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Piperaquine
A Resurgent Antimalarial Drug

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Contents

Abstract

Piperaquine is a bisquinoline antimalarial drug that was first synthesised in the 1960s, and used extensively in China and Indochina as prophylaxis and treatment during the next 20 years. A number of Chinese research groups documented that it was at least as effective as, and better tolerated than, chloroquine against falciparum and vivax malaria, but no pharmacokinetic characterisation was undertaken. With the development of piperaquine-resistant strains of *Plasmodium falciparum* and the emergence of the artemisinin derivatives, its use declined during the 1980s.

However, during the next decade, piperaquine was rediscovered by Chinese scientists as one of a number of compounds suitable for combination with an artemisinin derivative. The rationale for such artemisinin combination therapies (ACTs) was to provide an inexpensive, short-course treatment regimen with a high cure rate and good tolerability that would reduce transmission and protect against the development of parasite resistance. This approach has now been endorsed by the WHO.

Piperaquine-based ACT began as China-Vietnam 4 (CV4®: dihydroartemisinin [DHA], trimethoprim, piperaquine phosphate and primaquine phosphate),

which was followed by CV8® (the same components as CV4 but in increased quantities), Artecom® (in which primaquine was omitted) and Artekin® or Duo-Cotecxin® (DHA and piperaquine phosphate only). Recent Indochinese studies have confirmed the excellent clinical efficacy of piperaquine-DHA combinations (28-day cure rates >95%), and have demonstrated that currently recommended regimens are not associated with significant cardiotoxicity or other adverse effects.

The pharmacokinetic properties of piperaquine have also been characterised recently, revealing that it is a highly lipid-soluble drug with a large volume of distribution at steady state/bioavailability, long elimination half-life and a clearance that is markedly higher in children than in adults. The tolerability, efficacy, pharmacokinetic profile and low cost of piperaquine make it a promising partner drug for use as part of an ACT.

1. Historical Background

Piperaquine is a bisquinoline antimalarial drug that was synthesised independently by both the Shanghai Pharmaceutical Industry Research Institute in China and Rhone Poulenc in France in the 1960s.[1] Because of its relative potency and tolerability, it superseded chloroquine as the antimalarial recommended by the Chinese National Malaria Control Programme in 1978, and the equivalent of 140 million adult treatment doses were subsequently manufactured and distributed.[2] With the development of piperaquine-resistant strains of *Plasmodium falciparum* and the appearance of the artemisinin derivatives, piperaquine use diminished in the 1980s.

In 1990, Chinese scientists 'rediscovered' piperaquine as one of a number of components of short-course artemisinin-based combination therapies formulated to achieve a high cure rate without significant adverse effects. The first of these was China-Vietnam 4 (CV4®)[1], which contained dihydroartemisinin (DHA), trimethoprim, piperaquine phosphate and primaquine phosphate.[2] Initial small-scale, nonrandomised trials in China and Indochina led to the reformulation of CV4® as CV8®,[3] which was the same drug combination as CV4® but with different quantities of the components. CV8® was evaluated in further trials in Vietnam[4,5] and introduced into the Vietnamese National Malaria Control Programme in 2000. However, there were three concerns with CV8®. First, the role of trimethoprim was questionable since, like other antibacterial agents, it does not have prompt and potent antimalarial activity. Secondly, there is a high rate of glucose-6-phosphate dehydrogenase deficiency amongst Asian populations, especially in ethnic minorities in countries such as Vietnam.[6] Red cell haemolysis, sometimes fatal, can occur in this situation if primaquine is given. Thirdly, the dose of DHA in the original formulation of CV8® (10mg per tablet) resulted in a total dose that was much lower than recommended when DHA alone is used for the initial treatment of acute malaria (80mg over 2 days vs 480mg over 7 days).

The most recent changes to CV8® have seen primaquine excluded (Artecom®)[7] and, more recently, both primaquine and also trimethoprim excluded (Artekin 2®, which was renamed as Artekin®).[2,8] In the case of Artecom®, Artekin® and the most recent formulations of CV8®, the DHA content of each tablet has been increased to a total dose of 256mg over 2–3 days.

2. Chemical Structure and Physicochemical Properties

Piperaquine is available as the base ($C_{29}H_{32}Cl_2N_6$; 4,4′-(1,3-propaneiyldi-4,1-piperazinediyl)bis[7-

1 The use of trade names is for product identification purposes only and does not imply endorsement.

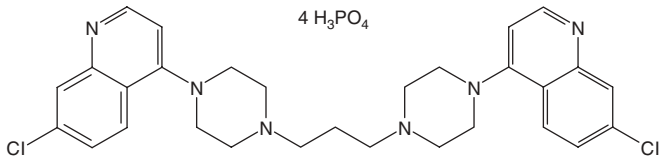

Fig. 1. Chemical structure of piperaquine phosphate (1,3-bis[1-(7-chloro-4′-quinolyl)-4′-piperazinyl] phosphate).

chloro]quinoline; molecular weight 535.51) and also as its water soluble tetra-phosphate salt, piperaquine phosphate (figure 1; C_{29} H_{32} Cl_2 N_6. 4 H_3PO_4; molecular weight 927.48; Rhone Poulenc 13228). Piperaquine base is a pale white to yellow crystalline powder with a melting point of 212–213°C[9] and UV absorption peaks at 225, 239 and 340nm.[10] It is a basic compound (dissociation constant [pKa] = 8.92) that is only sparingly soluble in water at neutral and alkaline pH, but has high lipid solubility ($log_{10}P = 6.16$).[11] Piperaquine phosphate is a white to pale yellow crystalline power, readily soluble in water, slightly bitter, sensitive to light and has a melting point 246–252°C.[12] Although commercially available in China and listed in the Chinese Pharmacopoeia,[13] piperaquine phosphate is not yet included in Western pharmacopoeias. A synthetic 7-hydroxylated derivative of piperaquine (4-(7-chloro-4-quinolinyl)-α-[[4-(7-chloro-4-quinolinyl)-1-piperazinyl]methyl]-1-piperazineethanol;C_{29} H_{32} Cl_2 N_6O; molecular weight 551.51) and its tetra-phosphate salt (C_{29} H_{32} Cl_2 N_6O. 4 H_3PO_4; molecular weight 943.46) have also been synthesised,[14] and shown to have antimalarial activity *in vitro* and in animals and humans.[15-18]

3. Pharmacodynamic Profile

3.1 *In Vitro* Antimalarial Activity

Bisquinolines as a class have received renewed interest in the last decade, with numerous studies showing good antimalarial activity against chloroquine-resistant *Plasmodium* strains.[19-21] The bulky bisquinoline structure may be important for activity against chloroquine-resistant strains, and may act by inhibiting the transporters that efflux chloroquine from the parasite food vacuole.[21,22] In 1992, Vennerstrom et al.[21] examined the activity of piperaquine and 13 other bisquinolines from an N,N-bis(7-chloroquinolin-4-yl) alkanediamines series against chloroquine-resistant *P. falciparum* strains *in vitro* and *P. berghei* strains in mice. Piperaquine and 12 of the 13 other bisquinolines showed a significantly lower resistance index *in vitro* than chloroquine, and good *in vivo* activity against *P. berghei* in mice without significant toxicity. The theory that steric inhibition of transporter-mediated drug efflux mechanisms protects piperaquine from chloroquine resistance is also supported by the activity of other bulky aminoquinoline compounds (including tetra- and trisquinolines) against chloroquine-resistant parasite strains.[23]

A study using electron microscopy showed differing morphological changes in the trophozoites of piperaquine-sensitive and piperaquine-resistant *P. berghei* ANKA strains.[24] Clumps of pigment were seen inside the food vacuole of the piperaquine-sensitive strains (also seen in sensitive parasites treated with chloroquine). Hence, these data suggest that the food vacuole is also the site of action of piperaquine.[25] A recent study in mice infected with *P. berghei* ANKA strain and treated with DHA and/or piperaquine phosphate, showed similar findings in intraerythrocytic trophozoites and gametocytes. Food vacuole membranes and mitochondria became swollen, and multilamellate whirls and abnormal pigment grains were seen within an hour of exposure.[26] Since piperaquine contains the 7-chloro-4-aminoquinoline structure found in all 4-aminoquinoline drugs, it is likely that piperaquine and aminoquinolines such as chloroquine have similar targets. Evidence suggesting the inhibition of the heme-digestion pathway in the parasite food vacuole is most convincing.[22] Haemoglobin, an essential source of nutrient for the parasite, is normally cleaved into the toxic globin and ferric heme (ferriprotoporphyrin IX) and is then detoxified

Table I. Concentration that produces 50% inhibition (IC_{50}) values for piperaquine and chloroquine from *in vitro* resistance studies

Origin of *Plasmodium falciparum* strain	Piperaquine IC_{50} (nmol/L)	Chloroquine IC_{50} (nmol/L)	Reference
South Yunnan, China	320	125	33
Southeast Yunnan, China	228	136	33
Yunnan Province, China	240	60	34
China	320	119	35
Laos	167	114	35
South Yunnan, China	243	125	36
Hainan Island, China	1720	910	37
Chloroquine-sensitive FCC-1/HN	59, 58[a]	81, 64[a]	31
Chloroquine-resistant Cambodian I	61, 89[a]	563, 220[a]	31
Chloroquine-sensitive *P. falciparum*	8.3	8.9	21
Chloroquine-resistant *P. falciparum*	16	100	21
Madagascar	26	70	38
Chloroquine-sensitive *P. falciparum*, Cameroon	36	42	39
Chloroquine-resistant *P. falciparum*, Cameroon	41	201	39

a Values are means of two separate studies.

by polymerisation or biocrystallisation to form structures known as 'haemozin' or malarial pigment.[27] Chloroquine, a highly lipophilic diprotic base, enters the food vacuole by traversing the membrane in its unprotonated form before becoming trapped in the acidic environment within the vacuole. It is hypothesised to act by either directly binding to ferriprotoporphyrin IX and/or by inhibiting enzymatic polymerisation of haemozin.[22,28-30] The tertiary nitrogens are possible sites of protonation on piperaquine and may allow the drug to act in a similar way to chloroquine.

3.2 Parasite Resistance to Piperaquine

Table I summarises *in vitro* parasite sensitivity data for piperaquine and chloroquine. The 50% inhibitory concentration (IC_{50}) of parasite isolates from China suggests that widespread unregulated use of piperaquine as a monotherapy in China since the late 1970s has played a significant role in the development of parasite resistance. Although methodological differences in the *in vitro* test methods make comparisons between studies difficult, IC_{50} values from Chinese isolates during the early 1980s were comparable with those of wild strains from Madagascar where piperaquine had not been used.[31,32] However, the IC_{50} values of isolates collected from southern China increased subsequently in areas where piperaquine was used widely.[31,33-37]

There are conflicting views in the literature on cross-resistance between piperaquine and other antimalarials, in particular chloroquine and artemisinin derivatives.[16,22,31,40-42] In two piperaquine-resistant strains developed *in vitro* by increasing drug pressure (*P. berghei* ANKA and K173 PR strains), cross-resistance was found between piperaquine and hydroxy-piperaquine, artesunate, artemisinin and mefloquine. In other strains, cross-resistance between piperaquine and pyronaridine was reported to be moderate. Studies showing cross-resistance between piperaquine and primaquine are inconclusive, with one reporting complete lack of cross-resistance while another showed minimal cross-resistance.[40,41] A recent study of bisquinolines by Basco and Ringwald[39] found that piperaquine was highly active against *P. falciparum*, with a mean IC_{50} of 39 nmol/L (range 8–78 nmol/L), and was also equally active against chloroquine-sensitive and -resistant strains from Cameroon.

The presence of cross-resistance between piperaquine and chloroquine is supported by other *in vitro* studies of isolates collected from the Chinese provinces of Yunnan and Hainan and the China-Laos border.[33,35-37] Yang et al.[35] reported that >95% of chloroquine-resistant *P. falciparum* exhibited cross-resistance to piperaquine and amodiaquine. How-

ever, there are no clinical or *in vitro* studies to suggest the occurrence of cross-resistance between piperaquine and artemisinin derivatives and/or mefloquine in *P. falciparum*. *In vitro* studies that report the absence of cross-resistance between piperaquine and chloroquine also report a general lack of cross-resistance with other 4-aminoquinolines, including amodiaquine.[22,31,32]

To our knowledge, there are no published studies assessing *in vitro* the possibility of an interaction between piperaquine and artemisinin derivatives. Significant synergism of the combination would help to overcome parasite resistance to either component. In the case of chloroquine, there is evidence of mild antagonism with the artemisinin drugs,[43] but the clinical relevance of this is questionable.

3.3 Animal Studies

Studies in mice infected with chloroquine-sensitive or -resistant strains of *P. berghei* found piperaquine and piperaquine phosphate to have different potencies in prophylactic and therapeutic roles. The doses of piperaquine and piperaquine phosphate required to suppress infections were found to be significantly different (piperaquine base 87 ± 4 mg/kg and piperaquine phosphate equivalent to 65 ± 3mg of base/kg; $p < 0.01$).[42] In the same study, *in vivo* curative doses of piperaquine and piperaquine phosphate against chloroquine-sensitive and -resistant *P. berghei* strains were also determined. ED_{50} (dose producing a 50% effective response) values were 5.0 ± 0.2 mg/kg (piperaquine base) and 4.5 ± 0.1 mg/kg (base equivalent of piperaquine phosphate) in chloroquine-sensitive strains, and 22.3 ± 1.2 mg/kg and 19.1 ± 0.8 mg/kg (piperaquine base and base equivalent of piperaquine phosphate, respectively) in chloroquine-resistant strains. These data suggest low-level cross-resistance between piperaquine and chloroquine.[42] Another study also showed piperaquine phosphate to be more potent than piperaquine base in protecting rodents from *P. berghei* malaria over 45 days (50 and 200 mg/kg).[44] In our view, the differences in potency between piperaquine and piperaquine phosphate seen in some studies are most likely to be related to differences in solubility.

3.4 Clinical Studies in Humans

Piperaquine phosphate was first used for human antimalarial prophylaxis in China in 1979. More than 20 000 residents in six provinces were given piperaquine alone or as piperaquine phosphate in the 'preventive tablet number 3' (equivalent to piperaquine base 150mg with sulfadoxine 50mg).[1,45] In Hainan province, >3000 residents were given 600mg of piperaquine base or four tablets of 'preventive tablet number 3' (piperaquine base 600mg plus sulfadoxine 200mg) each month. Malaria incidence steadily decreased from 2.8% to 1.4% over 3 months, while untreated residents recorded an incidence ranging from 5.8% to 10.3%.[46] These results are consistent with another study that used three or four tablets of 'preventive tablet number 3' (piperaquine base 450–600mg plus sulfadoxine 150–200mg). Malaria incidence over 28 days was significantly different between 973 treated residents (0.13–2.8%) and 7608 untreated controls (9.8–23.2%).[1,47]

After 1979, piperaquine replaced chloroquine as first-line treatment of chloroquine-resistant *P. falciparum* in China. Hence, piperaquine was often used as a comparison arm in studies evaluating efficacy of new drugs and/or formulations (table II).[16,48-52] The doses in the standard clinical regimen mostly varied between the equivalent of piperaquine base 1.5–3.0g given in divided doses over 2 or 3 days.[48] Trials utilising dosages in this range had recrudescence rates ranging from 0% to 37%, and one trial that used a lower dose equivalent to piperaquine base (0.75g) had a recrudescent rate of 80%. With doses of piperaquine base 1.5 or 1.8g, mean fever clearance time ranged from 17 to 36 hours and mean parasite clearance times from 61 to 105 hours. It is important to note that some studies reporting fever and parasite clearance time values outside of these ranges used lower than usual dosages.[49] However, these studies should be interpreted cautiously as sample sizes varied widely (n = 5–56), and the doc-

Table II. Clinical efficacy of piperaquine (base) monotherapy in humans

No. of patients	Total dose (g)	FCT (h) [mean ± SD]	PCT (h) [mean ± SD]	Follow-up (d)	Recrudescence (%)	Resistance	Reference
51	1.5	17 ± 6	105 ± 17	28	37	10 RI; 11 RII; 13 RIII	52
5	0.75	46 ± 53	122 ± 48	28	80	2 RI/RII; 1 RIII	50
53	1.5	36 ± 21	70 ± 21	28	13	7 RI	49
53	1.5	28 ± 22	100 ± 20	28	17	7RI; 2RII	51
15	1.8	NA	60 ± 10	28	27	NA	48
15	3.0	NA	61 ± 19	28	20	NA	48
3	1.5	24–48	48–72	0	NA	NA	53
43	1.8	<72	34–48 (n = 24) 58–72 (n = 19)	7	0	0%	1

FCT = fever clearance time; **NA** = not applicable; **PCT** = parasite clearance time; **RI** = prompt and sustained asexual parasite clearance to day 7 but reappearance before day 28; **RII** = >75% fall in asexual parasitaemia by 48h but not clearance, plus persistent parasitaemia on day 7; **RIII** = <25% fall in asexual parasitaemia by 48h, plus persistent parasitaemia on day 7.

umentation of patient randomisation and follow-up procedures was limited.

The efficacy of piperaquine has also been evaluated in the treatment of vivax malaria.[54] In a study of 280 patients, a total dose of piperaquine phosphate (1.5g over 2 days) was compared with a combination of chloroquine base (1.2g) and primaquine (30mg). The authors concluded that the two regimens had similar efficacy.

3.5 Piperaquine as a Part of Artemisinin-Based Combination Therapy

While the widespread introduction of the potent artemisinin derivatives has proved to be highly successful, it is imperative that they be used in conjunction with a second antimalarial drug in order to prevent the high recrudescence rates seen with short-course therapy.[55,56] This therapeutic strategy is termed artemisinin combination therapy (ACT) and has been widely advocated as the most appropriate strategy for antimalarial treatment.[57-59] However, the choice of a partner drug depends primarily on cost, tolerability and pre-existing drug resistance. Piperaquine rates well on all three of these issues and, therefore, appears to be an excellent partner drug for ACT.[2,8,60]

Combinations of piperaquine phosphate with DHA produced and marketed by Chinese and Vietnamese pharmaceutical companies are summarised in table III. Although DHA has had limited clinical use as monotherapy, its oral absorption is comparable with that of artesunate, which is most often used in combination with mefloquine.[3,61]

CV8® was the first DHA and piperaquine phosphate combination to be incorporated into national treatment recommendations in Vietnam. There are plans for extensive postmarketing surveillance.[3] In central and southern Vietnam, a 3-day regimen is used as the first-line treatment and local journals have reported a 28-day cure rate of at least 96%.[4,5,62,63] A recently published study has shown it to be as effective as atovaquone-proguanil for treatment of uncomplicated malaria (28-day cure rates 94% and 95% for CV8® [n = 84] and atovaquone/proguanil [n = 81], respectively).[64] With good tolerability and low cost,[65] the combination has advantages over artesunate-mefloquine, which is the usual ACT used in Vietnam.[66] Nevertheless, data on the pharmacokinetics and efficacy of the product are limited and safety in pregnancy, lactation and young children are lacking. Artecom®, which contains piperaquine phosphate, DHA and trimethoprim, is currently registered in China and Vietnam[67] but has had limited use. It has been superseded by Artekin® and Duo-Cotecxin®.

The first published report of the efficacy of Artekin® in humans was of a study of 106 Cambodian patients (76 children and 30 adults) with uncomplicated falciparum malaria.[8] The study showed excellent efficacy using a four-dose regimen (mean total doses according to age were DHA 6.6–10.1 mg/kg and piperaquine phosphate 52.9–81.2 mg/kg)

delivered over 32 hours, with 98.6% and 92.3% 28-day cure rates in children and adults, respectively.[8] A subsequent study using the same regimen in a further 62 Cambodian patients (32 adults, including ten with *P. vivax*, and 30 children with *P. falciparum*) performed in an area of high chloroquine resistance demonstrated 100% 28-day cure rates in all groups.[68] The findings of these two studies were subsequently confirmed in a large, randomised trial of Artekin®, Artecom® and artesunate-mefloquine.[2] The 56-day cure rate was 98.7% in 166 patients treated with Artekin® using similar dosages to those in the first report[8] administered over 48 hours. Artecom® also proved to be highly effective in this study, with a 56-day cure rate of 97.4% in a group of 157 patients. The 56-day cure rate for artesunate-mefloquine was 98.7%

4. Pharmacokinetic Profile

4.1 Animal Studies

A study in 1979 using ^{14}C-labelled piperaquine phosphate found that absorption from the gut in mice was rapid with a high systemic availability (80–90%).[69] During the 1-month observation period, piperaquine accumulated preferentially in the liver, kidney and spleen and the calculated half-life ($t_{1/2}$) was 9 days. However, these studies measured total ^{14}C and the results are of limited value as radiolabeled metabolites may also have been present, which contributed differentially to the total ^{14}C measurements over time.[69] A preliminary investigation using solvent extraction and paper chromatography on urine from two mice fed ^{14}C-piperaquine found no evidence for the presence of radiolabeled metabolites.[69] In a WHO report reintroducing piperaquine as a candidate antimalarial agent,[3] and in an animal toxicology study by Sheng et al.,[70] its $t_{1/2}$ in dogs was quoted as 9.4 days, but details of how this figure was derived were not given in either publication.

4.2 Clinical Studies in Humans

The first pharmacokinetic data of piperaquine in humans were published by Hung et al.[71] from studies in Cambodian children and adults with uncomplicated *P. falciparum* and *P. vivax* malaria treated with Artekin 2® tablets containing DHA 40mg and piperaquine phosphate 320mg or Artekin 2® granules for dissolution in water (DHA 15mg, piperaquine phosphate 120mg per sachet; used in younger children). Four equal doses were administered at 0, 6, 24 and 32 hours with mean total doses of piperaquine base of 32–35 mg/kg. Using a population pharmacokinetic approach, a two-compartment open model with first-order absorption, with or without a lag time, was fitted to the data. Simulated concentration-time plots (using the population parameters reported by Hung et al.[71]), for both adults and children with malaria are shown in figure 2. Absorption was slow, with mean absorption half-times ($t_{1/2,abs}$) of 9.1 and 9.3 hours in adults and children, respectively. The mean terminal elimination half-life ($t_{1/2,z}$) was long in both adults (543 hours) and children (324 hours), while the mean volume of distribution at steady state/bioavailability (V_{ss}/F) was very large in adults (574 L/kg) and children (614 L/kg). Clearance/bioavailability (CL/F) was approximately twice as high in children (1.85 L/h/kg) compared with adults (0.9 L/h/kg). It is of interest that the Artekin® formulations used in these studies achieved plasma piperaquine concen-

Table III. Contents of piperaquine phosphate and other ingredients in combination antimalarial tablets

Trade name	Piperaquine phosphate (mg)	Dihydroartemisinin (mg)	Trimethoprim (mg)	Primaquine (mg)
CV-8®[a]	320	32	90	5
Artecom®[a]	320	32	90	0
Artekin®[b], Duo-Cotecxin®[c]	320	40	0	0

a Central Pharmaceutical Factory 26, Ho Chi Minh City, Vietnam; Tonghe Pharmaceutical Co. Ltd, Chongqing, PR China (http://www.artecom.com.cn).

b Holleykin Pharmaceutical Co. Ltd, Guangzhou, PR China.

c Beijing Holley-Cotec Pharmaceuticals Co. Ltd, Beijing, PR China (http://www.cotecxin.com).

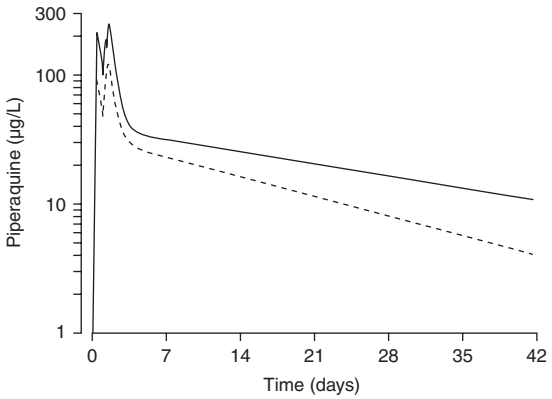

Fig. 2. Simulated piperaquine concentration-time profiles in adults (solid line), and children (dotted line) following oral piperaquine doses at 0, 6, 24 and 32 hours (total dose 32 mg/kg as base). Profiles are based on the population pharmacokinetic model and mean pharmacokinetic parameters for patients with falciparum or vivax malaria, as reported by Hung et al.[71]

trations that were generally above reported IC_{50} values for piperaquine (4.4 and 8.6 mg/L in sensitive and resistant *P. falciparum* strains, respectively).[21]

Piperaquine concentrates moderately in red blood cells with a mean red blood cell **:** plasma ratio of 1.5 at 46% haematocrit and over the plasma concentration range 50–500 µg/L.[9] Plasma protein binding of piperaquine has not been measured directly but is estimated to be around 97%.[9] Lindegårdh[72] has shown that piperaquine concentrations in EDTA and heparinised plasma are equivalent, while serum concentrations prepared from the same blood are approximately 58% higher.[72] This may be because the clotting process releases drug concentrated in leucocytes and/or thrombocytes.[72]

A recent crossover study in healthy Caucasian volunteers compared the bioavailability of piperaquine tablets (piperaquine phosphate 500mg = piperaquine base 289mg) fasting and after a standard high-fat breakfast.[73] After the high-fat meal, both maximum serum concentration and area under the concentration-time curve from 0 to last timepoint increased significantly ($p < 0.016$), by 172% and 134%, respectively, suggesting that the absorption of piperaquine may be facilitated by the presence of fat in the diet.

The metabolism of piperaquine in humans has not been studied in detail. Hung et al.[9] found no evidence for conversion to 7-hydroxy piperaquine, while a recent study of piperaquine oral bioavailability in volunteers reported that a minor unidentified putative metabolite peak in plasma (high-performance liquid chromotography [HPLC] retention time 11.5 minutes versus 4.8 minutes for piperaquine) from some individuals.[73] Lindegårdh et al.[72,74] have also documented the presence of a possible metabolite that appears in blood samples taken about 2 hours after oral piperaquine administration and is detected as a small peak with a longer HPLC retention time than piperaquine. Chromatograms published by Lindegårdh et al.[74] suggest that there may be a more polar metabolite as well. Since piperaquine has no primary functional groups that could form phase 2 polar metabolites, it seems likely that a phase 1 oxidative process somewhere on the ring structures may be a necessary prerequisite step for production of this putative metabolite.

Similar pharmacokinetic characteristics are seen with other highly lipid-soluble antimalarials. For example, Vd_{ss}/F is large and $t_{½,z}$ relatively long for chloroquine (115 L/kg, 3–14 days, respectively),[75] halofantrine (125 L/kg; 1–3 days)[76] and mefloquine (19 L/kg; 17 days).[77] Administration of mefloquine,[77] atovaquone[78] or halofantrine[76] with a fatty meal has also been shown to significantly increase the area under the respective plasma concentration-time curves. The high molecular weight of piperaquine, together with its pharmacokinetic properties suggests that it may undergo enterohepatic recycling. Interestingly, Chen et al.[69] have presented radiolabeled distribution and excretion data that they interpreted as supporting enterohepatic recycling as the main pathway for piperaquine excretion in mice.

5. Toxicity and Tolerability

5.1 Animal Studies

Sheng et al.[70] determined the LD_{50} (dose that is lethal to 50% of animals tested) and ED_{50} for

chloroquine and piperaquine in mice. On a molar basis, the data suggest that piperaquine is less toxic than chloroquine, with the putative therapeutic index for piperaquine being over five times greater than that for chloroquine (table IV).[70] In dogs administered piperaquine phosphate 100 or 200 mg/kg orally (n = 9 and 4, respectively), vomiting occurred in 67–75% and tremor in 11–25%, while one of four animals died after the 200 mg/kg dose.[70] Acute toxicity was also investigated in 13 monkeys administered doses of piperaquine phosphate ranging from 25 to 200 mg/kg. Tremor was absent and there were no deaths, while vomiting was frequently seen at the 100 and 200 mg/kg doses.[70]

In rabbits, acute cardiovascular toxicity of piperaquine phosphate was compared with that of chloroquine phosphate by determining the cumulative intravenous doses that caused a fall in blood pressure or electrocardiographic abnormalities.[70] In general, piperaquine had a significantly better cardiovascular toxicity profile than chloroquine. The dose needed to produce a 40mm Hg lowering of blood pressure was 2.6 times higher for piperaquine (42 mg/kg) than chloroquine (16 mg/kg). Prolongation of the electrocardiographic PR interval and QRS duration was commonly observed (70% and 40% of rabbits for piperaquine, and 100% and 100% of rabbits for chloroquine, respectively), but the cumulative dose of piperaquine associated with this toxicity (50–56 mg/kg) was about 5-fold higher than that for chloroquine (9–11 mg/kg). Atrioventricular block was present in 100% of rabbits after a cumulative chloroquine dose of 19 mg/kg, whereas only two animals showed this effect with piperaquine at a cumulative dose of 50 mg/kg. ST segment depression was seen in 90% of rabbits with piperaquine (35 mg/kg) but was not seen with chloroquine.

Table IV. LD_{50} (dose that is lethal to 50% of animals tested), ED_{50} (dose that produces a 50% effective response) and therapeutic index of piperaquine phosphate and chloroquine phosphate in mice

Drug	LD_{50} (μmol/kg)	ED_{50} (μmol/kg)	Therapeutic index
Piperaquine phosphate	1184	4.3	275
Chloroquine phosphate	849	20	42

The chronic toxicity of piperaquine phosphate was also investigated by Sheng et al.[70] in two studies in dogs and one in monkeys. In the first study in dogs, piperaquine was given at a dose of 100 mg/kg weekly for 14 weeks. Serum ALT, a sensitive indicator of hepatocellular damage, was consistently elevated in one of four treated dogs and occasionally raised in the other three, but these abnormalities resolved rapidly after cessation of the drug. Blood urea nitrogen was not altered by piperaquine treatment.

In a second study,[70] weekly haematological and blood chemistry studies were performed in six dogs during and after piperaquine 6 mg/kg weekly for 6 months. Hepatotoxicity was the main feature, with raised serum ALT levels in two dogs. Minor reversible depression of total white blood cell and neutrophil counts was also observed. These latter changes were not attributed to bone marrow depression. There were no urinary abnormalities and histopathological examinations of kidney sections were also normal at the end of the study. By contrast, histopathological examination of the liver at study end showed changes in all animals. There were areas of cell proliferation and fibrosis as well as phagocytes and Kupffer cells with brown inclusions. These changes were less evident 3 months after the dosing was ceased and were also less evident for the same dose administered over a 2-week period.

In the study in monkeys,[70] piperaquine was given as 25 mg/kg weekly for 3 months or 50 mg/kg every 2 weeks for 5 months. Histopathological changes in the liver at the end of these periods were less evident than in the dog experiments. As in dogs, there was a mild reversible depression of total white cell and neutrophil counts but no suppression of bone marrow.

Consistent with studies in dogs and monkeys, a study of liver ultrastructure in mice treated acutely with piperaquine phosphate 1000 mg/kg and sulfadoxine 100 mg/kg revealed immediate (4 hours after dosing) evidence of damage to hepatic mitochondria, and both smooth and rough endoplasmic reticulum.[79] These changes gradually decreased over time and 2–3 months later most cells were

normal, although some hepatocytes still contained fatty inclusions.

In vitro, piperaquine phosphate had no mutagenic effects in the Ames test, chromosome analysis assays or in sister chromatid exchange rates.[70] The offspring of pregnant mice administered piperaquine phosphate 40–360 mg/kg for 6 days, starting at day 9–14 of gestation, showed no evidence of embryotoxicity or teratogenicity.[70]

5.2 Clinical Studies in Humans

Overall, studies have shown piperaquine monotherapy to be well tolerated with few patients reporting adverse events.[2,48,50-52] The situation is similar in studies of piperaquine administered as part of ACT.[2,8,64] Common minor complaints have included mild headache, dizziness, nausea, abdominal pain and vomiting, although these symptoms are often difficult to distinguish from symptoms resulting from malaria itself.[48,50-52,71] In early Chinese studies, no haematological, biochemical, cardiac or hepatic abnormalities were described, but it is unclear whether they were specifically assessed.[48,50-52]

Given the toxicity profile described in animal studies of piperaquine[70] and the pattern of toxicity observed in humans from other closely chemically related aminoquinoline antimalarial drugs, the most important potential toxicity of piperaquine would relate to effects on cardiac conduction, blood pressure regulation and on glucose metabolism. However, a detailed safety and tolerability evaluation performed by Karunajeewa et al.[68] in 62 Cambodians (including 32 adults and 30 children) with uncomplicated malaria showed no significant electrocardiographic changes (including the corrected QT interval), changes in plasma glucose or postural hypotension following treatment with Artekin® at the manufacturer's recommended doses. Concurrent pharmacokinetic data from these patients suggest that peak piperaquine concentrations of <800 μg/L are not associated with clinically significant cardiotoxicity. There are no published data relating to the safety of piperaquine in pregnancy, lactation or children younger than 2 years. Although extensive experience with other closely related aminoquinoline drugs such as chloroquine in these patient groups suggests that piperaquine is also likely to be safely used, use of piperaquine during pregnancy and lactation cannot be recommended at this time.

6. Conclusion and Current Status of Piperaquine as an Antimalarial Drug

Piperaquine is an aminoquinoline antimalarial with a favourable safety and toxicity profile. It is effective against *P. vivax* and *P. falciparum*, including strains of *P. falciparum* resistant to chloroquine. However, when used extensively as monotherapy, parasite resistance to piperaquine can develop. Its tolerability, effectiveness, pharmacokinetic profile and low cost make it a promising partner drug for use as part of short-course ACT. Co-formulations of piperaquine with DHA (Artekin® and Duo-Cotecxin®) have proved highly effective, well tolerated and are available at a cost as low as $US1.00/adult treatment course (2004 value).[2] This makes them potentially affordable to many countries where the burden of disease due to malaria is greatest. Indeed, piperaquine is part of national anti-malarial drug policy as monotherapy (China) or ACT (CV8® in Vietnam), while Artekin® (Duo-Cotecxin®) is currently registered in a number of South-East Asian countries.

It has been recommended that ACT incorporate a short $t_{1/2}$ artemisinin derivative and an effective partner drug with a relatively long $t_{1/2}$ (>4 days).[2] Although long $t_{1/2}$ antimalarial agents such as chloroquine are particularly likely to induce resistance in *P. falciparum,* there is evidence that ACT can protect against this. In a study from Thailand, the addition of artesunate to mefloquine halted the progression of established mefloquine resistance;[80] however, this study was from a low transmission area. In areas of high transmission, such as found in sub-Saharan Africa, the frequency of malaria infection and low blood piperaquine concentrations in the tail of the elimination phase could, through selection of resistant parasite strains, limit the lifespan of treatments such as Artekin®.

The raw material piperaquine is not presently manufactured to Good Manufacturing Practice

(GMP) standards, and no piperaquine-containing preparations are registered with the US FDA or similar international drug regulatory bodies. Artemisinin derivatives have been in a similar position and yet are widely used throughout the tropics. Although this does not justify the development of non-GMP piperaquine-based ACT, it is not surprising that formulations such as Artekin® have been developed and distributed in the same way. However, it is encouraging to note that the Medicines for Malaria Venture organisation is actively involved in the development of piperaquine-based ACT which includes production of product to GMP standards.[81]

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