Review Article

Indian J Med Res 127, April 2008, pp 305-316

Chloroquine: Novel uses & manifestations

R.G. Cooper & T. Magwere*

*Division of Physiology, Faculty of Health, Birmingham City University & *University of Leeds, Faculty of Medicine & Health, St. James' University Hospital, Cancer Research Building, Leeds, UK*

Received March 13, 2007

Chloroquine (CHQ) is a cheap, relatively well tolerated drug initially developed for the treatment of malaria in the 1930s. CHQ has, however, since accrued a plethora of uses in the treatment and amelioration of several other diseases and conditions because of its lysosomotropic properties. It also has characteristic physiological and systemic effects. This review gives an overview of the history and pharmacology of CHQ, and progresses to consider some of the mechanisms that may underlie its biochemical and physiological effects. Additionally, an overview of some of the novel uses of CHQ in the treatment of viral infections and cancer are presented. The antimalarial mechanisms of CHQ were not discussed in this review. The message is that CHQ, despite its well-documented toxicity and adverse side effects may have important future uses that are associated with its lysosomotropic and immunomodulatory mechanisms. The possibility exists therefore that CHQ might be re-introduced into regular malaria treatment.

Key words Anti-inflammatory - antimalarial - chloroquine - malaria - pharmacology - toxicology

Introduction

The popularity of chloroquine [7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline, CHQ] for malaria treatment in many Third World countries emanates from it being cheap, widely available, relatively well tolerated, and having a rapid onset of action[1-5]. CHQ is commonly sold as an over-the-counter medication and is also used as an anti-inflammatory drug in the treatment of rheumatoid arthritis[6-8], discoid lupus erythematosus[9-10] and amoebic hepatitis[11]. CHQ inhibits pro-inflammatory cytokine release into human whole blood and may be of therapeutic benefit not only during chronic inflammation, but also in diseases that are related to bacteria-induced inflammation[12].

CHQ synthesis

CHQ was first synthesised in Germany by Bayer Corporation in 1934 as a cheaper alternative to the costly naturally occurring quinine, but was then considered toxic for any significant biological use[13]. However, as the demand for cheaper, readily available antimalarial drugs escalated during World War II, CHQ received a new lease of life and was subsequently discovered to be more effective than the costly quinine or quinidine against intra-erythrocytic malarial parasites[13]. For the following two decades thereafter (1946-1966), CHQ emerged as the drug of choice for treatment and prophylaxis of malaria in most disease-endemic tropical countries[14]. The disadvantages of

quinine/quinidine are that they are really toxic and have a short half-life[15].

Properties of CHQ and its usage

CHQ is a bitter, colourless, dimorphic crystalline powder soluble in water at pH 4.5, but less so at more neutral or alkaline *p*H. It therefore dissolves rapidly in the stomach (*p*H 2.0). CHQ's bitter taste may be masked following administration in drug-loaded hydrogel beads enclosed in hard gelatin capsules[16]. CHQ has a quinoline ring like that of quinine and a side chain identical to that of quinacrine; and the chloride atom in the seventh position appears to be crucial to its antimalarial activity[17]. It specifically inhibits the malaria parasite's digestive pathway for haemoglobin[9]. There are two enantiomers, the (-)-chloroquine being less active than (+)-chloroquine enantiomer against chloroquine-resistant strains of *Plasmodium falciparum*[9]. Comparative antimalarial drug trials in humans revealed that CHQ was a more effective antimalarial than quinidine and quinine[13]. Subsequently, it was developed as the first choice drug for prophylaxis and treatment of all types of malaria due to susceptible strains of *P. falciparum, P. ovale, P. vivax* and *P. malariae*[17].

The major vector of malaria in Africa is the *Anopheles gambiae* complex[18]. Malaria remains a major cause of mortality and morbidity in Africa, and there is a need to utilize effective prevention and intervention methods to combat the spread of the infection[19,20].

From 1966 onwards an emergence of CHQ resistence in malaria parasites was seen worldwide[20-24]. Indeed, four countries in Africa (Malawi, Kenya, Botswana and South Africa) now deploy pyrimethamine-sulphadoxine as their first-line antimalarial[25]. It is believed that factors such as inadequate dosing, incomplete courses of therapy, indiscriminate and inappropriate use, and reliance on less effective medications, have contributed to the emergence and spread of resistant parasites[26,27]. Even in the presence of CHQ resistance the drug may still be quite useful especially in areas with high communal immunity[28]. In a study from Ghana, a significantly higher proportion of inappropriateness of CHQ use was a factor influencing the lower sensitivity of *P. falciparum*[29].

To ensure its efficacy, and when alternative drug combinations are inaccessible, CHQ may be co-administered with calcium channel blockers, tricyclic anti-depressants and anti-histamines resulting in maintenance of CHQ levels[30]. Other studies using cyproheptadine have shown it to reverse resistance to CHQ in strains of *P. falciparum* both *in vivo* and *in vitro*[31]. Significant protection against CHQ-resistant malaria in mice has been shown using Menhades-fish oil[32]. Bio (benzyl) polyamine analogues have also been shown to inhibit both CHQ-resistant and CHQ-sensitive strains of *P. falciparum in vitro*[33]. Other studies report the effectiveness of gold-CHQ complexes against resistant strains[34]. Antimalarial drug combinations of CHQ and primaquine have been reported to reduce therapeutic failure in CHQ-resistant *P. vivax* infection[21], as has pyrimethamine-sulphadoxine-CHQ combinations[25]. The judicious use of such drug combinations with CHQ may help to avoid development of resistance and combat resistant infections[35,36]. Indeed, if the combined treatment translates into a 3-5 yr extension in the useful lifespan of CHQ, the overall cost would be less than that of developing the next, more expensive alternatives (mefloquine and quinine). As a response to increasing levels of resistance to antimalarial medicines, the WHO recommended that all countries experiencing resistance to conventional monotherapies, such as chloroquine, amodiaquine or sulphadoxine–pyrimethamine, should use combination therapies, preferably those containing artemisinin derivatives (ACTs – artemisinin-based combination therapies) for falciparum malaria[37]. Although combination therapies can be effective in reducing/reversing incidences of CHQ resistance in parasites[38], the WHO recommends that combination therapies be limited to: *(i)* artemether/lumefantrine; *(ii)* artesunate plus amodiaquine (in areas where the cure rate of amodiaquine monotherapy is greater than 80%); *(iii)* artesunate plus mefloquine (insufficient safety data to recommend its use in Africa); and *(iv)* artesunate plus sulphadoxine/pyrimethamine (in areas where the cure rate of sulphadoxine/pyrimethamine is greater than 80%).

Amodiaquine plus sulphadoxine/pyrimethamine may be considered as an interim option where ACTs cannot be made available, provided that efficacy of both is high.

Absorption, metabolism and excretion of CHQ

When administered orally, CHQ is rapidly and almost completely absorbed from the gastrointestinal tract with a bioavailability of 75-80 per cent[39,40]. Other routes of CHQ administration include subcutaneous, intramuscular and rectal[41]. Maximum plasma concentrations are reached in 1-2 h[3,8,42] and remain up to 3.6 hours[40] after administration. CHQ has a large

apparent volume of distribution in the body (160-800 l/kg)[25]. It has a half-life ranging from 2-3 days in rats[39,43,44] and up to 3-6 days in humans[11]. The elimination half-life of CHQ however varies greatly between plasma and tissue in humans, ranging from 2-3 days in plasma to over 300 h in tissues where the drug is more avidly bound[45]. Up to 70 per cent of an ingested dose of CHQ is excreted unchanged in the urine and the parent drug can still be detected in the urine up to 120 days following a single 300 mg dose in humans[46]. The total plasma clearance of CHQ is approximately 600 ml/min[47] and the renal clearance is about 400-450 ml/min[40]. The distribution of CHQ within human blood is also important because the malaria parasite is intraerythrocytic during schizogony[48]. CHQ-induced redistribution of a neutral aminopeptidase may be the cause of haemoglobin accumulation in endocytic vesicles of malaria parasites[49].

CHQ is a weakly basic tertiary amine and is metabolized by oxidative deamination, elimination of the side chain, and N-oxidative pathways. It is oxidatively de-ethylated to give mono-desethylCHQ (30-40% of blood CHQ concentration) and then to bis-desethylCHQ (5-10% of blood CHQ concentration)[50]. Available data suggest that the enzymes responsible for CHQ metabolism in humans are the cytochrome P-450 (CYP) isoforms CYP3A, CYP2C8, and CYP2D6[51-53]. The liver transforms approximately 30-50 per cent of the administered CHQ, although extrahepatic sites of microsomal metabolism could also be of clinical significance in view of the extensive tissue distribution of CHQ and the extrahepatic distribution of CYP3A isoenzymes[51]. Mono-desethylCHQ is the main metabolite of CHQ and it has been shown to have the same anti-malarial activity against CHQ-susceptible *P. falciparum* as the parent compound[54]. Bis-desethylCHQ is metabolized to a 4'-hydroxy-compound, which is further oxidised to its 4-carboxylic acid derivative. Further dealkylation of the CHQ side chain results in the production of 7-chloro-4-aminoquinoline[55-56]. In studies conducted on intravenous administration of CHQ at 2.5 mg/kg in rats, the excretion of mono-desethylCHQ and bis-desethylCHQ was 25 and 64 per cent, respectively with maximum urinary excretion on the first day[39]. Renal clearance of mono-desethylCHQ accounts for 65 per cent of the apparent total clearance of CHQ[57].

Renal effects of CHQ

Current evidence suggests that CHQ may affect kidney function when taken either during treatment or prophylaxis of malaria or administered acutely or chronically in rats[58-60] probably due to its accumulation in kidney cells[56]. The accumulation of CHQ in tissues may result from inhibition of anti-malarial microsomal metabolism in kidney cells and potentiate its uptake in lysosomes in the cytoplasm[61]. CHQ, which is also deposited in the adrenal glands[62], may indirectly affect kidney function by modulating the secretory patterns of aldosterone to cause a reduction in tubular Na^+ handling. The deposition of CHQ in the epithelial cells of the kidney may result in a possible interference with ion movements[43,63]. CHQ also causes vasodilatation and cardiac depression in rats[64]. This may alter perfusion pressure of the kidney and renal haemodynamics, and affect renal fluid and electrolyte handling. The lowering of Na^+-K^+-ATPase activity by CHQ is evidenced in the inhibition of renal brush border enzyme mediated carrier transport[65]. The influence of CHQ on renal fluid and electrolyte handling necessitates monitoring of kidney function in patients who consume the antimalarial in malaria endemic areas.

Chronic administration of CHQ has been reported to cause Na^+ retention possibly via increase in plasma aldosterone concentrations[59,66] and renal Na^+-K^+-ATPase activity[67]. It is not uncommon for people on CHQ prophylaxis to consume ethanol and/or analgesic drugs. The co-administration of CHQ with other drugs or substances that are substrates of the CYP enzymes (*e.g.*, ethanol and paracetamol) can result in adverse effects to the kidney[68-70]. It was shown that concurrent administration of CHQ and ethanol induced extensive damage to the proximal tubules and collective duct cells of the kidney[70]. Recently it has also been proposed that the impairment of renal function by CHQ may be due to its modulatory effects on the renal tubular response to vasopressin, either directly by inhibiting cyclic AMP generation or indirectly via induction of nitric oxide (NO) production[71]. If NO is involved in the mediation of CHQ-improved insulin sensitivity, then the administration of inducible nitric oxide synthase (iNOS) blockers might halt or reverse glucose-induced insulin secretion in pancreatic β-cells[72]. The critical role of nitric oxide in renal failure is underscored by findings that inhibition of iNOS in rats[73] or iNOS knockout mice[74] protects against acute renal failure.

Slow intravenous infusion of CHQ has been reported to alter kidney function by increasing urinary Na^+ excretion[58]. Plasma arginine vasopressin (AVP) concentrations increase in rats following acute CHQ administration presumably to increase urinary Na^+

excretion[60,74-77]. Natriuresis may also occur due to CHQ-induced synthesis of nitric oxide, which inhibits renal Na^+-K^+-ATPase activity[78,79]. Renal ion handling may be further potentiated by CHQ-induced, nitric oxide mediated inhibition of endothelial cell proliferation[80].

Three consecutive days of oral CHQ administration has been reported to cause Na^+ retention possibly via increases in plasma aldosterone concentrations[59,66,69,70,76,81] and renal Na^+-K^+-ATPase activity[67].

Pathomorphological influence of CHQ on the liver and kidney

CHQ is a potent autophagic drug that may lead to cellular degradation of hepatocytes in the liver with the concurrent production of vacuoles[82-84]. An initial decrease in the number and volume of mitochondria has also been reported[83] due to their sequestration in autophagic vacuoles. Observed increases in the numbers of lysosomes suggest further cellular degradation. This is accompanied by fusion of lysosomes with autophagic vacuoles resulting in the biogenesis of new lysosomes[84]. CHQ accumulates especially in the Kupffer cells of the liver with resultant lysosomal damage including overloading of the liver lysosomes with non-digestible material, and an increase in their size and number[85]. The reported accumulation of CHQ in lysosomes[86,87] has an apparent destabilising effect on lysosomal membranes[88,89].

Colombo and Bertini[90] argued that the biological and pharmacological actions of CHQ are directly related to its interaction with lysosomal membranes. CHQ, however, decreases the density of hepatocyte lysosomes, although it has no effect on sinusoidal cell lysosome density. Such a difference could result from the fact that sinusoidal cell lysosomes do not accumulate CHQ to the same extent as hepatocyte lysosomes, despite the former contributing to more than 40 per cent of the volume occupied by lysosomes in the liver[91]. The density decrease of lysosomes caused by CHQ has been reported to be due to their accumulation of the drug and their subsequent osmotic swelling[92,93]. Singh *et al* [94] reported additional effects of CHQ on organelles in rat hepatocytes as shown by increases in volume densities of mitochondria, lysosomes, rough and smooth endoplasmic reticula, and Golgi apparatus, and with a concomitant decrease in functional activity[95].

Currently, there are only a few investigations on the effect of CHQ on kidney morphology[70,96,97]. CHQ may exert its renal effects indirectly via histopathological and ultrastructural cardiac damage[98] through reductions in glomerular filtration rate (GFR). Given the importance of hepatic microsomal degradation of CHQ, an alteration in liver morphology by the antimalarial[56] is likely to result in an impairment of its metabolism and an increase in its circulating levels. This is likely to result in an impairment of kidney function due to its reported accumulation in cells therein[43,63]. Investigating the effects of long-term oral CHQ administration on possible alterations of kidney structure may help to partially explain previously observed renal effects of CHQ on fluid and electrolyte balance[58,59].

Effects of CHQ on cellular enzymes

The mechanisms underlying the physiological and systemic effects of CHQ are poorly understood. However, there is a growing body of literature to suggest that some of these effects may be exerted through interactions with cellular enzymes. Antimalarial drugs including CHQ were first documented to inhibit glucose 6-phosphate dehydrogenase activity *in vitro*[99]. Inhibition of drug metabolizing enzyme systems both *in vivo* and *in vitro* were described later[67] and it was subsequently demonstrated that additional effects of CHQ included alterations in phospholipid compositions of microsomes[100]. Decreases in CYP-mediated microsomal aminopyrine-*N*-demethylase, aniline hydroxylase, and cytosolic glutathione S-transferase activities were also observed in rats following administration of CHQ. Other enzyme systems such as phospholipase A1 and A2 and lysophospholipase activities are also inhibited by CHQ and related drugs *in vitro*[101]. Mitochondrial NADH dehydrogenase, succinate dehydrogenase, and cytochrome C oxidase activities are reduced following CHQ treatment in rats[95]. Recent studies have shown that antimalarial drugs including CHQ decrease cytochrome *aa*3 and *b* content and adversely affect mitochondrial energy transduction *in vivo* by acting as uncouplers of oxidative phosphorylation[102]. The uncoupling effect of CHQ and other antimalarials was shown to be specific for sites II and III of phosphorylation but did not affect site I[102]. Modulation of drug metabolizing enzymes by CHQ can lead to significant drug-drug interactions *in vivo* that lead to the psychotic side effects of some antidepressants and neuroleptic drugs[103].

Administration of CHQ to rats was shown to also cause alterations in several hepatic and renal antioxidant enzymes thereby inducing an oxidative stress in these organs[104,105]. When given to rats orally at 20 mg/kg once a week for 4 wk, CHQ caused an oxidative stress in rat

liver as shown by elevated activity of superoxide dismutase and decreases in H_2O_2-decomposing enzymes such as catalase and glutathione peroxidase. Increased markers of lipid peroxidation were increased in these organs confirming the extent of oxidative damage following the CHQ administration[104]. CHQ thus increased the intracellular levels of H_2O_2, a condition that exacerbated the susceptibility of rat organs to lipoxidative damage from subsequent oxidative challenges with menadione (30 mg/kg) or CCl_4 (1.25 ml/kg)[105]. In recent studies, it has been proposed that the retinopathy[106] and genotoxicity[107] exhibited by CHQ is due to its ability to induce intracellular and intra-organ oxidative stress/damage; and it is plausible that CHQ-induced organ failure could be exerted through such mechanisms.

CHQ induces the expression of iNOS[108], a property that is responsible for many of its physiological effects in organs such as the kidneys. It was shown that CHQ at non toxic concentrations (10-100 μM) could activate tyrosine kinase and protein kinase C to induce p38MAPK activation resulting in induction of iNOS expression and increased NO production in glioma C6 cells[108]. The stimulatory effects of CHQ on NO production were also demonstrated in mouse, pig, and human endothelial cells *in vitro*[80] and are thought to be mediated via a CHQ-induced impairment of iron metabolism. In patients with rheumatoid arthritis, CHQ has hormone-like effects in that NO production stimulates glucose-induced insulin secretion as well as preventing degradation of insulin[109]. The effects of CHQ on NO production, however, seem to be cell type-dependent. In murine peritoneal macrophages that have been stimulated with either interferon-gamma (IFN-γ) or bacterial lipopolysaccharide (LPS), CHQ inhibited iNOS activity and NO synthesis in a dose-dependent manner[110]. The inhibition of NO production by CHQ in macrophages occurred at both mRNA[110] and protein[111] levels where decreases in these cellular components were observed following exposure to the drug.

Effects of CHQ on cytokines and the immune system

The lysosomotropic effects of CHQ are widely believed to be responsible for its anti-inflammatory properties and effectiveness in the treatment of some autoimmune diseases[112]. CHQ was shown to decrease the production of the pro-inflammatory cytokines IFN-γ, tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in LPS- or phytohemagglutinin-stimulated peripheral blood mononuclear cells[113], and also augmented LPS-induced expression of TNF-α, IL-1α, IL-1β and IL-6 in monocytic and microglial cells[114]. When administered alone however, CHQ induced, rather that inhibited, the production of pro-inflammatory cytokines in astroglial cells through activation of the transcription factor NF-κB[114]. Park and colleagues[114] concluded that CHQ could induce either anti-inflammatory or pro-inflammatory responses in the CNS depending on the cellular context.

CHQ also exerts anti-inflammatory effects via non-lysosomotropic mechanisms[115]. CHQ was shown to inhibit TNF-α release in macrophages through inhibition of TNF-α mRNA synthesis, thereby showing it can also disrupt gene transcription[115-117] but does so without interfering with posttranslational modification or release of the cytokine from macrophages[118]. Jang and colleagues showed that CHQ also interfered with macrophage function by blocking the conversion of cell-associated TNF-α to mature protein, and reduced the levels of IL1β and IL-6 mRNA by altering their stability in a pH-dependent manner[118]. In human histiocytic U-937 cells, CHQ was shown to decrease cell surface expression of TNF-α receptors by retarding their transport to the cell surface[119]. The blocking of pro-inflammatory cytokines by CHQ was shown to be protective against LPS- and *Escherichia coli* DNA-induced inflammatory responses and/or sepsis in mice[120]. CHQ also inhibits cytokine release into human whole blood, an effect that could be beneficial in diseases that are related to bacterial-induced inflammation[12]. These anti-inflammatory properties of CHQ could have photoprotective effects in conditions such as lupus erythematosus[121] and could be exploited in the amelioration of conditions such as post-transfusion graft-versus-host disease[122].

The immunomodulatory effects of CHQ could theoretically have deleterious implications for diseases whose pathogenicity relies on suppression of the immune system. Seth and colleagues[123] showed that CHQ administration exacerbated the severity of Semliki Forest virus infections in mice by upregulating the mRNA levels of pro-inflammatory cytokines such as IL-1, IL-6, and IFN-γ -inducing factor, among others. However, this is the only incidence in the literature to date that has shown induction of pro-inflammatory cytokines by CHQ. As such, it is not known how CHQ treatment could affect the course of viral infections such as HIV in humans. However, the evolution of AIDS-causing HIV strains has recently been postulated to be related to CHQ use in humans[124-126].

A new lease of life for CHQ

The forgone discussion has shown that the physiological, cellular, and biochemical effects of CHQ are exerted through pleiotropic mechanisms involving both lysosomotropic-dependent and independent effects. This cornucopia of mechanisms of action has seen CHQ persist on therapeutic regimens for several diseases and conditions despite its systemic toxicity and the emergence of drug resistance in malaria parasites.

CHQ for treatment of viral infections?

CHQ is currently under clinical trials as a potential, antiretroviral drug in humans. Malariotherapy is basically safe for HIV infection and it improves some immunological parameters of HIV positive patients[127] resulting in an increased CD4 count[128]. Therapeutically induced acute vivax malaria was shown to be well tolerated in 20 HIV-positive subjects who represented a range of CD4 cell lines from 15-1868 per microlitre[127]. Paton *et al*[129] argued that drug combinations of HCHQ and/or hydroxycarbamide and didanosine may be suitable for poorer countries. HCHQ has been shown to suppress HIV-1 replication in T cells and monocytes *in vitro* by inhibiting post-transcriptional modification of the virus[130-132]. Early in 1995, Sperber *et al*[131] reported that administration of CHQ for 8 wk to HIV-1 positive patients resulted in decreases in copy number of HIV-1 mRNA as well as reductions in plasma levels of the pro-inflammatory cytokine IL-6 compared to placebo. In 1998, Pardridge *et al*[132] reported that CHQ could inhibit replication of HIV-1 in human peripheral lymphocytes at concentrations similar to those achievable in humans *in vivo*, with minimal effects on host cell DNA replication. The mechanism of action of CHQ on HIV-1 and -2 was later shown by Savarino and colleagues[133,134] to include structural alterations in newly formed viral envelope glycoproteins (gp120) which led to impairment of the infectivity and ability to form syncytia by the newly formed viruses.

It is now believed that CHQ, through its lysosomotropic effects of increasing intra-organellar *p*H, could impair the catalytic function of the glucosyltransferases involved in processing of HIV glycoproteins[135]. Thus CHQ has potential for use as an adjunct in standard antiretroviral drug therapy. Some research groups have since demonstrated that CHQ has synergistic effects with zidovudine, didanosine, and hydroxyurea[133,134,136] as well as with protease inhibitors such as indinavir, ritonavir, and saquinavir[136]. The presence of CHQ in breast milk[137] and in blood[138] has been postulated to be related to a reduction in the risk of vertical transmission of HIV in humans. CHQ is associated with low levels of HIV RNA in breast milk[137]. To date, CHQ is among several drugs that have been shown to have *in vitro* activity against the replication of SARS or coronavirus infections[139].

CHQ - a part in anticancer strategies?

In 1992, Djordevic and colleagues[140] reported that treatment of mouse melanoma cells with CHQ potentiated the effectiveness of radiation-induced cell killing. Human MDA-MB231 cancer cells were influenced by CHQ via radiosensitizing effects through a destabilisation of lysosomes and plasma membranes[89]. They showed that treatment of MDA-MB231 cells with CHQ resulted in the latter accumulating into lysosomes thereby causing their volume to increase; the swelling of the lysosomes was associated with translocation of ceramide to the lysosomal surface thus inducing massive necrotic cell death when the cells were exposed to radiation[89]. Other research groups have also demonstrated, using a wide range of concentrations (0.25-128 μM) for 24-72 h, that low doses of CHQ inhibited growth of A549 human lung cancer cells in culture and that higher doses of CHQ induced A549 cell death by necrosis[141].

CHQ also has potential for use as a chemosensitizer in cancer in conjunction with some conventional antineoplastic agents. CHQ has recently been shown to inhibit the function of membrane-associated proteins belonging to the p-glycoprotein and multi-drug resistance (MDR) protein families[89,142,143]. These proteins are at the forefront as mediators of chemotherapy resistance in a wide range of cancers because they pump drugs out of cells and are usually overexpressed in most chemoresistant cell phenotypes[144]. The inhibition of drug efflux from the cell by CHQ and related antimalarials could help in sensitizing resistant cells to the cytotoxic effects of anticancer drugs by maintaining high intracellular concentrations of the chemotherapeutic agent. Indeed, some research groups have shown that CHQ enhances the toxicity of doxorubicin in some resistant cancer cell lines[143] as well as augment the antiviral effects of some agents[136]. However, the use of CHQ *in vivo* is not without its attendant problems. Radiotherapy given after a course of CHQ treatment led to unexpected skin reactions[145], and this has prompted other workers to suggest a rigorous revaluation and delineation of all undesirable side effects before CHQ can be used safely in any new treatments[146].

A comeback for the malaria miracle drug?

In 1993 Malawi, an African country, withdrew CHQ from use as a treatment for malaria in favour of the sulphadoxine-pyrimethamine combinations. A decade later, there was a return of CHQ-sensitive *P. falciparum* malaria in Malawi[147]. In Uganda, a study demonstrated that there were no pharmacokinetic interactions between CHQ, sulphadoxine and pyrimethamine when given together[148].

In their study Kublin and colleagues[147] measured the prevalence of the *pfcrt* 76T genotype (a molecular marker for CHQ-resistance) and observed that after 10 yr of CHQ withdrawal, the prevalence of this genotype decreased from a peak of 85 per cent in 1992 to only 13 per cent in 2000. A subsequent trial in 2001 showed that CHQ was able to clear 100 per cent of infections in a group of asymptomatic *P. falciparum* infections and no incidence of the *pfcrt* genotype was detected[147]. These findings were later corroborated by the another group who showed that CHQ cured 99 per cent of 80 malaria cases in Blantyre (Malawi) and the cure rate was even superior to that of the sulphadoxine-pyrimethamine combination[149]. However, the authors urged a cautious return to the use of CHQ in malaria endemic areas and suggest that it be used in combination with other drugs to prevent the recurrence of drug resistance in parasites.

Conclusion

CHQ is one of the most successful and widely used medications and with obvious health precautions, saving countless lives from the scourge of malaria. Its relatively simple manufacturing methods mean that it is affordable in many countries of the world. It has numerous other uses that could prove significant as measures are sought desperately to combat the spread of some viral diseases and cancer. Indeed, there is the real potential for CHQ to be restored to the antimalarial armamentarium; and, efforts must focus on understanding the mode of action of antimalarial agents and on the mechanism by which the *P. falciparum* impedes the action of these drugs[150].

References

1. Salomone S, Godfraind T. Drugs that reverse chloroquine resistance in malaria. *Trends Pharmacol Sci* 1990; *11* : 475-6.
2. Veignie E, Moreau S. The mode of action of chloroquine. Non-weak base properties of 4-aminoquinolines and antimalarial effects on strains of *Plasmodium. Ann Trop Med Parasitol* 1991; *85* : 229-37.
3. de Vries PJ, Oosterhuis B, van Boxtel CJ. Single-dose pharmacokinetics of chloroquine and its main metabolite in healthy volunteers. *Drug Invest* 1994; *8* : 143-9.
4. Wetsteyn JCFM, de Vries PJ, Oosterhuis B, van Boxtel CJ. The pharmacokinetics of three multiple dose regimens of chloroquine: implications for malaria chemoprophylaxis. *Br J Clin Pharmacol* 1995; *39* : 696-9.
5. Verhoef H, Hodgins E, Eggelte TA, Carter JY, Lema O, West CE, *et al*. Anti-malarial drug use among pre-school children in an area of seasonal malaria transmission in Kenya. *Am J Trop Med Hyg* 1999; *61* : 770-5.
6. Titus EO. Recent developments in the understanding of the pharmacokinetics and mechanism of action of chloroquine. *Ther Drug Monit* 1989; *11* : 369-79.
7. Augustijns P, Geusens P, Verbeke N. Chloroquine levels in blood during chronic treatment of patient with rheumatoid arthritis. *Eur J Clin Pharmacol* 1992; *42* : 429-33.
8. Augustijns P, Verbeke N. Stereoselective pharmacokinetic properties of chloroquine and de-ethyl-chloroquine in humans. *Clin Pharmacokinet* 1993; *24* : 259-69.
9. Krishna S, White NJ. Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. *Clin Pharmacokinet* 1996; *30* : 263-99.
10. Meinαo IM, Sato EI, Andrade LEC, Fenaz MB, Atra E. Controlled trial with chloroquine diphosphate in systemic lupus erythematosus. *Lupus* 1996; *5* : 237-41.
11. Abdi AY, Gustafsson LL, Ericsson O, Hellgren U. *Handbook of drugs for tropical parasitic infections*. 2nd ed. London: Taylor & Francis Ltd; 1995.
12. Karres I, Kremer JP, Dietl I, Steckholzer U, Jochum M, Ertel W. Chloroquine inhibits proinflammatory cytokine release into human whole blood. *Am J Physiol* 1998; *274* : R1058-64.
13. Bruce-Chwatt LJ, editor. *Chemotherapy of malaria*. 2nd ed. WHO Monograph Series 27. Geneva: World Health Organization; 1981.
14. Radl S. From chloroquine to antineoplastic drugs? The story of antibacterial quinolones. *Arch Pharm Med Chem Res* 1996; *329* : 115-9.
15. Sowunmi A. Disposition of oral quinine in African patients suffering from acute uncomplicated falciparum malaria. *East Afr Med J* 1996; *73* : 519-23.
16. Munjeri O, Hodza P, Osim EE, Musabayane CT. An investigation into the suitability of amidated pectin hydrogel beads as a delivery matrix for chloroquine. *J Pharm Sci* 1998; *87* : 905-8.
17. Pandey AV, Bisht H, Babbarwal VK, Srivastava J, Pandey KC, Chauhan VS. Mechanism of malarial haem detoxification inhibition by chloroquine. *Biochem J* 2001; *355* : 333-8.
18. Snow RW, Craig M, Deichmann U, Marsh K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bull World Health Organ* 1999; *77* : 624-40.
19. Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A, *et al*. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* 1997; *349* : 1650-4.

20. Baird JK. Resurgent malaria at the millennium: control strategies in crisis. *Drugs* 2000; *59* : 719-43.
21. Baird JK, Basri H, Subianto B, Fryauff DJ, McElroy PD, Leksana B, *et al*. Treatment of chloroquine-resistant *Plasmodium vivax* with chloroquine and primaquine or halofantrine. *J Infec Dis* 1995; *171* : 1678-82.
22. Frappier F, Jossang A, Soudon J, Calvo F, Rasoanaivo P, Ratsimamanga-Urverg S, *et al*. Bisbenzylisoquinolines as modulators of chloroquine resistance in *Plasmodium falciparum* and multidrug resistance in tumor cells. *Antimicrob Agents Chemother* 1996; *40* : 1476-81.
23. Phillips-Howard PA Confronting the challenge of antimalarial drug resistance in Africa. Managing the introduction of new antimalarial drugs to Africa: theory and practice. *PPH Antimal Drug Policy* 1998; *27* : 1-24.
24. Molyneux DH, Floyd K, Barnish G, Fevre EM. Transmission control and drug resistance in malaria: a crucial interaction. *Parasitol Today* 1999; *15* : 238-40.
25. White NJ, Nosten F, Looareesuwan S, Watkins WM, Marsh K, Snow RW, *et al*. Averting a malaria disaster. *Lancet* 1999; *353* : 1965-7.
26. Shapira A.. The resistance of falciparum malaria in Africa to 4-aminoquinolines and antifolates. *Scand J Infect Dis* 1990; *75* (Suppl) : 1-64.
27. Bloland PB, Lackritz EM, Kazembe PN, Were JBO, Steketee R, Campbell CC. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *J Infect Dis* 1993; *167* : 932-7.
28. Karbwang J, Harinasuta T. Overview: clinical pharmacology of antimalarials. *Southeast Asian J Trop Med Public Health* 1992; *23* (Suppl 4): 95-109.
29. Abuaku BK, Koram KA, Binka FN. Antimalarial drug use among caregivers in Ghana. *Afr Hlth Sci* 2004; *4* : 171-7.
30. Basco LK, Ringwald P, Le Bras J. *In vivo-in vitro* test for chloroquine potentiation by cyproheptadine against *Plasmodium falciparum*. *Trans R Soc Trop Med Hyg* 1991; *85* : 206-7.
31. Peters W, Ekong R, Robinson BL, Warhurst DC, Pan X-Q. Antihistaminic drugs that reverse chloroquine resistance in *Plasmodium falciparum*. *Lancet* 1989; *2* : 334-5.
32. Levander OA, Ager AL Jr, Morris VC, May RG. Menhaden-fish oil in a vitamin E-deficient diet: protection against chloroquine-resistant malaria in mice. *Am J Clin Nutr* 1989; *50* : 1237-9.
33. Bitonti AJ, Dumont JA, Bush TL, Edwards ML, Stemerick DM, McCann PP, *et al*. Bis(benzyl)polyamine analogs inhibit the growth of chloroquine-resistant human malaria parasites (*Plasmodium falciparum*) *in vitro* and in combination with α-difluoromethylornithine cure murine malaria. *Proc Natl Acad Sci USA* 1989; *86* : 651-5.
34. Dadachova E. Preparation of ^{198}Au(I)-labelled gold-chloroquine complex [^{198}Au(PPh_3)(CQ)]PF_6 as a potential antimalarial agent. *J Label Compd Radiopharm* 1999; *42* : 287-92.
35. McIntosh HM, Greenwood BM. Chloroquine or amodiaquine combined with sulfadoxine-pyrimethamine as a treatment for uncomplicated malaria - a systematic review. *Ann Trop Med Parasitol* 1998; *92* : 265-70.
36. Warhurst DC. Drug resistance in *Plasmodium falciparum* malaria. *Infection* 1999; *27* (Suppl. 2) : S55-8.
37. WHO Antimalarial drug combination therapy: Report of WHO technical consultation, 4-5 April. World Health Organization (WHO/CDS/RBM/2001.35), Geneva, Switzerland; 2001.
38. Facts on ACTs (Artemisinin-based combination therapies). January update. World Health Organization, Geneva, Switzerland; 2006. Available at: *http://www.rbm.who.int/cmc_upload/0/000/015/364/RBMInfosheet_9.htm*
39. Adelusi SA, Salako LA. Tissue and blood concentrations of chloroquine following chronic administration in the rat. *J Pharm Pharmacol* 1982; *34* : 733-5.
40. Gustafsson LL, Walker O, Alvαn G, Beermann B, Estevez F, Gleisner L, *et al*. Disposition of chloroquine in man after single intravenous and oral doses. *Br J Pharmacol* 1983; *15* : 471-9.
41. Minker E, Ivan J. Experimental and clinicopharmacological study of rectal absorption of chloroquine. *Acta Phys Academiae Sci Hung* 1991; *77* : 237-48.
42. Walker O, Dawodu AH, Adeyokunnu AA, Salako LA, Alvαn G. Plasma chloroquine and desethylchloroquine concentrations in children during and after chloroquine treatment for malaria. *Br J Pharmacol* 1983; *16* : 701-5.
43. McChesney EW, Banks WF Jr., Fabian RJ. Tissue distribution of chloroquine, hydroxychloroquine, and desethylchloroquine in the rat. *Toxicol Appl Pharmacol* 1967; *10* : 501-13.
44. White NJ. Antimalarial pharmacokinetics and treatment regimens. *Br J Clin Pharmacol* 1992; *34* : 1-10.
45. Desjardins RE, Doberstyn EB, Wernsdorfer WH. The treatment and prophylaxis of Malaria. In: Wernsdorfer WH, McGregor I, editors. *Malaria: Principles and practice of malariology.* Vol. I. London: Churchill Livingstone; 1988 p. 827-59.
46. Pussard E, Verdier F. Antimalarial 4-aminoquinolines: mode of action and pharmacokinetics. *Fund Clin Pharmacol* 1994; *8* : 1-17.
47. Walker O, Alvαn G, Beerman B, Gustafsson LL, Lindstöm BL, Sjöqvist F. The pharmacokinetics of chloroquine in healthy volunteers. *Br J Clin Pharmacol* 1982; *14* (Suppl) : 624.
48. Frisk-Holmberg M, Bergkvist Y, Domeij-Nyberg B, Hellstrom L, Jasson F. Chloroquine serum concentration and side-effects: evidence for dose-dependent kinetics. *Clin Pharmacol Ther* 1979; *25* : 345-50.
49. Fitch CD, Cai GZ, Chen YF, Ryerse JS. Relationship of chloroquine-induced redistribution of a neutral aminopeptidase to hemoglobin accumulation in malaria parasites. *Arch Biochem Biophys* 2003; *410* : 296-306.
50. Houzé P, de Reynies A, Baud FJ, Benatar MF, Pays M. Simultaneous determination of chloroquine and its three metabolites in human plasma, whole blood and urine by ion-pair high-performance liquid chromatography. *J Chromatogr* 1992; *574* : 305-12.
51. Ducharme J, Farinotti R. Clinical pharmacokinetics and metabolism of chloroquine. Focus on recent advancements. *Clin Pharmacokinet* 1996; *31* : 257-74.

52. Kim KA, Park JY, Lee JS, Lim, S. Cytochrome P450 2C8 and CYP3A4/5 are involved in chloroquine metabolism in human liver microsomes. *Arch Pharm Res* 2003; *26* : 631-7.

53. Projean D, Baune B, Farinotti R, Flinois JP, Beaune P, Taburet AM, *et al*. *In vitro* metabolism of chloroquine: identification of CYP2C8, CYP3A4, and CYP2D6 as the main isoforms catalyzing N-desethylchloroquine formation. *Drug Metab Disposition* 2003; *31* : 748-54.

54. Aderounmu AF. *In vitro* assessment of the antimalarial activity of chloroquine and its major metabolites. *Am J Trop Med Parasitol* 1984; *78* : 581-5.

55. Kuroda K. Detection and distribution of chloroquine metabolites in human tissues. *J Pharmacol Exp Ther* 1962; *137* : 156-61.

56. McChesney EW, Conway WD, Banks WF, Rogers JE, Shekosky JM. Studies of the metabolism of some compounds of the 4-amino-7-chloroquinoline series. *J Pharmacol Exp Ther* 1966; *151* : 482-93.

57. Adjepon-Yamoah KK, Ofori-Adjei D, Woolhouse NM, Lindström B. Whole-blood single-dose kinetics of chloroquine and desethylchloroquine in Africans. *Ther Drug Monit* 1986; *8* : 195-9.

58. Musabayane CT, Ndhlovu CE, Mamutse G, Bwititi P, Balment RJ. Acute chloroquine administration increases renal sodium excretion. *J Trop Med Hyg* 1993; *96* : 305-10.

59. Musabayane CT, Ndhlovu CE, Balment RJ. The effects of oral chloroquine administration on kidney function. *Renal Fail* 1994; *16* : 221-8.

60. Musabayane CT, Windle RJ, Forling ML, Balment RJ. Arginine vasopressin mediates the chloroquine induced increase in renal sodium excretion. *Trop Med Int Health* 1996; *1* : 542-50.

61. Back DJ, Purba HS, Park BK, Ward SA, Orme ML. Effect of chloroquine and primaquine on antipyrine metabolism. *Br J Clin Pharmacol* 1983; *16* : 497-502.

62. Gustafsson LL, Lindström B, Grahnén A, Alvαn G. Chloroquine excretion following malaria prophylaxis. *Br J Clin Chem* 1987; *24* : 221-4.

63. Knox JM, Owens DW. The chloroquine mystery. *Arch Dermatol* 1966; *94* : 205-14.

64. Sofola OA, Olude IO, Adegoke F. The effects of chronic chloroquine toxicity on blood pressure of rats. *J Trop Med Hyg* 1981; *84* : 249-52.

65. Chesney RW, Budreau AM. Chloroquine, a novel inhibitor of amino acid transport by rat renal brush border membrane vesicles. *Amino Acids* 1995; *8* : 141-58.

66. Ogilvie KM, Lee S, Weiss B, Rivier C. Mechanisms mediating the influence of alcohol on the hypothalamic-pituitary-adrenal axis responses to immune and non-immune signals. *Alcohol Clin Exp Res* 1998; *22* : 2435-75.

67. Novoa E, Rodrigo R. Renal handling of electrolytes and (Na+K)-ATPase activity after unilateral nephrectomy during long-term ethanol feeding. *Acta Physiol Pharmacol Ther Latinoamer* 1989; *39* : 15-26.

68. Ahmed MH, Ashton N, Balment RJ. Renal function in a rat model of analgesic nephropathy: effect of chloroquine. *J Pharmacol Exp Ther* 2003; *305* : 123-30.

69. Musabayane CT, Cooper RG, Osim E, Balment RJ. Renal electrolyte and fluid handling in the rat following chloroquine and/or ethanol administration. *Gen Pharmacol - Vasc Syst* 2000; *34* : 43-51.

70. Musabayane CT, Cooper RG, Prasada Rao PVV, Balment RJ. Effects of ethanol on the changes in renal fluid and electrolyte handling and kidney morphology induced by long-term chloroquine administration to rats. *Alcohol* 2000; *22* : 129-38.

71. Ahmed MH, Osman MM. Why does chloroquine impair renal function? Chloroquine may modulate the renal tubular response to vasopressin either directly by inhibiting cyclic AMP generation, or indirectly via nitric oxide. *Med Hypotheses* 2007; *68* : 140-3.

72. Ahmed MH. Insulin resistance and nitric oxide and associated renal injury: innocent bystanders or accessories to the crime? *NZ Med J* 2006; *119* : U2112.

73. Noiri E, Peresleni T, Miller F, Goligorsky MS. *In vivo* targeting of inducible NO synthase with oligodeoxynucleotides protects rat kidney against ischemia. *J Clin Invest* 1996; *97* : 2377-83.

74. Ling H, Edelstein C, Gengaro P, Meng X, Lucia S, Knotek M, *et al*. Attenuation of renal ischemia-reperfusion injury in inducible nitric oxide synthase knockout mice. *Am J Physiol* 1999; *277* : F383-90.

75. Cooper RG, Musabayane CT. The acute effects of combined chloroquine and ethanol on renal electrolyte handling. *Proceedings of the 2nd International MIM African Malaria Conference*; 1999, March 14-19; Durban, South Africa; 1999. p. C-42-3.

76. Cooper RG, Musabayane CT. Effects of ethanol on plasma chloroquine, arginine vasopressin (AVP) concentrations and renal hydro-electrolyte handling in the rat. *Renal Fail* 2000; *22* : 785-98.

77. Musabayane CT, Musvibe A, Wenyika J, Munjeri O, Osim EE. Chloroquine influences renal function in rural Zimbabweans with acute transient fever. *Renal Fail* 1999; *21* : 189-97.

78. Liang MY, Knox FG. Nitric oxide activates PKC alpha and inhibits Na^+-K^+-ATPase in opossum kidney cells. *Am J Physiol-Renal Physiol* 1999; *277* : F859-65.

79. Kang DG, Kim JW, Lee J. Effects of nitric oxide synthesis inhibition on the Na, K-ATPase activity in the kidney. *Pharmacol Res* 2000; *41* : 121-5.

80. Ghigo D, Aldieri E, Todde R, Costamagna C, Garbarino G, Pescarmona G, *et al*. Chloroquine stimulates nitric oxide synthesis in murine, porcine, and human endothelial cells. *J Clin Invest* 1998; *102* : 595-605.

81. Cooper RG, Osim E, Musabayane CT. Ethanol reduces the natriuretic effect of chloroquine. In: Samkange C, editor. *Proceedings of the 1997 Annual Medical Research Day*; 1997 October 25; Harare, Zimbabwe; 1997. p. 9.

82. Abraham R, Hendy R, Grasso P. Formation of myeloid bodies in rat liver lysosomes after chloroquine administration. *Exp Mol Path* 1968; *9* : 212-29.

83. Wisner-Gebhart AM, Brabec RK, Gray RH. Morphometric studies of chloroquine-induced changes in hepatocytic organelles in the rat. *Exp Mol Path* 1980; *33* : 144-52.

84. Ericsson JL. Mechanism of cellular autophagy. In: Dingle JT, Fells HB, editors. *Lysosomes in biology and pathology*, Vol. 2. New York: Wiley; 1969. p. 345-94.

85. Schneider P, Korolenko TA, Busch U. A review of drug-induced lysosomal disorders of the liver in man and laboratory animals. *Microsc Res Tech* 1997; *36* : 253-75.

86. Allison AC, Young MR. Uptake of dyes and drugs by living cells in culture. *Life Sci* 1964; *3* : 1407-14.

87. MacIntyre AC, Cutler DJ. Kinetics of chloroquine uptake into isolated rat hepatocytes. *J Pharm Sci* 1993; *82* : 592-600.

88. Weissmann G. Lysosomes and joint disease. *Arthritis Rheum* 1966; *9* : 834-40.

89. Zhao H, Cai Y, Santi S, Lafrenie R, Lee H. Chloroquine-mediated radiosensitization is due to the destabilization of the lysosomal membrane and subsequent induction of cell death by necrosis. *Radiat Res* 2005; *164* : 250-7.

90. Colombo MI, Bertini F. Properties of binding sites for chloroquine in liver lysosomal membranes. *J Cell Physiol* 1988; *137* : 598-602.

91. Blouin A, Bollender RP, Weibel ER. Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. A stereological study. *J Cell Biol* 1977; *72* : 441-55.

92. Wibo M, Poole B. Protein degradation in cultured cells. II. The uptake of chloroquine by rat fibroblasts and the inhibition of cellular protein degradation and cathepsin B1. *J Cell Biol* 1974; *63* : 430-40.

93. Limet JN, Quintart J, Schneider YJ, Courtoy PJ. Receptor-mediated endocytosis of polymeric IgA and galactosylated serum albumin in rat liver. Evidence for intracellular ligand sorting and identification of distinct endosomal compartments. *Eur J Biochem* 1985; *146* : 539-48.

94. Singh KP, Krause W, David H, von Zglinicki J. Effects of chloroquine on hepatocyte organelles in rat. *Exp Pathol* 1985; *28* : 119-24.

95. Deepalakshmi PD, Parasakthy K, Shanthi S, Devaraj NS. Effect of chloroquine on rat liver mitochondria. *Indian J Exp Biol* 1994; *32* : 797-9.

96. Hostetler KY, Richman DD. Studies on the mechanism of phospholipid storage induced by amantadine and chloroquine in Madin Darby canine kidney cells. *Biochem Pharmacol* 1982; *31* : 3795-9.

97. Prasada Rao PVV, Cooper RG, Musabayane C. Histopathological changes in kidneys of rats exposed to chloroquine and ethanol in combination. In: Sharp B, editor. *Proceedings of the 2nd International MIM African Malaria Conference; 1999*. March 14-19; Durban, South Africa; 1999. p. C-140-1.

98. Teixeira RA, Filho MM, Benvenuti LA, Costa R, Pedrosa AA, Nishióka SAD. Cardiac damage from chronic use of chloroquine. A case report and review of the literature. *Arq Bras Cardiol* 2002; *79* : 85-8.

99. Cotton DW, Sutorius AH. Inhibiting effect of some antimalarial substances on glucose-6-phosphate dehydrogenase. *Nature* 1971; *233* : 197.

100. Emerole GO, Thabrew MI. Changes in some rat hepatic microsomal components induced by prolonged administration of chloroquine. *Biochem Pharmacol* 1983; *32* : 3005-9.

101. Loffler BM, Bohn E, Hesse B, Kunze H. Effects of antimalarial drugs on phospholipase A and lysophospholipase activities in plasma membrane, mitochondrial, microsomal and cytosolic subcellular fractions of rat liver. *Biochim Biophys Acta* 1985; *835* : 448-55.

102. Katewa SD, Katyare SS. Treatment with antimalarials adversely affects the oxidative energy metabolism in rat liver mitochondria. *Drug Chem Toxicol* 2004; *27* : 41-53.

103. Alisky JM, Chertkova EL, Iczkowski KA. Drug interactions and pharmacogenetic reactions are the basis for chloroquine and mefloquine-induced psychosis. *Med Hypotheses* 2006; *67* : 1090-94.

104. Magwere T, Naik YS, Hasler JA. Effects of chloroquine treatment on antioxidant enzymes in rat liver and kidney. *Free Radical Biol Med* 1997; *22* : 321-7.

105. Magwere T, Hasler JA. The role of xenobiotics in the modulation of antioxidant status and susceptibility to oxidative damage. In: Bahorun T, Gurib-Fakim A, editors. *Molecular and therapeutic aspects of Redox biochemistry*, London: OICA International (UK) Limited; 2003. p. 78-86.

106. Toler SM. Oxidative stress plays an important role in the pathogenesis of drug-induced retinopathy. *Exp Biol Med* (Maywood) 2004; *229* : 607-15.

107. Farombi EO. Genotoxicity of chloroquine in rat liver cells: protective role of free radical scavengers. *Cell Biol Toxicol* 2006; *22* : 159-67.

108. Chen TH, Chang PC, Chang MC, Lin YF, Lee HM. Chloroquine induces the expression of inducible nitric oxide synthase in C6 glioma cells. *Pharmacol Res* 2005; *51* : 329-36.

109. Ahmed MH. Nitric oxide might be involved in the pathogenesis of chloroquine-induced lipidosis. *Clin Ther* 2005; *27* : 509-10.

110. Park YC, Pae HO, Yoo JC, Choi BM, Jue DM, Chung HT. Chloroquine inhibits inducible nitric oxide synthase expression in murine peritoneal macrophages. *Pharmacol Toxicol* 1999; *85* : 188-91.

111. Hrabak A, Sefrioui H, Vercruysse V, Temesi A, Bajor T, Vray B. Action of chloroquine on nitric oxide production and parasite killing by macrophages. *Eur J Pharmacol* 1998; *354* : 83-90.

112. Wozniacka A, Lesiak A, Narbutt J, Kobos J, Pavel S, Sysa-Jedrzejowska A. Chloroquine treatment reduces the number of cutaneous HLA-DR$^+$ and CD1a$^+$ cells in patients with systemic lupus erythematosus. *Lupus* 2007; *16* : 89-94.

113. van den Borne BE, Dijkmans BA, de Rooij HH, le Cessie S, Verweij CL. Chloroquine and hydroxychloroquine equally affect tumor necrosis factor-alpha, interleukin 6, and interferon-gamma production by peripheral blood mononuclear cells. *J Rheumatol* 1997; *24* : 55-60.

114. Park J, Kwon D, Choi C, Oh JW, Benveniste EN. Chloroquine induces activation of nuclear factor-kappaB and subsequent expression of pro-inflammatory cytokines by human astroglial cells. *J Neurochem* 2003; *84* : 1266-74.

115. Weber SM, Levitz SM. Chloroquine interferes with lipopolysaccharide-induced TNF-alpha gene expression by a nonlysosomotropic mechanism. *J Immunol* 2000; *165* : 1534-40.

116. Zhu X, Ertel W, Ayala A, Morrison MH, Perrin MM, Chaudry IH. Chloroquine inhibits macrophage tumour necrosis factor-alpha mRNA transcription. *Immunology* 1993; *80* : 122-6.

117. Weber SM, Chen JM, Levitz SM. Inhibition of mitogen-activated protein kinase signalling by chloroquine. *J Immunol* 2002; *168* : 5303-9.

118. Jang CH, Choi JH, Byun MS, Jue DM. Chloroquine inhibits production of TNF-alpha, IL-1beta and IL-6 from lipopolysaccharide-stimulated human monocytes/ macrophages by different modes. *Rheumatology* 2006; *45* : 703-10.

119. Jeong JY, Choi JW, Jeon KI, Jue DM. Chloroquine decreases cell-surface expression of tumour necrosis factor receptors in human histiocytic U-937 cells. *Immunology* 2002; *105* : 83-91.

120. Hong Z, Jiang Z, Liangxi W, Guofu D, Ping L, Yongling L, *et al*. Chloroquine protects mice from challenge with CpG ODN and LPS by decreasing proinflammatory cytokine release. *Int Immunopharmacol* 2004; *4* : 223-34.

121. Wozniacka A, Lesiak A, Narbutt J, McCauliffe DP, Sysa-Jedrzejowska A. Chloroquine treatment influences proinflammatory cytokine levels in systemic lupus erythematosus patients. *Lupus* 2006; *15* : 268-75.

122. Nishimura M, Hidaka N, Akaza T, Tadokoro K, Juji T. Immunosuppressive effects of chloroquine: potential effectiveness for treatment of post-transfusion graft-versus-host disease. *Transfusion Medicine* 1998; *8* : 209-14.

123. Seth P, Mani H, Singh AK, Banaudha KK, Madhavan S, Sidhu GS, *et al*. Acceleration of viral replication and up-regulation of cytokine levels by antimalarials: implications in malaria-endemic areas. *Am J Trop Med Hyg* 1999; *61* : 180-6.

124. Parris GE. The timing is right: Evolution of AIDS-causing HIV strains are consistent with history of chloroquine use. *Med Hypotheses* 2006; *67* : 1258-9.

125. Parris GE. Update on hypothesis linking chloroquine-resistant malaria to disease-causing HIV. *Med Hypotheses* 2006; *67* : 670-1.

126. Parris GE. AIDS: caused by development of resistance to drugs in a non-target intracellular parasite. *Med Hypotheses* 2007; *68* : 151-7.

127. Chen X, Xiao B, Xu H, Shi W, Gao K, Rao J. Procedure and clinical assessments of malariotherapy: recent experience in 20 HIV patients. *Chin Med J (Engl.)* 2003; *116* : 1016-21.

128. Heimlich HJ, Chen XP, Xiao BQ, Liu SG, Lu YH, Spletzer EG, *et al*. Malariotherapy for HIV patients. *Mech Ageing Dev* 1997; *93* : 79-85.

129. Paton NI, Aboulhab J, Karin F. Hydroxychloroquine, hydroxycarbamide, and didanosine as economic treatment for HIV-1. *Lancet* 2002; *359* : 1667-8.

130. Ornstein MH, Sperber K. The anti-inflammatory and antiviral effects of hydroxychloroquine in two patients with acquired immunodeficiency syndrome and active inflammatory arthritis. *Arthritis Rheum* 1996; *39* : 157-61.

131. Sperber K, Louie M, Kraus T, Proner J, Sapira E, Lin S, *et al*. Hydroxychloroquine treatment of patients with human immunodeficiency virus type 1. *Clin Ther* 1995; *17* : 622-36.

132. Pardridge WM, Yang J, Diagne A. Chloroquine inhibits HIV-1 replication in human peripheral blood lymphocytes. *Immunol Lett* 1998; *64* : 45-7.

133. Savarino A, Gennero L, Chen HC, Serrano D, Malavasi F, Boelaert JR, *et al*. Anti-HIV effects of chloroquine: mechanisms of inhibition and spectrum of activity. AIDS 2001; *15* : 2221-9.

134. Savarino A, Gennero L, Sperber K, Boelaert JR. The anti-HIV-1 activity of chloroquine. *J Clin Virol* 2001; *20* : 131-5.

135. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003; *3* : 722-7.

136. Boelaert JR, Sperber K, Piette J. Chloroquine exerts an additive *in vitro* anti-HIV type 1 effect when associated with didanosine and hydroxyurea. *AIDS Res Hum Retrovirus* 1999; *15* : 1241-7.

137. Semrau K, Kuhn L, Kasonde P, Sinkala M, Kankasa C, Shutes E, *et al*. Impact of chloroquine on viral load in breast milk. *Trop Med Int Health* 2006; *11* : 800-3.

138. Neely M, Kalyesubula I, Bagenda D, Myers C, Olness K. Effect of chloroquine on human immunodeficiency virus (HIV) vertical transmission. *Afr Health Sci* 2003; *3* : 61-7.

139. de Clercq E. Potential antivirals and antiviral strategies against SARS coronavirus infections. *Expert Rev Anti Infect Ther* 2006; *4* : 291-302.

140. Djordevic B, Lange CS, Rotman M. Potentiation of radiation lethality in mouse melanoma cells by mild hyperthermia and chloroquine. *Melanoma Res* 1992; *2* : 321-6.

141. Fan C, Wang W, Zhao B, Zhang S, Miao J. Chloroquine inhibits cell growth and induces cell death in A549 lung cancer cells. *Bioorg Med Chem* 2006; *14* : 3218-22.

142. Vezmar M, Georges E. Direct binding of chloroquine to the multidrug resistance protein (MRP): possible role for MRP in chloroquine drug transport and resistance in tumor cells. *Biochem Pharmacol* 1998; *56* : 733-42.

143. Vezmar M, Georges E. Reversal of MRP-mediated doxorubicin resistance with quinoline-based drugs. *Biochem Pharmacol* 2000; *59* : 1245-52.

144. Kvackajova-Kisucka J, Barancik M, Breier A. Drug transporters and their role in multidrug resistance of neoplastic cells. *Gen Physiol Biophys* 2001; *20* : 215-37.

145. Rustogi A, Munshi A, Jalali R. Unexpected skin reaction induced by radiotherapy after chloroquine use. *Lancet Oncol* 2006; *7* : 608-9.

146. Savarino A, Lucia MB, Giordano F, Cauda R. Risks and benefits of chloroquine use in anticancer strategies. *Lancet Oncol* 2006; *7* : 792-3.

147. Kublin JG, Cortese JF, Njunju EM, Mukadam RA, Wirima JJ, Kazembe PN, *et al*. Re-emergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *J Infect Dis* 2003; *187* : 1870-5.

148. Obua C, Ntale M, Lundblad MS, Mahindi M, Gustafsson LL, Ogwal-Okeng JW, *et al*. Pharmacokinetic interactions between chloroquine, sulfadoxine and pyrimethamine and their bioequivalence in a generic fixed-dose combination in healthy volunteers in Uganda. *Afr Health Sci* 2006; *6* : 86-92.

149. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, Takala SL, *et al*. Return of chloroquine antimalarial efficacy in Malawi. *N Engl J Med* 2006; *355* : 1959-66.

150. Lelievre J, Berry A, Benoit-Vical F. Artemisinin and chloroquine: do mode of action and mechanism of resistance involve the same protagonists? *Curr Opin Investig Drugs* 2007; *8* : 117-24.

Reprint requests: Dr R.G. Cooper, Senior Lecturer, Division of Physiology, Faculty of Health, Birmingham City University, 704 Baker Building, Franchise Street, Perry Barr, Birmingham B42 2SU, UK
e-mail: rgcooperuk@yahoo.com