Other 4-Methanolquinolines, Amyl Alcohols and Phentathrenes: Mefloquine, Lumefantrine and Halofantrine

Francois Nosten, Penelope A. Phillips-Howard, and Feiko O. ter Kuile

Abstract This chapter describes mefloquine, pyronaridine, halofantrine, piperaquine and lumefantrine under the broader title of the 4-methanolquinolines, amyl alcohols and phentathrenes. We provide a brief resume of each drug, in terms of their chemical properties, formulae, pharmacokinetics, clinical indications for use, and their efficacy and safety. Recognizing the limited number of antimalarials available, and in the developmental pipeline, attention is focussed on describing the history of each drug and how their indications have evolved as data on safety in human populations accumulates over time, and how patterns of use have changed with growing multiple drug resistance. Their combined use with the artemisinin derivatives is briefly described and readers are recommended to consult other chapters in this book which more fully describe such combinations.

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1 Mefloquine

1.1 Structure and Action

Mefloquine hydrochloride is a 4-quinolinemethol derivative synthesised as a structural analogue (2-aryl substituted chemical) of quinine. Its full chemical name is (R*, S*)-(±)-α-2-piperidinyl-2,8-bis (trifluoromethyl)-4-quinolinemethanol hydrochloride. Its formula is $C_{17}H_{16}F_6N_2O$ (Fig. 1). Mefloquine was discovered by the Experimental Therapeutics Division of the Walter Reed Army Institute of Research (WRAIR) in the 1970s for chemoprophylaxis (250 mg weekly) and therapy (15–25 mg/kg) and was approved by the U.S. Food and Drug Administration in 1989.

Mefloquine is a blood schizonticide, active against the erythrocytic stages of *Plasmodium falciparum* and *P. vivax*, with no effect on the exoerythrocytic (hepatic) stages of the parasite, and with limited information of its effect on *P. ovale, P. malariae* and *P. knowlesi*. Studies indicate mefloquine interferes with the transport of haemoglobin and other substances from the host erythrocyte to the food vacuole of the malaria parasite, causing swelling and cytotoxicity [1]. Mefloquine strongly inhibits endocytosis in the D10 strain of *P. falciparum* using several lines of evidence: a reduction in haemoglobin levels in the parasite as assessed by Western blotting, decreased levels of accumulation of biotinylated dextran by the parasite in preloaded erythrocytes, significantly lower concentrations of fluorescent dextran in the food vacuole, and a reduced percentage of parasites with multiple transport vesicles [2].

Mefloquine is a chiral molecule; it has two asymmetric carbon atoms and exists in two racemic forms (erythro and threo), each of which is composed of a pair of optical isomers, i.e. (±)-erythro-enantiomers and the (±)-threo-epimers. Clinically, the racemic mixture of the erythro-enantiomers is used [3]. Unlike other antimalarial drugs such as chloroquine, halofantrine, and lumefantrine, there is stereoselectivity

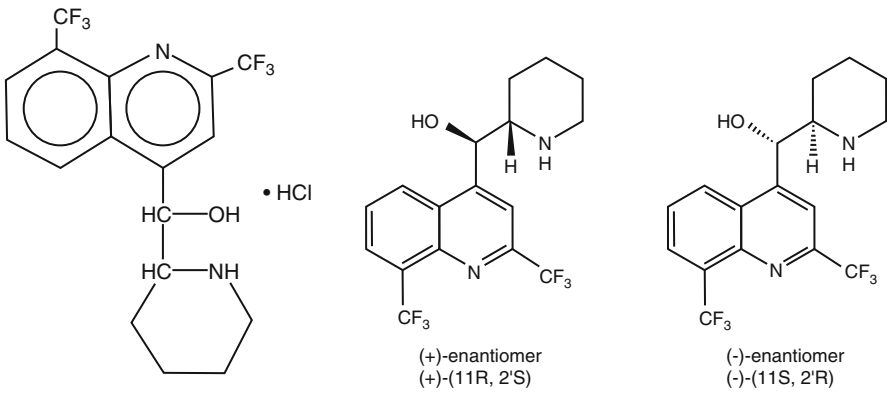

Fig. 1 Structure of mefloquine hydrochloride and its two enantiomers

in its antimalarial activity, with the (+)-isomer ~1.7 times more potent than the (−)-isomer in vitro [4, 5].

1.2 Pharmacokinetics

Mefloquine is moderately well absorbed orally and extensively distributed and is 98% bound to plasma proteins. Splitting 25 mg/kg mefloquine into 2 or 3 doses given 16–24 h apart reduces vomiting, improves oral bioavailability and the therapeutic response in the treatment of acute falciparum malaria [6]. Food increases its bioavailability by up to 40%. The parent compound is metabolized by the cytochrome P450 enzyme CYP3A4 to two major metabolites: carboxy- and hydroxyl-mefloquine, which are inactive against *P. falciparum*. Mefloquine is eliminated slowly and has a terminal elimination half-life of ~3 weeks in volunteers and 2 weeks in patients. Total clearance, which is essentially hepatic, is 30 ml/min in volunteers. A steady-state plasma concentration of 1,000–2,000 μg/l is reached after 7–10 weeks following weekly 250 mg prophylaxis and it is therefore recommended to start medication at least 2 weeks before travel. There are stereoselective differences in their pharmacokinetics and the ability of the mefloquine enantiomers to cause certain adverse effects [5]. In humans, the plasma concentration of the (−) enantiomer is approximately threefold higher than the (+) enantiomer, reflecting the stereoselectivity in the clearance and volume distribution [5, 7–11]. Co-administration with artemisinin does not appear to influence mefloquine enantiomer pharmacokinetics [12].

1.3 Clinical Use

One tablet of 250 mg mefloquine hydrochloride per week (adult dose; equivalent to 228 mg of the free base) has been used for prophylaxis in travellers, including for young children and pregnant women. The limited data available on the use of mefloquine in human pregnancy are reassuring and do not indicate an increased teratogenic risk [13]. A retrospective study of 208 women on the Thai–Burmese border treated with mefloquine found an increased risk of stillbirths [14], however, this finding was not confirmed in a large prospective trial of mefloquine prophylaxis in Malawian pregnant women [15] and remains unexplained. As a treatment, it is now mainly used in combination with artesunate, a water-soluble artemisinin derivative. It is available as a loose combination (as part of a blister pack containing both drugs), and as a new fixed-dose combination developed with support from the Drugs for Neglected Diseases Initiative (DNDi) and produced by Farmanguinhos/Fiocruz, Brazil and Cipla, India. The treatment dose is 25 mg/kg of mefloquine and 12 mg/kg of artesunate given as 8.3 and 4 mg/kg/day over 3 days, respectively. Tablets of the new fixed-dose combination come in adult and child “strengths”,

with several co-blistered formulations of the loose combinations made by different manufacturers. Mefloquine is also being explored for a new indication as intermittent preventive therapy (IPT) against malaria. Two trials evaluating the role of mefloquine as IPT in infants (IPTi) [16] and in pregnant women (IPTp) [17] found mefloquine to be very effective, but the low tolerability limited its acceptance for use as IPTi. Further IPTp studies are ongoing in five countries in Africa with the lower 15 mg/kg dose in pregnancy.

1.4 Resistance

Most experience with mefloquine as monotherapy and later in combination with the artemisinin derivatives has been gained from areas of multiple drug resistance in Southeast Asia, such as on the Thai–Burmese (Myanmar) border. Thailand was the first country to use mefloquine for first-line treatment of acute malaria. From 1985 to 1990, it was recommended in combination with sulfadoxine and pyrimethamine, as "MSP" in a fixed-dose combination, at a single dose of ~15/30/1.5 mg/kg, providing a 98% cure rate after its introduction in 1985, however, this dropped to <50% in children by 1990 [18]. Because of high levels of existing parasite resistance to SP, and lack of additional therapeutic efficacy over mefloquine alone, the SP component was dropped [18, 19] and replaced by mefloquine monotherapy; initially at a single dose of 15 mg/kg. High levels of treatment failure with this dose [18, 20] prompted 25 mg/kg dosing [21–23], split (750 and 500 mg, 16–24 h apart) to reduce vomiting [24]. Within 8 years, mefloquine monotherapy failure rates on the Thai–Burmese border reached 60% and, following extensive testing, the combination of mefloquine 25 mg/kg with artesunate 12 mg/kg given over 3 days (MAS_3) became the new standard therapy [25]. This therapy, combined with early diagnosis and use of insecticide treated nets, reduced *P. falciparum* malaria incidence, and halted, and later reversed the progression of mefloquine resistance [26–28]. The combination offered a potential public health solution for multiple drug-resistant *P. falciparum*, and allowed time for the development of other new drugs [1].

In vivo resistance to mefloquine, mediated mainly by an increase in gene copy number and expression of the *P. falciparum* multi-drug resistance (MDR) gene-1 (*pfmdr1*), a gene encoding a parasite-transport protein [29, 30], has been confirmed. This has been reported on the borders of Thailand with Burma (Myanmar) and Cambodia, in the western provinces of Cambodia, the eastern states of Burma (Myanmar) and its border with China, along the Laos and Burma borders, the adjacent Thai–Cambodian border and in southern Vietnam. It is likely the initial deployment of low dose of mefloquine may have encouraged resistance. Theoretical evidence suggests that initial use of higher doses, preferably in combination with an artemisinin derivative, is less likely to lead to resistance [31].

1.5 Tolerability

Preclinical studies demonstrate mefloquine to be safe and effective [32], and extensive clinical experience to date supports this. Nevertheless, widespread deployment of mefloquine for treatment and prophylaxis has been hampered by concerns about its tolerability. Side effects following treatment are common; they are usually mild and restricted to dizziness/vertigo and gastro-intestinal disturbances [24]. Vomiting after mefloquine is a problem in young children, but can be mitigated by splitting the dose over 2 or 3 days, and by fever reduction [24, 33]. In older children and adults, mild neuro-psychiatric events (headache, dizziness, insomnia and vivid dreams) are reported in ~25% of patients treated with 25 mg/kg mefloquine.

Mefloquine is also associated with a self-limiting acute neuropsychiatric syndrome manifest by encephalopathy, convulsions or psychosis [34–36], apparent in international travellers taking 250 mg mefloquine each week for prophylaxis [35, 37]. Mefloquine is thus contraindicated for prophylaxis in patients with active depression, a recent history of depression, generalised anxiety disorder, psychosis, or schizophrenia or other major psychiatric disorders, or with a history of convulsions [38]. These contraindications are prevalent in 9–10% of the military [39] and civilian [40] populations presenting for malaria chemoprophylaxis [41], but are not documented in endemic populations. While the mechanism is not yet fully understood, neuropsychiatric events have been demonstrated to be associated with dose in humans [42, 43]. The rates in travellers are estimated to be 1:10,000 persons, equally frequent with chloroquine prophylaxis, but higher than in similar populations that used other forms of prophylaxis [35, 44]. The incidence following treatment doses is 1:1,000 in Asian patients [45], 1:200 in Caucasian or African patients with uncomplicated malaria and 1:20 in patients recovering from severe malaria [46]. Previous history of psychiatric illness or epilepsy is a risk factor. Females and individuals of low body mass index are also at apparent greater risk. Neuropsychiatric reactions are more common if mefloquine was used in the previous 2 months, and thus should not be used to treat recrudescent infections within 2 months of treatment.

Total (racemic) concentrations of mefloquine are ~30-fold higher in brain than in plasma [47]. In man, approximately threefold higher concentrations of the (−)-enantiomer is observed in plasma, and 1.5-fold higher in brain, but post-mortem studies demonstrated stereoselective brain penetration, greater for the (+)-enantiomer, with (−) and (+) concentrations at ~23- versus 56-fold higher in the brain's white matter compared with plasma (the reverse is found in rat models where the penetration of the (−)-enantiomer is greater than that of its antipode) [7, 47–49]. There is a growing body of evidence on the mechanisms of possible neurotoxicity (see reviews [50] and [41]). The high level of accumulation of mefloquine in brain tissue may be associated with direct neurotoxic damage and cell death, with binding to neuroreceptors and cholinesterases, inhibition of sarco (endo)plasmic reticulum Ca^{2+} ATPase (SERCA) activity and interference with

cellular Ca^{2+} homeostasis and reductions in central nervous system efflux in individuals possessing certain (human) MDR1 polymorphisms [50].

1.6 The Future

Using mefloquine as a scaffold, WRAIR has constructed a library of 200 potential next generation quinoline methanol compounds to identify leads that possess biological properties consistent with the target product profile for malaria chemoprophylaxis but less susceptible to passage across the blood–brain barrier (to reduce adverse neurological effects) [51, 52]. During a programme to examine the biochemical basis of side effects, investigators discovered that the (−)-(R,S)-enantiomer is a potent adenosine A2A receptor antagonist, resulting in a programme to develop novel adenosine A2A antagonists for the management of Parkinson's disease [53, 54]. Mefloquine is effective against JC virus and is reported to have successfully treated progressive multifocal leuko-encephalopathy (a progressive, usually fatal, demyelinating disease caused by the JC virus) [55, 56]. Mefloquine is undergoing in vitro and in vivo studies to evaluate its effectiveness for the treatment of helminth infections [57], including those caused by *Schistosoma* [58], *Clonorchis* and *Paragonimus* [59].

2 Pyronaridine

2.1 Structure and Action

Pyronaridine is a Mannich base with a pyronaridine nucleus synthesised from mepacrine (9 amino acridine). Its formula is $C_{29}H_{32}ClN_5O_2$ (Fig. 2). It was synthesised in 1970 in China and is available as a free base and as a tetraphosphate, the salt used in current formulations. It was used in China as a monotherapy in the 1980s and 1990s but has now been developed as a combination therapy with

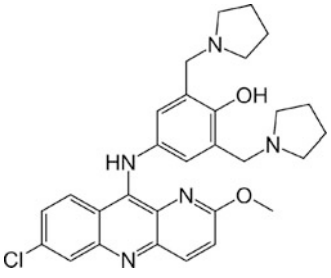

Fig. 2 Structure of pyronaridine

artesunate by Shin Poong Pharmaceutical (Korea) and Medicines for Malaria Venture (MMV, Switzerland).

Pyronaridine is active against asexual forms of *Plasmodium* by forming complexes with ferriprotoporphyrin IX. Growth studies of *P. falciparum* K1 in culture demonstrate the ability of pyronaridine to inhibit in vitro β-haematin formation, to form a complex with a stoichiometry of 1:2, to enhance haematin-induced red blood cell lysis, and to inhibit glutathione-dependent degradation of haematin [60]. However, observations that pyronaridine exerted this mechanism of action in situ, based on showing antagonism of pyronaridine in combination with antimalarials (chloroquine, mefloquine, and quinine) that inhibit β-haematin formation, were equivocal. Interestingly pyronaridine is also active against young gametocytes (stage II and III) in vitro [61], although recent clinical studies did not detect a difference in gametocyte carriage following treatment with the fixed combination artesunate–pyronaridine when compared with artemether– lumefantrine [62]. The compound is also a poor substrate and an inhibitor of the Permeability glycoprotein (P-gp) ATP-dependant transporter, a product of the human multidrug resistance-1 (MDR1;ABCB1) gene that influences the passage of many drugs across epithelial barriers. The P-gp-mediated efflux could attenuate oral absorption of the drug when the luminal concentration falls; however, this is likely to play a minimal role in the initial absorption of the drug.

2.2 Pharmacokinetics

The pharmacokinetic properties of pyronaridine are not well characterised. The drug is readily absorbed from the small intestine following oral administration and is widely distributed in most tissues [63]. The peak value of the drug in the blood is reached at around 8 h post-administration, and it shows a poor permeability across the blood–brain barrier. Pyronaridine concentrates preferentially in infected red blood cells and its distribution and elimination are influenced by age and disease status. It is eliminated slowly, with the half-life currently estimated at 18 days in patients with malaria [64]. Improvements to the assay that measures blood concentrations are likely to reveal a longer half-life.

2.3 Resistance

Resistance is known to have developed in *P. falciparum* when the drug was used in China but the molecular mechanism is unknown. High recurrence rates were noted in early clinical trials in Thailand [65] and cross-resistance with chloroquine was suggested in vitro [66]. Pyronaridine is not used as a single agent anymore but only in combination with artesunate to prevent the emergence of de novo resistance.

3 Halofantrine

3.1 Structure, Action and Resistance

Halofantrine belongs to the phenanthrene methanol group and was developed by WRAIR in the 1960s and then by Smith Klein, now GlaxoSmithKlein. It is a small chiral molecule and the chemical formula is $C_{26}H_{30}Cl_2F_3NO$ (Fig. 3). The oral formulation is in tablets containing 250 mg of hydrochloride salt. Like other quinoline derivatives, the mode of action of halofantrine appears to be in the inhibition of the formation of β-haematin crystals but the precise mechanism of action is unclear. Recently it was shown that halofantrine forms complexes with ferriprotoporphyrin IX and that the inhibition of the haemozoin formation occurs principally at the lipid–aqueous interface, an environment more compatible with the crystal structure of halofantrine–ferriprotoporphyrin IX [67]. Halofantrine is poorly and erratically absorbed due to its low solubility. Absorption is enhanced by fat co-administration. The principal active metabolite is *N*-desbutyl-halofantrine. The terminal half-life in patients with malaria is ~4.7 days, making it a relatively rapidly eliminated antimalarial when compared with other drugs of the same group. As halofantrine was withdrawn from use due to the discovery of halofantrine cardiotoxicity (see below), there are few studies on the development of resistance to this drug. Molecular studies have demonstrated that mutations in *pfmdr1* result in altered halofantrine transport, suggesting a role for this efflux transport mechanism in resistance to this drug [68]. Cross-resistance with mefloquine was shown in clinical studies in South East Asia [69] and it is likely that the drug shares common resistance mechanisms with mefloquine and lumefantrine due to their chemical similarities.

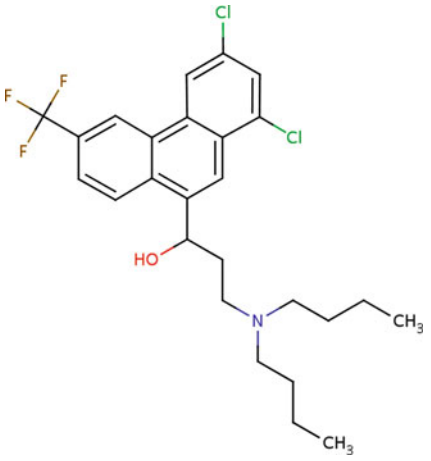

Fig. 3 Structure of halofantrine

3.2 Tolerability

Initially, halofantrine looked like a promising drug for the treatment of uncomplicated falciparum infections caused by chloroquine-resistant parasites. It rapidly cleared parasites and was well tolerated. The first report of the cardiotoxicity of halofantrine in 1993 [70], came as a surprise since the drug had been developed in full compliance with GCP standards. Halofantrine and its principal quinidine-like metabolite have a Class III effect on cardiac repolarization [71]. It causes a dose-dependent blockade of the I_{kr} channel (through hERG) by binding to the open or inactivated state. This translates on an ECG to a marked prolongation of the QT interval and is more marked when halofantrine is given after mefloquine, probably as a result of the inhibition of the slow delayed rectifier potassium channel I_{ks} [72]. This QT prolongation, seen at therapeutic doses, increases the risk of the potentially fatal Torsade de Pointes and since the first report, several sudden deaths have been related to the drug. Because of this, halofantrine has been withdrawn in many countries and from international guidelines on the treatment of malaria.

4 Piperaquine

4.1 Structure and Action

Piperaquine is a bis-amino 4 quinoline synthesised more than 50 years ago by Rhone-Poulenc (France). It was abandoned and rediscovered in China by the Shanghai Research Institute of Pharmaceutical Industry. The drug was used on a large scale (140,000,000 doses) for prophylaxis and treatment of chloroquine resistant *P. falciparum* between 1978 and 1994, but resistance developed in the 1990s. Piperaquine is a lipophilic compound and its chemical formula is $C_{29}H_{32}Cl_2N_6$ (Fig. 4). The full mechanism of action is unknown but piperaquine concentrates in the parasite food vacuole and inhibits the dimerisation of haematin by binding. Recent work by Warhurst and colleagues has shed more light on the possible mode of action [73]. The high activity of piperaquine against chloroquine-resistant falciparum could be explained by its high lipid accumulation ratio (LAR) leading to an increase in β-haematin inhibition in vacuolar lipids where the crystals of haemozoin are produced. The drug may also act by blocking efflux from the food

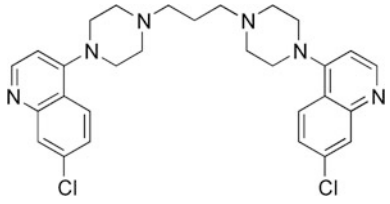

Fig. 4 Structure of piperaquine

vacuole by hydrophobic interaction with the parasite chloroqine-resistance transporter, *pfcrt* [73].

4.2 Pharmacokinetics

Piperaquine is lipophilic and exhibits considerable inter-individual variability in pharmacokinetics. It accumulates preferentially in infected red blood cells and this affects the plasma/blood concentration ratio. Like chloroquine, it has a large apparent volume of distribution and a slow elimination. The terminal elimination half-life is probably longer than previously thought and could exceed 30 days [74]. This is valuable for ensuring a prolonged post-treatment prophylactic effect and when the drug is used for IPT. In children, studies have shown that, dose adjustment may be needed [75]. Likewise, pregnant women may have lower piperaquine exposure than non-pregnant women, and the dosage in pregnancy may also need to be adjusted. Piperaquine has two major metabolites: a carboxylic acid and a mono-*N*-oxidated piperaquine product.

4.3 Resistance

Resistance to piperaquine is known to have developed in vivo in China when it was used as monotherapy but there is no indication that it has spread elsewhere. There is no specific molecular marker of resistance to piperaquine and the role of the *P. falciparum* transport proteins *pfmdr1* and *pfcrt* remains unclear [76, 77]. In clinical use, piperaquine is now used in a fixed combination with dihydroartemisinin (DHA) developed by Holleypharm (China) and Sigma-Tau (Italy) in partnership with MMV (Switzerland). DHA-piperaquine is one of the most promising artemisinin-based combination therapies (ACTs) in the antimalarial armamentarium.

5 Lumefantrine

5.1 Structure and Action

Lumefantrine is a racemic 2,4,7,9-substituted fluorine derivative belonging to the arylamino-alcohol group of antimalarials with a molecular structure reminiscent of halofantrine. It was originally synthesised by the Academy of Military Sciences in Beijing (PRC) in the 1980s. Its chemical formula is $C_{30}H_{32}Cl_3NO$ (Fig. 5). It is insoluble in water and the two enantiomers have equal antimalarial activities.

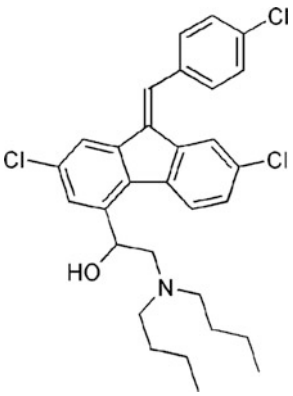

Fig. 5 Structure of lumefantrine

Lumefantrine is active against *P. falciparum* and *P. vivax* asexual stages but not against pre-erythrocytic liver stages, including hynozoites, or against gametocytes. The mode of action of lumefantrine is not known precisely but by similarity of structure with the antimalarials of the same group, it is assumed that lumefantrine kills parasites by inhibiting the polymerisation of heme.

5.2 Pharmacokinetics

The pharmacokinetics of lumefantrine have been extensively described in various populations (European, Asian and African) in adults, children and also in pregnant women. The drug is slowly absorbed (time to peak concentrations is approximately 6 h) and metabolised to desbutyl-lumefantrine via CYP3A4 but largely eliminated as parent compound via the liver in faeces and urine. The absorption is dose limited, so the total daily dose must be given on two separate occasions in order to be absorbed, the first serious impediment to observance [78]. The terminal elimination half-life is approximately 4.5 days, reminiscent of halofantrine and much shorter than mefloquine or piperaquine. Lumefantrine is highly lipophilic and has a low and variable bioavailability. This is a major contributor to the observed inter-individual variability in its kinetics. The relative fraction of the dose absorbed is also highly variable between patients and between doses. This is probably explained by the combined effects of illness on intestinal mobility, increased food intake with recovery and decreasing parasitaemia. Co-administration of food (or some fat) has a marked effect on the absorption of lumefantrine and this is the second major impediment to observance. Recent studies have shown that as little as 1.2 g of fat was needed for optimum lumefantrine absorption [71]. Unfortunately, this was not taken into account when the paediatric formulation of artemether–lumefantrine (Coartem) was developed. Initially, a 4-dose regimen of Coartem was recommended based on the initial Chinese trials. However this resulted in low cure rates in Thailand [79] but it helped in defining the lumefantrine exposure–cure

rate relationship. The most determinant factors of cure were found to be the initial parasite load and the Area Under the plasma concentration Curve (AUC) of lumefantrine. A useful surrogate of the AUC is the day 7 lumefantrine concentration. A plasma lumefantrine concentration of 280 ng/ml was found to be a useful discriminating cut-off to determine subsequent risk of recrudescence [80] and, in the absence of resistance, a day 7 concentration of 500 ng/ml would be expected to cure >90% of patients [81]. After too many years of delay, the 6-dose regimen became universally recommended. For pregnancy, however, this standard regimen is associated with lower plasma concentrations because of the increased volume of distribution and faster elimination, which will lead to treatment failures [82]. Modelling suggests that longer courses are needed to achieve lumefantrine concentrations comparable to that in non-pregnant patients [75].

5.3 Resistance

Resistance to lumefantrine can be readily obtained in vitro and in animal models. In vitro, single-nucleotide polymorphisms in the *pfmdr1* gene have been associated with increased IC_{50} values for lumefantrine [83]. An increase in *pfmdr1* copy number also resulted in decreased in vitro susceptibility and increased risk of failure in patients receiving the 4-dose regimen [80]. Interestingly, resistance to lumefantrine in *P. falciparum* could be associated with the loss of chloroquine resistance (i.e. the loss of the *pfcrt* K76T mutation) [84]. Lumefantrine is not used as a single drug but only in combination with artemether (see Coartem). Its future depends on controlling the emergence of resistance to the artemisinin derivatives and/or to the emergence of resistance to lumefantrine itself or through cross resistance with mefloquine.

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