Abstract

Solithromycin (CEM-101) is a new fluoroketolide antibiotic under clinical development for the treatment of community-acquired bacterial respiratory tract infections. In this study, we evaluated its in vitro and in vivo activity against different species of *Plasmodium* parasites to see if there is a potential for use in the treatment of malaria.

Solithromycin was tested by the semi-automated microdilution assay against intra-erythrocytic forms of *P. falciparum* derived from asynchronous cultures of the strain NF54, essentially as described (Matile 1990). Parasite growth over 120h was measured by the incorporation of radiolabelled [^{3}H] hypoxanthine (in hypoxanthine-free culture medium) added after 96h of drug incubation and 24h prior to the termination of the test. Because of its slow mode of action, solithromycin was followed for 120 hrs vs. the usual 72h assay. The test was run 3 times, and at lower concentrations, because it was more active than expected. In vitro *P. falciparum* data for solithromycin in the 120h assay (96h + 24h) showed an IC_{50} against NF54 of 2.4 compared to artesunate with an IC_{50} of 3.3, clindamycin 5.3 and chloroquine 4.7 ng/ml.

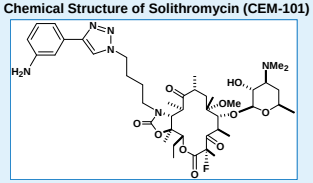
Based on its promising in vitro activity, solithromycin was tested in the murine *P. berghei* model as described (Vennerstrom 2004). Solithromycin was first studied after a single dose of 100 mg/kg either in DMSO or HPMV. In the second experiment, solithromycin was given at a dose of 100 mg/kg daily for 4 days in both vehicles. Following the single dose, solithromycin showed antiparasitic activity of 80.05% and 81.45% and mouse survival in days was 15.2 and 12.7 days, respectively. Following daily doses of 100 mg/kg for 4 days solithromycin showed antiparasitic activity of 99.79% in both vehicles. The mice survived for 30 days in both experiments and thus solithromycin is considered to be fully curative in this model.

Solithromycin has been shown to have excellent in vitro and in vivo activity against *Plasmodium* species. This data would support future studies to determine solithromycin's potential for the treatment of blood stage malaria in combination with a fast-acting antimalarial. It may also have additional benefits because of its activity as an antibiotic.

Introduction

- New drugs are needed for treating malaria because of limitations of the existing drugs and signals that the malaria parasite is becoming resistant. Because of this, new agents with a different mechanism of action are needed.
- Solithromycin (CEM-101) belongs to the known class of macrolides, which includes azithromycin (AZ) which is a broad-spectrum antibacterial that inhibits protein synthesis, and also manifests reasonable efficacy as an antimalarial. Macrolides have had broad therapeutic applications in infectious diseases and have a history of safety and efficacy.
- Solithromycin has been tested in microbiology, pharmacology, and toxicology studies. It has progressed through formal pre-clinical regulatory studies, advanced into Phase I clinical studies during 2008, and is presently in a phase II community-acquired bacterial pneumonia study. It is available in both oral and intravenous formulations. Here we describe solithromycin as a potential antimalarial drug development candidate based on in vitro and in vivo studies.

Chemical Structure of Solithromycin (CEM-101)



Materials and Methods

In vitro Antimalarial Activity

Compounds were tested against intra-erythrocytic forms of *P. falciparum* derived from asynchronous stock cultures of the strain NF54 (airport strain of unknown origin), essentially as described (Desjardins 1979, Matile 1990). The culture medium was a modification of that previously described (Dorn 1995, Trager 1976Human erythrocytes served as host cells. The cultures were kept at 37^{0}C in an atmosphere of 3% O_2, 4% CO_2 and 93% N_2 in humidified modular chambers. Drug testing was carried out in 96-well microtiter plates. The compounds were dissolved prediluted in hypoxanthine-free culture medium and tested in duplicate over a 64-fold range. After addition of an equal volume of parasite culture with an initial parasitemia of 0.3% in a 5% erythrocyte suspension, the test plates were incubated under the conditions described above for 72 hours (classical approach) or 120 hours.

Materials and Methods

Parasite growth was measured by the incorporation of radiolabelled [^{3}H] hypoxanthine (0.5 µCi in a volume of 50 µL hypoxanthine-free culture medium) added 24 hours prior to the termination of the test.

Cultures were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted the results recorded as counts per minute (cpm) per well at each drug concentration and expressed as a percentage of the untreated controls. Fifty percent inhibitory concentrations (IC_{50}) were estimated by linear interpolation (Huber 1993).

In vivo Antimalarial Efficacy Studies

The animal experiments described here were carried out at the Swiss Tropical Institute (Basel, Switzerland), adhering to local and national regulations of laboratory animal welfare in Switzerland. Compounds were tested in the murine *P. berghei* model essentially as previously described (Vennerstrom 2004, Ridley 1997, Peters 1987).

Method of Infection and Treatment of Animals

In vivo antimalarial activity was assessed for groups of three to five female NMRI mice (20–22 g) intravenously infected on day 0 with *P. berghei* strain ANKA (donation from AP Waters and CJ Janse, Leiden University). From donor mice with approximately 30% parasitemia, heparinized blood was taken and diluted in physiological saline to 10^8 parasitized erythrocytes/mL. An aliquot (0.2 mL) of this suspension was injected intravenously into experimental and control groups of mice. In control mice, parasitemia typically rose to 30% by day 3 after infection, and control mice died between day 6 and day 7 after infection. In the described experiments, however, control animals (n=5) were sacrificed on Day 4 post-infection.

Administration of Compounds

Compounds were administered orally to groups of 3 mice either as a single dose (24 hours post-infection) or as 4 doses (3, 24, 48, and 72 hours post-infection).

Results

Results in vitro

In the in vitro tests, activity was assessed in both the 72 hour assay (**Table 1**) and the 120 hour assay (**Table 2**). The results of the studies indicate that solithromycin seems to be slow acting, because the IC_{50} value in the 120 hour assay is much lower compared to the value in the "classical" 72 hour assay. The parasites in the 120 hour assay, but not the 72 hour assay, seem to grow again up to about 10% growth (data not shown). The 120 hour assay 50 ng/mL were used as a starting concentration, whereas in the 72 hour assay 10,000 ng/mL was used as the highest concentration.

Table 1 - In vitro *P. falciparum* Data for Solithromycin in the 72 h Assay (48 + 24)

Drug	IC_{50} NF54			
	Test 1	Test 2	Test 3	Test 4
Solithromycin (CEM-101)	919*	1536*	1487*	1258*
Chloroquine	4.4	5.4	4.4	4.8
Artesunate	3.0	3.2	3.0	2.8

* Not active at 72 hours

Table 2 - In vitro *P. falciparum* Data for Solithromycin in the 120 h Assay (96 + 24)

Drug	IC_{50} NF54		
	Test 1	Test 2	Test 3
Solithromycin (CEM-101)	<156[1]	<15[1]	**2.4**
Clindamycin	<7.8	5.3	5.3
Chloroquine	4.6	4.6	4.7
Artesunate	4.2	3.9	3.3

[1] Initial concentration tested was too high

Evaluation of the Results in vivo

With the single-dose regimen, the degree of infection (parasitemia expressed in % of infected erythrocytes) was determined by FACS analysis on Day 3 (72 hours post-infection). The blood samples from the quadruple-dose regimens were collected and analyzed by FACS on Day 4. The difference of the mean infection rate of the control group (= 100%) to the test group was calculated and expressed as percent reduction. As an example, activity determination with a mean of e.g., 2% parasitemia in treated mice and a mean of e.g., 40% parasitemia in the control animals is calculated as follows: (40%-2%)/40% *100= 95% activity. The survival time in days was recorded up to 30 days after infection. A compound was considered curative if the animal survived to Day 30 after infection with no detectable parasites (confirmed by light microscopy).

Results in vivo

Solithromycin (CEM-101) was tested in vivo against *P. berghei*. Control mice were sacrificed on Day 4 following infection with *P. berghei*. Treatment of mice with 1×100 mg/kg of solithromycin, clindamycin, and azithromycin resulted in survival to 7.0 days for clindamycin, 14.0 days for azithromycin and 13-16 days for solithromycin (**Table 3**).

Results

Solithromycin, clindamycin and azithromycin treatment at 4×100 mg/kg resulted in extended survival for clindamycin to 18-20 days Azithromycin extended survival to 24-30 days but was not considered curative as parasites were still present by microscopy (**Table 5**). Solithromycin was curative with both 30 day survival and negative microscope parasites, since a compound is considered curative if the animal survives up to Day 30 after infection with no detectable parasites by microscopy (**Tables 4 and 5**).

Table 3 - In vivo Antimalarial Activity of Solithromycin (CEM-101), Azithromycin, and Clindamycin (1x100 mg/kg)

Cages	Substances	Dosage mg/kg: 1x	Route	Visual observation	Parasitized RBC over 100				Avg.	% of control	% Activity	Mouse survival in days				Avg.
1	Clindamycin	100	DSMO	p.o	pos.	8.55	8.44	8.78		8.26	29.21	78.79	7	7	7	7.0
2	Clindamycin	100	HPMC	p.o	pos.	8.78	8.82	8.75		8.15	20.94	79.06	7	8	7	7.3
3	CEM-101	100	DSMO	p.o	pos.	7.71	8.88	8.72		7.76	19.95	80.05	14	20	13	15.7
4	CEM-101	100	HPMC	p.o	pos.	8.36	7.88	8.18		7.27	18.55	81.45	10	14	14	12.7
5	Azithromycin	100	DSMO	p.o	pos.	12.48	9.87	12.21		11.52	29.61	70.39	14	14	14	14
6	Azithromycin	100	HPMC	p.o	pos.	13.2	12.9	14.54		13.55	34.81	65.19	14	14	14	14
Co	Control Day 3				pos.	34.13	38.72	41.71	37.5	42.84	38.82					

Table 4 - In vivo Antimalarial Activity of Solithromycin (CEM-101), Azithromycin, and Clindamycin (4x100 mg/kg)

Cages	Substances	Dosage mg/kg: 4x	Route	Visual observation	Parasitized RBC over 100				Avg.	% of control	% Activity	Mouse survival in days				Avg.
7	Clindamycin	100	DSMO	p.o	pos.	0.22	0.24	0.17		0.21	0.25	99.75	15	20	20	18.3
8	Clindamycin	100	HPMC	p.o	pos.	0.19	0.29	0.54		0.34	0.41	99.59	20	15	16	20
9	CEM-101	100	DSMO	p.o	pos.	0.15	0.19	0.26		0.17	0.21	99.79	30	30	30	30
10	CEM-101	100	HPMC	p.o	pos.	0.18	0.2	0.14		0.17	0.21	99.79	30	30	30	30
11	Azithromycin	100	DSMO	p.o	pos.	0.87	0.6	0.72		0.73	0.89	99.11	30	30	30	30
12	Azithromycin	100	HPMC	p.o	pos.	0.6	0.79	0.76		0.72	0.87	99.13	17	30	25	24
Co	Control Day 4				pos.	73.95	83.64	87.29	81.58	83.72	83.44					4.0

Table 5 - Microscopic Evaluation at 30 days of Solithromycin (CEM-101) and Azithromycin (4x100 mg/kg) in vivo Antimalarial Activity

Cages	Substances	Dosage mg/ml: 4X	Route	Visual Observation	Parasitized RBC in one drop of blood		
9	CEM-101	100	DSMO	p.o.	neg	0	0	0
10	Cem-101	100	HPMC	p.o.	neg	0	0	0
					Parasitized RBC over 100 made by FACS		
11	Azithromycin	100	DSMO	p.o.	pos.	34.32	2.2	35.31
12	Azithromycin	100	HPMC	p.o.	pos.	74.04		

Discussion/Conclusions

- Solithromycin (CEM-101) showed delayed killing in vitro with little activity in the 72 hour and 96 hour assay.
- Solithromycin showed better activity than clindamycin, chloroquine, and artesunate in the 120 hour assay.
- Solithromycin dosed daily at 100 mg/kg for four days cured mice of *P. berghei* infection, unlike clindamycin or azithromycin.
- Solithromycin has activity similar to artesunate, which has demonstrated 30 day cures with a 4×100 mg/kg dose in this model.
- Solithromycin is active, in vitro, against multidrug resistant- and azithromycin-resistant strains of *P. falciparum*. (data on file – unpublished)
- Solithromycin is the most active protein synthesis inhibitor tested against *P. falciparum* in vitro and *P. berghei* in vivo.

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