Non-Antifolate Antibiotics: Clindamycin, Doxycycline, Azithromycin and Fosmidomycin

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Abstract A range of antibiotics, in addition to those that target folate metabolism, have demonstrated antimalarial activity. They include those belonging to the lincosamides, tetracyclines and macrolides classes and fosmidomycin, a derivative of phosphonic acid. Predominantly, they target pathways within the apicoplast, a relict plastid found in most apicomplexan parasites including *Plasmodium*. In general, they are not highly active against malarial parasites and are slow acting but are clinically useful when used in combination with other antimalarial drug classes. In addition, some are safe to use in pregnancy and for the treatment of small children. Here, we review the current understanding of their mechanisms of action and clinical use.

1 Introduction

There are many non-antifolate antibiotics that have antimalarial activity including members belonging to the following classes: fluoroquinolones, lincosamides, tetracyclines and macrolides. The fluoroquinolones will not be discussed further here, as they were moderately active against *Plasmodium falciparum* in vitro [1, 2], but this property did not extend to useful antimalarial efficacy when tested in vivo [3, 4]. Fosmidomycin is a phosphonic acid derivative that is also included in discussion, as a potentially interesting new class of antimalarial. The lincosamides, tetracyclines and macrolides have established antimalarial properties and are clinically useful in particular circumstances. None of these antibiotics is highly active and rapidly acting; so, their usage requires combination with other rapidly effective classes of antimalarial, where they contribute to increasing cure rates even with

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shorter courses of treatment. Some antibiotics have the additional advantages of being safe to use in pregnancy or for small children (discussed below).

2 Clindamycin

The lincosamides are named after lincomycin that was extracted from *Streptomyces lincolnensis* in a soil sample in 1962. The structure of lincosamides is unusual, as are their antimicrobial properties. Clindamycin was the only semi-synthetic lincosamide to be developed for clinical use (Fig. 1). Here, it has activity against Gram-positive bacteria and many anaerobes, but not against Gram-negative aerobes. More relevant to its antimalarial properties, clindamycin is active against several apicomplexans including *Plasmodium* spp., *Toxoplasma gondii* and *Babesia microti*.

2.1 Mechanism of Action

Clindamycin acts at the same site on ribosomes as erythromycin and chloramphenicol. It inhibits protein synthesis in bacteria by binding to the 50S ribosomal unit and it can exert concentration-dependent bactericidal activity beyond its accepted bacteriostatic effects [5]. In its actions against apicomplexans, clindamycin targets their apicoplast organelles and interferes with function and survival after one or two rounds of parasite replication have taken place, resulting in a "delayed-death" phenotype. This is well described for *Toxoplasma* [6] as well as for *P. falciparum* with correlations between in vitro and in vivo pharmacodynamics being observed [7–9]. Further evidence for this proposed mechanism of action for clindamycin comes from observations of mutations in the apicoplast genome of parasites obtained from the Peruvian Amazon. A point-mutation in the apicoplast-encoded 23S rRNA gene that confers resistance to lincosamides in other organisms was identified in parasites that were 100-fold more resistant to clindamycin than wild-type parasites [10].

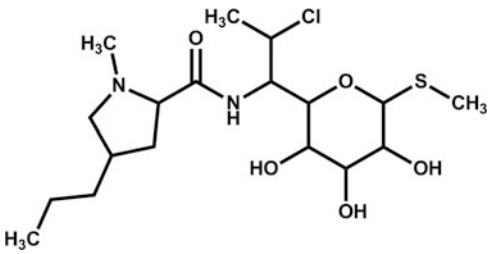

Fig. 1 Structure of clindamycin

2.2 Pharmacokinetics

Clindamycin has good (>90%) bioavailability after oral dosing, is protein bound (>90%) and widely distributed in tissues and has an elimination half-time of approximately 2–3 h. It is eliminated mainly by biliary excretion (with 20% excreted by kidneys) after metabolism to three major derivatives [11].

2.3 Usage in Malaria

The antimalarial properties of clindamycin have been confirmed in many studies, including earlier studies after the first one in 1975 that used clindamycin as a monotherapy before its more successful use in combination treatment regimens [11]. The interesting mechanism of action of clindamycin and its pharmacokinetic properties suggest that a minimum of 5 days treatment with at least twice-daily dosing is needed to achieve adequate cure rates in uncomplicated malaria [11], a regimen that is not currently recommended.

Table 1 summarises the antimalarial properties of clindamycin combined with quinine. Clindamycin dosages ranging from 5 mg/kg twice daily given with quinine, to 8 mg/kg three times a day or 12 mg/kg twice daily in general give acceptable cure rates. One small study [21] found that a 3-day treatment of the combination was as effective as a 7-day monotherapy regimen with quinine alone in travellers returning with uncomplicated malaria. Clindamycin can be used in children and pregnant women and may be combined with artesunate as a partner antimalarial, although short-course regimens may need further study to confirm efficacy [24].

Clindamycin may be less effective in treating *P. vivax* infections [11] even when prolonged courses of monotherapy are used, although a later review suggested that clindamycin is more effective than tetracyclines [25]. It also has no apparent synergism with quinine for *P. vivax* and nor does it have anti-relapse properties [11].

2.4 Tolerance and Safety

Non-specific and relatively common gastro-intestinal side effects of nausea, vomiting, abdominal pain and diarrhoea are associated with clindamycin use. Toxin-producing *Clostridium difficile* colitis can also complicate clindamycin therapy, most commonly in hospitalised patients treated with prolonged courses of clindamycin, and not with the antimalarial regimes that have been most studied in recent years. Rashes may be relatively common side effects, but severe eruptions are not. Transient reversible neutropenia and thrombocytopenia have also occurred.

Table 1 Clinical trials of clindamycin plus quinine against *P. falciparum* malaria

Study details					Regimen							Efficacy (%)	Ref.
Year	Place	Design[a]	Pop.[b]	*N*[c]	Clindamycin		Quinine		Route[d]	Days	Dosing[e]		
					Dosage, form[f]	No. of doses/day	Dosage	No. of doses/day					
1974	United States	WHO	A	5	450 mg, salt	4	540 mg	3	p.o.	3	Yes	100	[12]
1975	United States	WHO	A	5	450 mg, salt	3	560 mg	3	p.o.	3	Yes	60	[13]
1975	United States	WHO	A	2	600 mg, salt	1	560 mg	3	p.o.	3	No	50	[13]
1975	Thailand	WHO	A	4	450 mg, salt	3	540 mg	3	p.o.	3	Yes	100	[14]
1975	Thailand	WHO	A	5	150 mg, salt	3	270 mg	3	p.o.	3	No	60	[14]
1988	Brazil	WHO, RCT	A	40	10 mg/kg, base	2	12 mg/kg	2	p.o.	3	Yes	90	[15]
1994	Gabon	WHO, RCT	C	34	5 mg/kg, base	2	12 mg/kg	2	p.o.	3	Yes	88	[16]
1995	Gabon	WHO, RCT	C[g]	50	5 mg/kg, base	3	8 mg/kg	3	i.v.	4	Yes	96	[17]
1995	Gabon	WHO, RCT	A	40	5 mg/kg, base	2	12 mg/kg	2	p.o.	3[h]	Yes	92	[18]
1997	Gabon	WHO[i]	C	161	8 mg/kg, salt	2	8 mg/kg	2	p.o.	3	Yes	97	[19]
2000	Thailand	WHO, RCT	A	68	5 mg/kg, base	4	8 mg/kg	3	p.o.	7	Yes	100	[20]
2001	France	WHO, RCT	A	53	5 mg/kg, salt	3	8 mg/kg	3	i.v.	3	Yes	100	[21]
2001	Thailand	WHO, RCT	P	65	5 mg/kg, NS[j]	3	8 mg/kg	3	p.o.	7	Yes	100	[22]

[a]WHO, study conducted according to World Health Organisation guidelines [23]; *RCT* randomised controlled trial

[b]Pop., study population; A, adults; C, children; P, pregnant women

[c]*N*, number of subjects

[d]i.v., intravenous; p.o., oral

[e]Adequate dosing (i.e., clindamycin given at least twice daily and more than 3 days)

[f]8 mg of clindamycin hydrochloride salt is equivalent to 5 mg of base

[g]Severe malaria

[h]Quinine was administered for only 1.5 days

[i]Short follow-up (3 weeks)

[j]*NS* not specified. Taken from [11] with kind permission from the the authors and the American Society for Microbiology

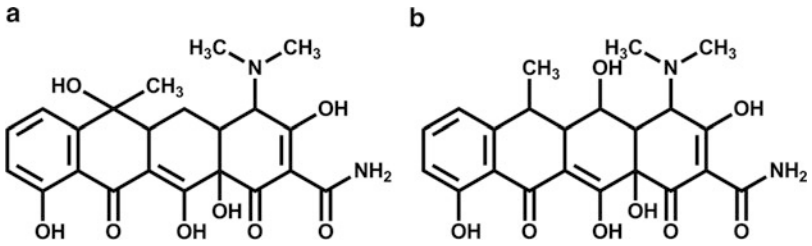

Fig. 2 Structures of tetracycline (**a**) and doxycycline (**b**)

3 Tetracyclines Including Doxycycline

The discovery of chlortetracycline in 1948 preceded that of clindamycin, although it was also from another soil organism *Streptomyces aureofasciens*. Catalytic dehydrogenation of chlortetracycline gave tetracycline in 1953. This class of antibiotic has wide antimicrobial properties, ranging from Gram-positive and Gram-negative bacteria, rickettsiae (where its use can be diagnostic), mycoplasmas, chlamydia and protozoa. Doxycycline is a semi-synthetic derivative of tetracycline first developed in 1967. The structures of tetracycline and doxycycline are given in Fig. 2.

3.1 Mechanism of Action

The tetracyclines including doxycycline act by inhibiting protein synthesis by binding to the 30S ribosomal subunit. They are relatively slow acting as antimalarial agents. Doxycycline is useful as a prophylactic agent on its own, as well as being used in combination treatment regimens to increase cure rates of conventional antimalarial agents [26]. It has limited casual prophylactic activity [26] and does not kill hypnozoites.

3.2 Pharmacokinetics

Doxycyline has ~90% oral bioavailability, which is reduced by food and delayed by alcohol, with peaks without delay occurring at ~2 h after administration [26]. Co-administration with cations such as iron supplements reduces absorption by chelation of the antibiotic, and milk may reduce absorption [26]. Plasma protein binding of doxycycline is 90% and elimination half-time is 18 h, making this one of the most convenient tetracyclines to use in practise. Most elimination of unchanged drug is through the gastrointestinal tract, with about a third being renally excreted.

3.3 Usage in Malaria

Tetracycline requires dosing 4 times as day and is therefore cumbersome to use as an antimalarial. Doxycyline (for adults up to 100 mg base twice daily, with another antimalarial) should only be used in combination with rapidly acting antimalarials such as quinine to increase cure rates, and not on its own. Table 2 summarises antimalarial treatment properties of doxycycline [26]. It can be used as a prophylactic antimalarial (for adults up to 100 mg base once daily starting before travel and continuing for 4 weeks after return), even in areas of multidrug-resistant parasites [26].

3.4 Safety and Toxicity

The tetracyclines are not recommended in pregnancy, and in children whose teeth can be stained (8 years or less). Doxycycline is a photosensitising antibiotic and can also cause oesophageal erosions and heartburn if taken incorrectly. Nausea and abdominal pain are relatively common, but frequency can be reduced by taking doxycycline with food [26].

4 Azithromycin

Azithromycin is a semi-synthetic derivative of erythromycin, whereby a methyl-substituted nitrogen atom is incorporated into the lactone ring system to improve acid stability (Fig. 3). In contrast to doxycycline, azithromycin can be used in younger children and in pregnancy, but its much increased cost compared with doxycycline may limit its applicability for management of malaria. Azithromycin is useful in the management of respiratory tract infections and has activity against *Toxoplasma* and can be used to treat uncomplicated babesiosis when combined with atovaquone in immunocompetent individuals [32].

4.1 Mechanism of Action

Azithromycin acts by binding to the 50S ribosomal sub-unit of susceptible microorganisms and inhibiting protein synthesis. Consistent with the activities of other antimalarial antibiotics, azithromycin is relatively slow acting and should be used in combination therapies for malaria. There may be some cross-resistance between antibiotics as observed for clindamycin-resistant *T. gondii*.

Table 2 Efficacy of doxycycline with or without quinine for treatment of *P. falciparum* malaria

Country (population)	Study type	Sample size/doxycycline dose	Duration of treatment	Cure rate (95% CI)	Ref.
United States (mixture of immune and non-immune)	Challenge, open-label	*P. vivax:*			[27]
		1/100 mg twice a day	4 days	0	
		1/100 mg twice a day	6 days	0	
		P. falciparum:			
		4/100 mg twice a day	5 days	0	
		9/100 mg twice a day	7 days	100%	
West Malaysia (varied, children aged 2 months–8 years)	Challenge, open-label	9/4 mg/kg daily	4 days	44.4%	[28]
		26/4 mg/kg daily	7 days	84.6%	
Irian Jaya, Indonesia (non-immune)	Randomised, open-label, comparator = CQ ($N = 30$) and doxycycline + CQ ($N = 39$)	20/100 mg twice a day	7 days	*P. falciparum*: 64.7% (42.0–87.4%) *P. vivax*: 33.3% (6.6–59.5%)	[29]
Brazil (semi-immune)	Randomised, open-label, comparator = AL ($N = 28$)	31/100 mg twice a day + 500 mg quinine every 8 h	5 days doxycycline 3 days quinine	100%	[30]
Pakistan (unknown immune status)	Challenge, open-label	100/100 mg twice a day + 10 mg/kg quinine every 8 h	7 days doxycycline 3 days quinine	100%	[31]

CQ chloroquine, *AL* artemether-lumefantrine. Taken from [26] with kind permission from the authors and the American Journal of Tropical Medicine and Hygiene

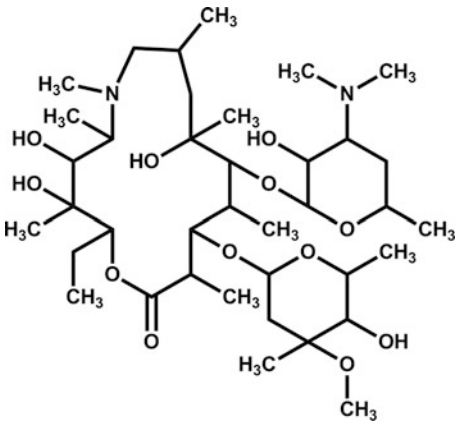

Fig. 3 Structure of azithromycin

4.2 Pharmacokinetics

Azithromycin has moderate oral bioavailability (37% after 500 mg) and this is reduced significantly (by 50%) if given with food; hence, it should be used either 1 h before food or 2 h after. Antacids also reduce availability. The degree of protein binding of azithromycin is dose-dependent, but not usually >50%. The drug is extensively distributed in tissues, which act as a depot with an elimination half-time of ~70 h. Most drugs are probably eliminated unchanged by biliary and gastrointestinal excretion.

4.3 Clinical Studies

Azithromycin by itself is not useful to treat uncomplicated *P. falciparum* or *P. vivax* infections [33] as failure rates can exceed 50% when conventional antibacterial doses for adults of 0.5 g per day for 3 days are used. Cure rates may be increased if the dose of azithromycin is also increased (to for example, 1 g per day) in combination therapies, but comparisons with other antimalarial combinations are not so encouraging (Fig. 4). Current evidence suggests that other combinations should be used to treat malaria unless there is no choice.

4.4 Safety and Toxicity

Azithromycin is relatively well tolerated, with mild gastrointestinal side effects being most commonly reported. Occasional abnormalities in liver function are observed and allergic reactions are rare. Nausea may be more common with higher dose regimens [33].

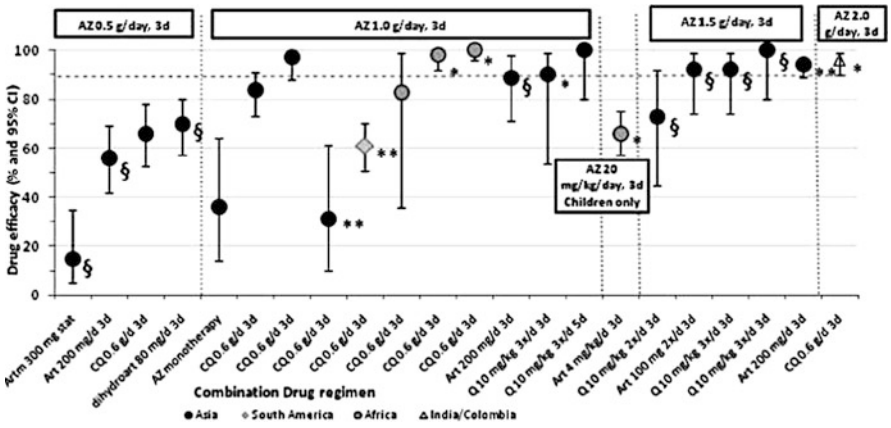

Fig. 4 Efficacy of azithromycin containing treatment regimens for *P. falciparum* (28 day follow-up). *Abbr*: *AZ* azithromycin, *CQ* chloroquine, *Artm* artemether, *Art* artesunate, *dihydroart* dihydroartemisinin, *Q* quinine, *CI* confidence interval, *d* days. *Symbols*: *Asterisk* PCR-corrected, *double asterisk* partially PCR-corrected, *section sign* study conducted in an area without malaria transmission (Bangkok). The AZ dose in the combination with artemisinin (300 mg) was 500 mg at start, followed by 250 mg after 24 h and 48 h. An interrupted line has been drawn at the 90% efficacy level, the minimum level for the 95% confidence interval for a potentially useful drug regimen as recommended by WHO [34]. Taken from [33] with kind permission from the authors and John Wiley & Sons, Ltd on behalf of The Cochrane Collaboration. Copyright Cochrane Collaboration.

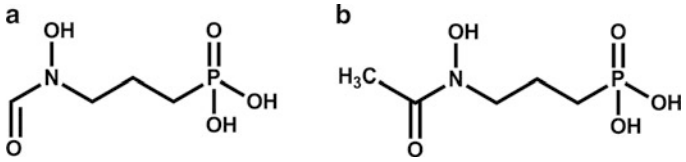

Fig. 5 Structure of fosmidomycin (**a**) and FR-900098 (**b**)

5 Fosmidomycin

Fosmidomycin is a natural antibiotic, originally derived from *Streptomyces lavendulae*. It is currently being investigated as a combination partner in antimalarial chemotherapy regimens, with the idea that it represents a non-artemisinin class of antimalarial with an unusual mode of action. Fosmidomycin's relatively simple chemical structure (Fig. 5) makes it amenable to complete synthesis, which is how it is currently made for investigational studies. Fujisawa Pharmaceutical Company in Osaka, Japan [35], originally developed fosmidomycin as an antibacterial agent [36–39] for treating urinary tract infections approximately three decades ago. It is most effective against enterobacteria and not against Gram-positive organisms or anaerobes. Since its discovery, cephalosporins have emerged as being more effective for recurrent infections and the development of fosmidomycin as an antibacterial

agent has not been taken further forward, until it has been repurposed as an antimalarial.

6 Mechanism of Action

The repurposing of fosmidomycin as an antimalarial depended on several advances in understanding the biology of the malarial parasite. One advance lay in the recognition of the plastid organelle as an excellent target for new drugs, as no similar structure exists in animal cells. Another advance was the identification of an alternative [nonmevalonate or 1-deoxy-D-xylulose 5-phosphate (DOXP)] pathway for isoprenoid synthesis in parasites that was hitherto described in plants and eubacteria. Key enzymes forming part of this synthesis pathway include DOXP reductoisomerase and 2*C*-methyl-D-erythritol-4-(cytidine-5-diphospho) transferase (Fig. 6) and are located in the parasite's apicoplast. This pathway contributes to many functions such as prenylation of membrane-bound proteins and synthesis of carotenoids and terpenoids.

Hassan Jomaa and colleagues [40] tested the idea that inhibiting this pathway would prove lethal to parasites by expressing a recombinant version of DOXP reductoisomerase and showing that it was inhibited by fosmidomycin (with an inhibitory constant of ~30 nM). They also showed that fosmidomycin and compound FR-900098 (Fig. 5), a compound that acts as a pro-drug for fosmidomycin, killed cultured *P. falciparum*, including highly chloroquine-resistant strains (IC_{50} values ranging from 300 to 1,200 nM), as well as being able to cure mice infected with *P. vinckei*. Recently, it has been reported that fosmidomycin also targets a second enzyme in the DOXP pathway, 2*C*-methyl-D-erythritol-4-(cytidine-5-diphospho) transferase (Fig. 6) [41]. Other recent studies have suggested that the uptake of fosmidomycin, which is highly charged, requires specific transport mechanisms [42, 43]. This includes the new permeability pathways (NPP) that are responsible for altered plasma membrane permeability of the host erythrocyte as intracellular plasmodial parasites mature [42, 44]. These new data not only explain the selectively of fosmidomycin for plasmodial parasites over closely related

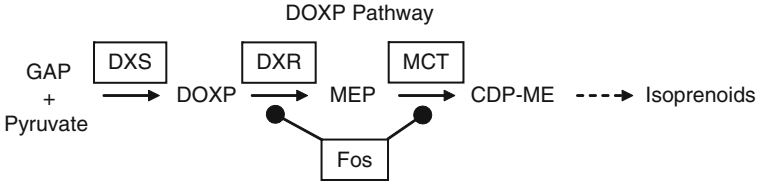

Fig. 6 The 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway for isoprenoid synthesis and the sites of action of fosmidomycin (Fos). *GAP* glyceraldehyde-3-phosphate, *DXS* DOXP synthase, *DXR* DOXP reductoisomerase, *MEP* 2*C*-methyl-D-erythritol-4-phosphate, *MCT* 2*C*-methyl-D-erythritol-4-(cytidine-5-diphospho) transferase, *CDP-ME* 4-(cytidine-5-diphospho)-2*C*-methyl-D-erythritol

Table 3 Fosmidomycin clinical trials data

Region of study	Study design	Regimen	Subjects	Safety and tolerability	Efficacy
Monotherapy					
Gabon/Thailand [48]	Uncontrolled, open-label study	1,200 mg every 8 h for 7 days	26 adults	Well tolerated with mild GI disturbances in 5 patients	At day 28, cure rates were 78% (7/9) in Gabon and 22% (2/9) in Thailand
Gabon [49]	Uncontrolled, open-label study	1,200 mg every 8 h for 5, 4 or 3 days	32 adults	Well tolerated with most frequent AEs being headache, weakness, myalgia, abdominal pain, and loose stools	At day 14, cure rates were 89% (8/9), 88% (7/8) and 60% (6/10) for 5, 4 and 3 day regimens
Combination therapy					
Gabon [50]	Randomised, controlled open-label study	30/5 mg/kg FC, 30 mg/kg F or 5 mg/kg C every 12 h for 5 days	36 children (7–14 years)	Well tolerated with no serious AEs (mostly GI disturbances)	At day 28, cure rates were 100% (12/12), 42% (5/12) and 100% (12/12) for FC, F and C regimens
Gabon [51]	Uncontrolled, dose-reduction study	30/10 mg/kg FC every 12 h for 5, 4, 3, 2, 1 days	52 children (7–14 years)	Well tolerated with most frequent AEs being GI disturbances [mostly loose stools (13 events) and abdominal pain (9 events)]	At day 28, cure rates were 100% (10/10), 100% (10/10), 90% (9/10), 70% (7/10) and 10% (1/10) for 5, 4, 3, 2 and 1 day regimens
Gabon [52]	Uncontrolled, dose-reduction study	30/2 mg/kg F/AS every 12 h for 5, 4, 3, 2, 1 days	50 children (6–12 years)	Well tolerated with most frequent AEs being GI events	At day 28, cure rates were 100% (9/9), 90% (9/10), 100% (10/10), 60% (6/10) and 40% (4/10) for 5, 4, 3, 2 and 1 day regimens
Gabon [53]	Uncontrolled, Phase IIb, single-arm study	30/10 mg/kg FC every 12 h for 3 days	51 children (1–14 years)	Well tolerated with GI disturbances but relatively high rates of treatment-associated	At day 28, cure rates were 89% (42/47) and 62% (5/8) for children 3–14 years and 1–2 years

(continued)

Table 3 (continued)

Region of study	Study design	Regimen	Subjects	Safety and tolerability	Efficacy
				neutropenia (8/51) and falls of haemoglobin concentrations of ≥2 g/dl (7/51)	
Gabon [54]	Randomised study	30/10 mg/kg FC every 12 h for 3 days or 25/1.25 mg/kg SP single dose	105 children (3–14 years)	Both treatments were well tolerated with slightly more AEs occurring in the SP group	At day 28, cure rates were 94% (46/49) and 94% (46/49) for FC and SP regimens
Thailand [46]	Uncontrolled, open-label study	A: 1,200 mg F every 12 h for 7 days or B: 900/600 mg FC every 12 h for 7 days	33 adults	Both treatments were well tolerated with no serious AEs. Mild events included headache and GI disturbance	At day 28, 22% (2/9) and 100% (12/12) for A and B regimens
Thailand [47]	Randomised, uncontrolled open-label study	A: 900/300 mg FC every 6 h for 3 days or B: 1,800/600 mg FC every 12 h for 3 days	114 Adults	Both regimens well tolerated with no serious AEs (GI events were most with abdominal pain developing in 10% of patients, diarrhoea in 5% and vomiting in 4%)	At day 28, 91% (21/23) and 90% (70/78) for A and B regimens

F fosmidomycin, *C* clindamycin, *SP* sulfadoxine-pyrimethamine, *AS* artesunate, *AE* adverse event, *GI* gastro-intestinal

species (e.g. *Toxoplasma*) but can also be used to test potential resistance mechanisms in *Plasmodium* spp.

6.1 Pharmacokinetics

Fosmidomycin has relatively poor oral bioavailability (~25%) and is poorly protein bound (<5%) with an elimination half-time of 1.6–1.8 h [45]. This elimination half time is somewhat prolonged in subjects with malaria to 3.4 h (range 1.4–11.8 h) and not altered importantly after co-administration with clindamycin in one study [46], although particular dosing regimens of this combination (fosmidomycin and clindamycin) may influence pharmacokinetic behaviour of each drug [47].

6.2 Fosmidomycin in Malaria

Table 3 summarises studies of fosmidomycin in children and adults with malaria. Monotherapy studies with fosmidomycin have confirmed its antimalarial activity in patients, and also that, unlike clindamycin, it has relatively rapid antimalarial actions. However, recrudescence rates are rather high, compelling the choice of an appropriate combination partner to achieve adequate clinical and parasitological cure rates. Clindamycin has been the most thoroughly studied partner drug and results of various studies are also included in Table 3. In general, a 3-day treatment regimen with 30 mg/kg fosmidomycin and 10 mg/kg clindamycin given in a 8 or 12 h interval and in the populations reported provided adequate responses in adults and children except for one study in children aged <3 years [53]. This combination was also well tolerated, with mild gastrointestinal side effects being some of the most frequent (Table 3).

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