J Antimicrob Chemother 2015; **70**: 1608–1621
doi:10.1093/jac/dkv018 Advance Access publication 17 February 2015

Journal of Antimicrobial Chemotherapy

Chloroquine analogues in drug discovery: new directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases

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Antimalarial drugs (e.g. chloroquine and its close structural analogues) were developed primarily to treat malaria; however, they are beneficial for many dermatological, immunological, rheumatological and severe infectious diseases, for which they are used mostly today. Chloroquine and hydroxychloroquine, two of the most fascinating drugs developed in the last 50 years, are increasingly recognized for their effectiveness in myriad non-malarial diseases. In advanced research, chloroquine and hydroxychloroquine have been shown to have various immunomodulatory and immunosuppressive effects, and currently have established roles in the management of rheumatic diseases, lupus erythematosus (different forms) and skin diseases, and in the treatment of different forms of cancer. Recently, chloroquine analogues have also been found to have metabolic, cardiovascular, antithrombotic and antineoplastic effects. This review is concerned with the lysosomotropic, anti-inflammatory and immunomodulatory mechanisms of chloroquine, hydroxychloroquine, quinacrine and related analogues, and the current evidence for both their beneficial effects and potential adverse manifestations in various diseases.

Keywords: hydroxychloroquine, quinacrine, SLE, therapies, lysosomotropic actions, toxicity profiles

Introduction

Historical perspective and development of chloroquine analogues

The first natural antimalarial agent, quinine, derived from the bark of the cinchona tree, helped to shape today's world by making it possible to live in tropical countries despite lethal tropical malaria. Chemical synthesis of chloroquine analogues originated in the work of Paul Ehrlich's group, who treated malaria patients in 1891 with methylene blue, a synthetic dye that is selectively absorbed by the parasites causing malaria. This was the first synthetic drug used for the treatment of human malaria. Subsequently, an analogue of methylene blue was synthesized by replacing one methyl group with a basic side chain, which significantly improved antimalarial activity. This positive result eventually led in 1925 to the synthesis of pamaquine. The next event was the attachment of the basic side chain of pamaquine to several different heterocyclic ring systems, leading to the synthesis of the acridine derivative quinacrine (having an extra benzene ring and thus an acridine nucleus when compared with chloroquine). Investigation of the structure of quinacrine led to the discovery of two chloroquine analogues, sontoquine and primaquine, which showed excellent antimalarial activity. Studies on these compounds then led to the discovery of resochin. This compound was ignored for a decade, since it was initially thought to be too toxic for clinical use. However, its toxicological properties were re-examined and it was found to be safe for human subjects. The Wehrmacht in North Africa used a resochin formulation ('sontoquine'); it was captured during World War II by the Americans, and this led to the synthesis of chloroquine.[1,2] From earlier empirical studies, it became clear that chloroquine was one of the most effective agents, and the study of further structural variations led to the discovery of hydroxychloroquine (which differs from chloroquine only by a hydroxyl group), which proved to be 3-fold less toxic.[3] The historical developmental events are summarized in Figure 1. Although chloroquine has been abandoned for prophylaxis in most countries due to the resistance of the pathogens *Plasmodium falciparum* and *Plasmodium vivax*, chloroquine analogues are still used in Korea, China, Turkey, Mexico, Paraguay, etc., for the prophylactic treatment of malaria.[4–6] A milestone in the fortunes of chloroquine analogues occurred during World War II; millions of soldiers took antimalarial prophylaxis, and observations indicated that antimalarial treatment improved the soldiers' rashes and inflammatory arthritis. This led to the first trial that showed the efficacy of quinacrine in systemic lupus erythematosus (SLE). Similar observations opened the door for regular treatment of patients with rheumatoid arthritis (RA) and SLE with chloroquine analogues. Nowadays, chloroquine analogues are used for the treatment of

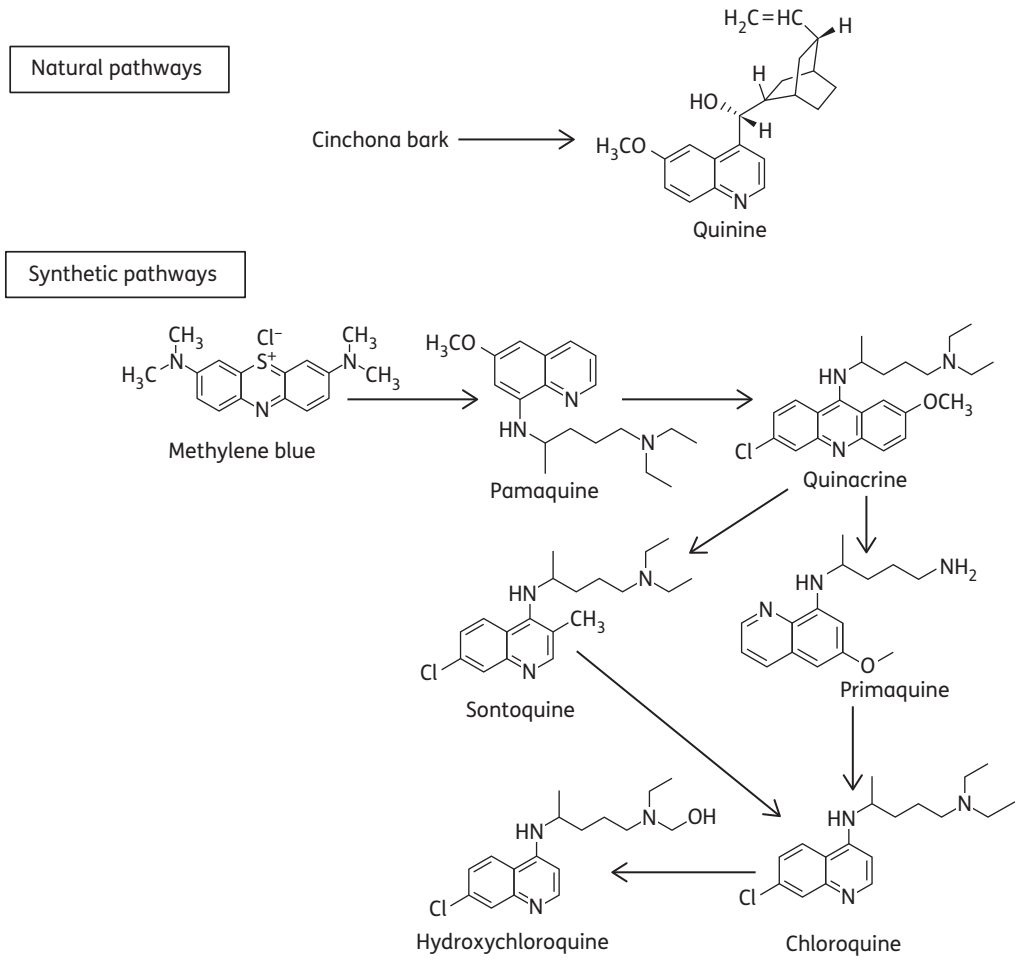


Figure 1. Historical developmental pathways of chloroquine analogues.

other rheumatic disorders, as well as a wide variety of dermatological, immunological, cancerous and infectious diseases.[7]

Pharmacokinetic considerations

Chloroquine analogues are water soluble and almost completely absorbed from the gastrointestinal tract. Both chloroquine and hydroxychloroquine reach the peak plasma concentration 4–12 h after an individual dose and achieve equilibrium plasma levels after 4–6 weeks of constant daily dosing, although there is considerable inter-individual variation. The half-lives of chloroquine and hydroxychloroquine are prolonged, ranging between 40 and 50 days. Chloroquine analogues have strong affinities for blood constituents, particularly thrombocytes and granulocytes, which reduces the plasma concentrations. In addition, a major fraction of chloroquine analogues in the plasma is bound to plasma proteins, mainly albumin and α-acid glycoprotein, and also avidly bound to several tissues in the body when given at therapeutic doses. As a result, excretion of chloroquine analogues is quite slow. Although small amounts are excreted in bile, sweat and saliva, the major elimination route of chloroquine analogues is via the renal system, and elimination may thus be affected by the pH of urine.[8–10]

Indications for chloroquine analogues

Chloroquine analogues have been shown to have potent beneficial effects in many non-malarial diseases. For practical purposes, the indications for chloroquine analogues can be summarized in several ways (Table 1). The current evidence for applications of chloroquine, hydroxychloroquine and, to a lesser extent, quinacrine is discussed below.

Lupus erythematosus (LE)

Chloroquine analogues prevent lupus flares clinically and increase the long-term survival of patients with systemic SLE, cutaneous LE (CLE) or discoid LE.[11–14] These drugs are also effective for the treatment of lupus patients who are pregnant, for neonates with lupus, or lupus patients who also have other diseases such as osteonecrosis and inflammatory bowel disease.[15–18] In patients with SLE, chloroquine and hydroxychloroquine improve certain systemic manifestations, such as arthralgia, myalgia, serositis and haematological abnormalities. Recently, prolactinoma and recurrent granulomatous mastitis in SLE patients have been successfully treated with hydroxychloroquine.[19] In patients with CLE, a combination of hydroxychloroquine and quinacrine is more appropriate as

Table 1. Summary of indications for chloroquine analogues

FDA-approved and FDA-labelled indications
Malaria (except resistant *P. falciparum* and *P. vivax* causing malaria)
Lupus erythematosus in different forms, such as discoid, systemic; also effective in pregnant SLE patients
RA, act as first-line disease-modifying antirheumatic drugs
Chloroquine analogues in clinical research trials
Lupus erythematosus (discoid and cutaneous) in different adjunct therapies
RA in combination with other drugs
Psoriatic arthritis
Prostatic cancer
Additional research trials
Local metastatic melanoma, chronic lymphocytic leukaemia and diffuse large B cell lymphoma
Unapproved but first-line uses include
PCT and chronic ulcerative stomatitis
Hepatic amoebic abscess
Refractory chronic urticaria[135,136]
Quinacrine is used as an effective contraception[137]
Second- and third-line treatments
Non-infectious skin diseases such as dermatomyositis, sarcoidosis, polymorphous light eruption and disseminated granuloma annulare
Miscellaneous conditions
Sjögren's syndrome, granuloma annulare, erosive lichen planus, frontal fibrosing alopecia,[138] necrobiosis lipoidica, chronic actinic dermatitis, actinic reticuloid, actinic prurigo, epidermolysis bullosa, Kikuchi–Fujimoto disease, graft-versus-host disease, chronic erythema nodosum, morphea and systemic sclerosis, pemphigus vulgaris, pemphigus foliaceus and pemphigoid gestationis[139]
Chloroquine analogues and current research
Bone diseases, different forms of cancers, hyperglycaemia, dyslipidaemia, thrombosis and severe infectious diseases
Chloroquine analogues as investigational drugs
AIDS and severe acute respiratory syndrome (SARS)
Human prion diseases (CJD) and LAM

initiation therapy than hydroxychloroquine or chloroquine monotherapy and improves the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) score and the response rate in non-responders.[20,21] Hydroxychloroquine is beneficial for patients with membranous lupus nephritis.[12] Furthermore, the use of hydroxychloroquine in SLE patients is associated with improved overall survival, decreased accrual of damage and lowered rates of infections.

RA

Both chloroquine and hydroxychloroquine inhibit antigen presentation in dendritic cells, cytokine production in macrophages, and calcium and Toll-like receptor (TLR) signalling in B, T and other immune cells. Thus, chloroquine analogues have become the most commonly prescribed drugs in the treatment of many rheumatic diseases, including RA, palindromic arthritis, psoriatic arthritis and juvenile idiopathic arthritis.[22–25] In RA, hydroxychloroquine is usually a component of medication combinations, including triple-drug therapy with methotrexate and sulfasalazine, a regimen that has been advocated as a safe, well-tolerated alternative to more expensive biological therapies. In the early stages of RA, chloroquine analogues reduce cardiovascular risks. Hydroxychloroquine also confers significant improvement in the symptoms of mild to moderate knee osteoarthritis, rheumatoid vasculitis and non-gout joint deposition diseases.[26–28]

Anticancer strategies

The incorporation of chloroquine, hydroxychloroquine, quinacrine and other chloroquine analogues, such as 8-hydroxyquinoline,[29,30] in chemotherapeutic regimens has become a therapeutic approach in oncology, because of their inhibitory actions on lysosomes or acceleration of the radio-sensitizing effects of some chemotherapeutic drugs used concomitantly with radiotherapy.[31,32] Therefore, chloroquine analogues are taken into consideration in clinical trials with radiotherapy and chemotherapy. The use of chloroquine analogues has been focused on the treatment of highly aggressive and metastatic cancers, including relapsed leukaemias, melanoma, osteosarcoma and cancers of the head and neck, brain, lung, breast, ovary, prostate and pancreas, as well as gastrointestinal cancers, which remain incurable in the clinic in spite of aggressive therapy.[31,33] In these cases, chloroquine analogues influence the potential biological effects of different cancer cells, such as by inhibiting cell growth and/or inducing cell death by autophagy-dependent modulation.[34–36] Some of these studies have used relatively high drug concentrations, doubling the usual dose in patients with SLE. While these doses have low levels of toxicity, especially in the setting of life-threatening illness, more efficient drug delivery systems, such as the use of targeted nanoparticles, have been proposed as methods of enhancing the efficacies of these agents. Several clinical trials using combinations of chloroquine analogues with different therapeutic agents for cancers are currently being carried out.[35] The results of these clinical trials are likely to be helpful in determining the directions of research on chloroquine analogue-mediated cancer treatments.

Contemporary cases of dermatological disorders

Porphyria cutanea tarda (PCT)

In PCT, reactive oxygen species are produced and damage the skin, resulting in severe mucosal erosions and epidermal friability and blistering. Repeated phlebotomy is the mainstay treatment for PCT. Nevertheless, in many of the cases in which patients do not respond this procedure is contraindicated. In these cases, a low-dose regimen of chloroquine analogues gives favourable results without untoward reactions.[37,38]

Chronic ulcerative stomatitis (CUS)

CUS is characterized by a course of painful ulceration in the oral mucosa of older patients, caused by the interaction of antinuclear antibodies with squamous epithelia. Lesions associated with CUS are refractory to local and systemic corticosteroids, but treatment with chloroquine analogues lead to remission and so these agents are the first-line treatment for CUS.[1,39]

Dermatomyositis

Chloroquine analogues are effective in the treatment of dermatomyositis (childhood or juvenile form). The improvement in muscle strength in patients receiving chloroquine analogues as adjunct therapies is (at least in part) a consequence of the improvement of skin manifestations.[40,41]

Sarcoidosis

It is reported that quinacrine improves cutaneous sarcoidosis. The beneficial outcome of chloroquine in the medication of pulmonary sarcoidosis was discovered in 1960. Since then, many reports have confirmed the effectiveness of chloroquine analogues in the treatment of subcutaneous, pulmonary and osseous sarcoidosis.[42–44]

Sjögren's syndrome

Hydroxychloroquine is of benefit in patients with primary Sjögren's syndrome.[45–47] Hydroxychloroquine lowers serum B-cell activating factor (BAFF) levels and improves the atherogenic index in primary Sjögren's syndrome.[48]

Lichen planus

Chloroquine analogues cure cutaneous lichen planus of the nails, oral mucosa and lower lip, and lichen planopilaris.[49–51] The efficacy of hydroxychloroquine has also been proved in the treatment of oral lichen planus, in which it acts by lowering the up-regulated expression of regulatory T cells (Tregs).[52]

Miscellaneous

This section summarizes some diseases in which chloroquine analogues are used randomly, the scientific evidence being insufficient and limited to isolated case reports. The use of chloroquine analogues is recommended in patients with disseminated granuloma annulare that does not respond well to topical corticosteroids or in whom corticosteroids cannot be used due to the extent of the lesions.[53] Chloroquine analogues (particularly hydroxychloroquine) are effective alternatives for the long-term treatment of some photosensitive disorders, such as chronic actinic dermatitis and actinic reticuloid.[54] Chloroquine analogues inhibit the development of graft-versus-host disease (GVHD) by suppressing T cell responses to foreign minor and major histocompatibility complex (MHC) antigens and alterations in T cell cytokine production.[55] Hydroxychloroquine also prevents acute GVHD in patients who have received bone marrow transplantation.[56]

Chloroquine analogues and current research

Bone diseases

Administration of chloroquine or hydroxychloroquine reportedly results in a slowing or even arrest of joint destruction as well as increased bone mineral density (BMD) in RA and SLE patients.[57] We and other groups have also investigated the direct inhibitory effects of chloroquine on osteoclast function[58] and the differentiation and bone-forming activity of osteoblasts.[59] In these studies, chloroquine suppressed the bone-resorbing activity of osteoclasts by inhibition of acidification in lysosomes, as well as osteoclast differentiation *in vitro* and *in vivo*. These studies demonstrate the importance of the effect of chloroquine analogues on the function as well as the differentiation of osteoclast-mediated bone diseases, including osteoporosis as well as RA.

Hyperglycaemia

The hypoglycaemic effect of chloroquine analogues increases insulin sensitivity in patients with insulin resistance.[60,61] A clinical trial in type II diabetic patients who were treated with a short course of chloroquine showed a significant improvement in glucose tolerance. Hydroxychloroquine also emerges as a well-tolerated therapeutic option for type II diabetic mellitus. When hydroxychloroquine was combined with insulin for the treatment of diabetes mellitus, glycated haemoglobin decreased significantly compared with patients receiving placebo, and the insulin dose had to be reduced by 30% in the hydroxychloroquine group.[62] The anti-diabetic mechanism of chloroquine analogues involves decreases in insulin clearance and degradation rates and an increase in the secretion of C-peptide.[63]

Anti-lipidaemic effects

Chloroquine analogues have plasma lipid-lowering effects in RA, SLE, dyslipidaemia and diabetes mellitus that are therapeutically relevant due to the increased risks of premature atherosclerosis in these diseases.[11,21,64,65] Treatment with hydroxychloroquine reduces the levels of cholesterol, triglycerides and LDL irrespective of concomitant steroid administration, diet or weight. In fact, dyslipidaemias are very frequent in SLE and certainly play a pivotal role in the 50-fold greater risk of developing coronary artery disease. Coronary diseases are important causes of mortality in SLE patients.[66] Mechanisms responsible for altered lipid profiles with chloroquine analogue treatment include a significant increase in lipid clearance rate and up-regulation of LDL receptors.

Coagulopathy and thrombosis

Hydroxychloroquine prevents significant thromboembolic events in the postoperative period following total hip arthroplasty or during pregnancy.[11,67,68] Several studies indicate that chloroquine analogues have an effect in the prevention of thrombotic phenomena.[69] The antithrombotic effect of chloroquine analogues has been attributed to a range of mechanisms, including reduction in red blood cell aggregation, inhibition of platelet aggregation and adhesion, reduction in blood viscosity and enhancement of antiplatelet activity.[70,71] Chloroquine and hydroxychloroquine exert beneficial effects in pulmonary arterial hypertension (PAH), pulmonary haemosiderosis and common variable immunodeficiency (CVID) granulomatous disease.[72–74] Chronic hydroxychloroquine treatment reduces hypertension, endothelial dysfunction and organ damage in patients with severe lupus.[75] These studies demonstrate the direct impact of chloroquine analogues on cardiac patient care.

Chloroquine analogues as investigational drugs in microbial infections

Chloroquine analogues have been found to be effective against bacterial infections such as endocarditis[76] and Q fever,[77] parasitic

infections such as giardiasis[78] and viral infections such as Ebola virus disease,[79] hepatitis C virus-related arthritis[80] and chikungunya.[81] Chloroquine analogues are being or have been used in clinical trials as investigational antiretroviral agents in humans with HIV-1/AIDS (registration numbers NCT01650558, NCT02004314 and NCT01067417). Combined treatment with hydroxychloroquine, hydroxyurea and didanosine in antiretroviral-naive HIV patients decreased viral replication and increased the CD4 count.[82] Human corona virus (hCoV) threatened to cause a pandemic of SARS. Chloroquine was shown to inhibit the replication and spread of corona virus *in vitro* and to prevent infection with hCoV in newborn mice, and this shows promise as a potential therapy for this resistant virus.[83,84] Human prion diseases are characterized clinically by cognitive, neuropsychiatric and motor dysfunction. The most common form of prion disease is sporadic Creutzfeldt–Jakob disease (CJD), which affects ~1–2 people per million annually worldwide. The accumulation of the pathogenic form of prion protein is a pivotal event in prion diseases. The chloroquine analogue quinacrine inhibits not only the accumulation of pathogenic prion protein but also the conversion of normal cellular prion protein to disease-associated forms. Clinical trials of quinacrine in patients with CJD are in progress.[85,86] Lymphangioleiomyomatosis (LAM) is associated with cystic lung destruction and lymphatic and kidney tumours and predominantly affects premenopausal women. Inhibition of autophagy with chloroquine analogues results in a decreased LAM cell load in the lungs and improvement in pulmonary function.[87]

Chloroquine analogues and antiphospholipid syndrome (APS)

The APS is a systemic autoimmune disorder characterized by recurrent thrombosis and/or pregnancy morbidity occurring in patients with persistent antiphospholipid antibodies (aPL). There is ample evidence of the protective effects of hydroxychloroquine in primary obstetrical APS.[88,89]

Mechanisms of action

Although it would be aesthetically pleasing to ascribe all therapeutic effects to a single mode of action, this is not the case with the actions of chloroquine analogues. There is certainly more than one mechanism for the actions of these drugs, and some of them are discussed here.

Rationales for lysosomotropic amines

Chloroquine is a diprotic weak base ($pK_{a1}=8.1$, $pK_{a2}=10.2$ at 37°C) that can exist in both protonated and unprotonated forms (Figure 2 and Table 2). Unprotonated chloroquine can diffuse freely and rapidly across the membranes of cells and organelles to acidic cytoplasmic vesicles (late endosomes and lysosomes). Once protonated, chloroquine is trapped in the acidic organelles (lysosomes) and can no longer freely diffuse out. Therefore, chloroquine analogues are known as lysosomotropic agents (i.e. they are taken up selectively into lysosomes).[90] Lysosomes are cellular compartments containing acid hydrolases that digest several macromolecules. For optimal activity of hydrolases, pH is maintained at ~5.0 by the action of lysosomal H^+-ATPases.[91] As more H^+ ions are pumped into the acidic vesicle by the ATP-dependent pumps of lysosomal H^+-ATPases, more chloroquine will diffuse from the cell's cytoplasm into the acidic vesicle to cause partition according to the difference between two pH gradients. This leads to an irreversible accumulation of chloroquine in lysosomes to >100-fold excess concentration and causes an elevation of pH due to trapping of H^+ ions by chloroquine.[87] Hydroxychloroquine, a related lysosomotropic amine, appears to be very similar to chloroquine in its effect on cellular function. Thus, chloroquine analogues interfere with lysosomal acidification, which in turn inhibits proteolysis, chemotaxis, phagocytosis and antigen presentation. As a result, cells are not able to proceed with endocytosis, exosome release and phagolysosomal fusion in an orderly manner.[92]

As antigen processing is an acidic, pH-dependent phenomenon, chloroquine turns off the process of antigen presentation by decreasing the number of autoantigenic peptides appearing on the cell surface. Because autoantigenic peptides have low affinity for self-MHC, elevation of pH in the acidic compartments selectively decreases the loading of autoantigenic self-peptides, while leaving intact the response to exogenous peptides with higher affinity. This decreased amount of self-peptide–MHC complex on antigen-presenting macrophages and other target cells results in decreased stimulation of CD4+ T cells. Thus, the production of a series of cytokines by both T cells and antigen-presenting cells also decreases. The increased pH induced by chloroquine in lysosomes also causes decreased activities of the aspartyl protease cathepsin D and the cysteine protease cathepsin B, which are responsible for early and late cleavage of invariant chains from the MHC class II molecule, respectively (Figure 2). Inhibition of antigen presentation by chloroquine appears to primarily affect professional antigen-presenting cells such as dendritic cells, B cells and macrophages, which have MHC class II-enriched compartments.[1,21,93]

Anti-inflammatory and immunomodulatory effects

Chloroquine analogues have well-recognized anti-inflammatory and immunomodulatory actions,[21] but their specific mechanisms in individual diseases are not clear. The major proposed mechanisms of actions of chloroquine analogues are summarized in Table 3 and Figure 3.

Anticancer effects

The anticancer mechanisms of chloroquine analogues are more complex, with many potential cellular targets. The most common approach in cancer therapy is the inhibition of autophagy and sensitization of malignant cells to radiation and chemotherapeutic agents by chloroquine analogues.[31,32] A number of clinical trials are in progress; the results obtained so far indicate that the use of chloroquine analogues may lead to changes in cancer therapeutic strategies.[35] The lysosomotropic properties of chloroquine analogues are their most important characteristics for alteration of the malignant progression of cancer cells. Chloroquine accumulates preferentially in lysosomes and raises intralysosomal pH, which in turn increases the permeability and volume of lysosomes. The increased intralysosomal pH produced by chloroquine analogues may not be sufficient to cause cellular damage specifically in tumour cells at therapeutically achievable concentrations. However, the analogues can damage tumour cells when lysosomal permeability is also increased by radiation, which causes the release of proteolytic enzymes and damages cellular functional proteins, including plasma membrane-associated proteins (Figure 4). Thus, chloroquine analogues sequester and modify

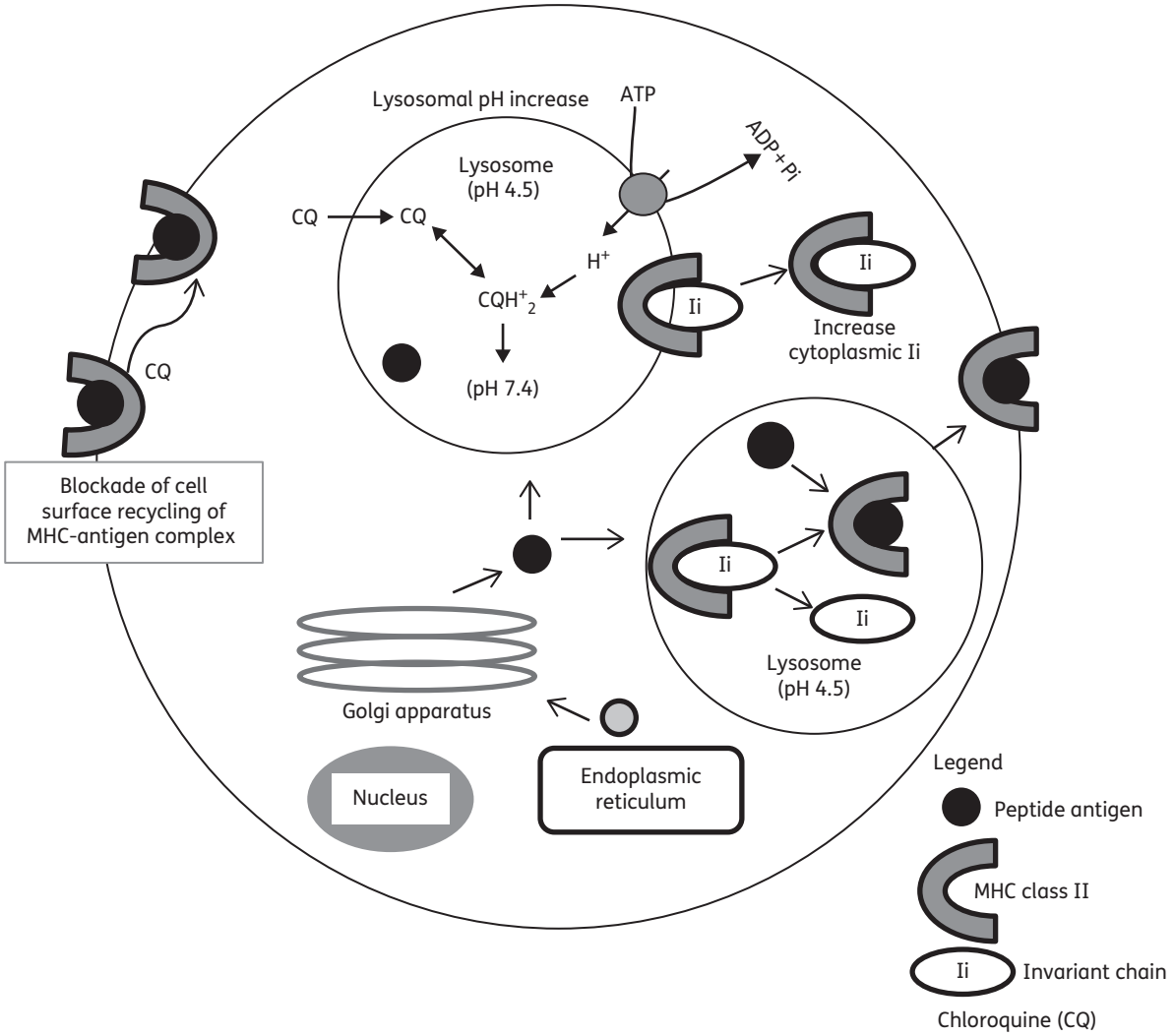


Figure 2. Major inhibitory roles of chloroquine in lysosomes. Intralysosomal pH is increased by chloroquine. Note that the raised pH is not involved in the antimalarial mechanism of action.

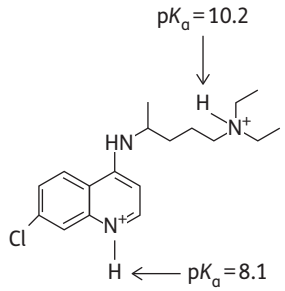
many important cell membrane constituents (ceramides in lysosomes), thus limiting plasma membrane repair and recycling. These events are therapeutically useful.[94]

The ATP-binding cassette (ABC) family of transmembrane proteins and the multidrug resistance proteins extrude chemotherapeutic drugs from targeted cancer cells. This drug-extruding activity contributes to drug resistance in cancer, and expression of one or more ABC proteins and multidrug resistance proteins is often up-regulated during anticancer chemotherapy. In chemosensitization, chloroquine inhibits anticancer drug extrusion by blocking transporters of the ABC family and multidrug resistance proteins. Thus, chloroquine modulates the tumour response to anticancer drugs (Figure 4). Chloroquine analogues, used at clinically achievable concentrations, are also known to sensitize cells to radiation and anticancer drugs.[95] Chloroquine enhances the radiosensitizing effects of some chemotherapeutic drugs used concomitantly with radiotherapy by increasing lysosomal permeability, by releasing membrane-damaging proteolytic enzymes or by inhibiting ABC-mediated drug extrusion (Figure 4). The other main actions of chloroquine analogues responsible for most intracellular actions are (i) the molecular intercalation of chloroquine into DNA[96] and (ii) the inhibition of lysosomal enzymes, particularly phospholipase A2.[97] Configurational changes in nucleic acids render neoplastic cells more susceptible to the cytotoxic effects of radiotherapy as well as chemotherapy. Therefore, potentiation of the effects of chloroquine analogues is taken into consideration in clinical trials with radiotherapy and chemotherapy.

Adverse effects

There are relatively few adverse effects at the standard doses of chloroquine analogues that are used for the prophylaxis of malaria and other systemic diseases. However, acute toxicity of chloroquine analogues is encountered most frequently when therapeutic or high doses are administered very rapidly by parenteral routes. The most serious complications of chloroquine analogues are retinopathy, cardiomyopathy, neuromyopathy and myopathy. The estimated frequency and reversibility of these complications is given in Table 4.

Table 2. Percentages of protonated forms of chloroquine at different pHs



pH	% CQ	% CQH^+	% CQH_2^{2+}
5.0	0.000	0.079	99.919
5.5	0.000	0.252	99.747
6.0	0.000	0.794	99.205
6.4	0.000	1.971	98.028
6.9	0.002	5.979	94.017
7.4	0.026	16.739	83.234
8.4	1.039	66.095	32.865

Neutral (CQ), mono-protonated (CQH^+) and di-protonated (CQH_2^{2+}) forms of chloroquine are included. The percentages are calculated using the Hendersen–Hasselbalch equation, with pK_a values of 8.1 for the quinoline nitrogen and 10.2 for the side chain diethylamine nitrogen.

Table 3. Anti-inflammatory and immunomodulatory actions of chloroquine analogues

- Inhibition of antigen processing and presentation
- Inhibition of stimulation of TLR9 cells, which participate in immune responses
- Inhibition of cytokine production and release by T cells: IL1, 2, 6 or 18, TNFα and IFNγ
- Increase in Treg activity and up-regulated levels of IFNα and IL2 and 10 cytokines
- Inhibition of activity of cytotoxic T lymphocytes and self-reactive CD4+ lymphocytes
- Reduced levels of chemokines CCL2 and CXCL10 in SLE
- DNA binding: competitive inhibition of anti-DNA antibodies
- Inhibition of phospholipase A2 and thereby antagonizing the effects of prostaglandins and leukotrienes
- Decreased DNA, RNA and protein synthesis in thymocytes
- Blockade of the actions of endogenous as well as exogenous histamine
- Inhibition of nitric oxide formation by macrophages and induced production of reactive oxygen species in astroglial cells
- Absorption and blocking of cutaneous reactions to UV light
- Inhibition of T and B cell receptor calcium signalling
- Inhibition of matrix metalloproteinases
- Inhibition of micro-RNA expression
- Decreased T_H17-related cytokines

Sensory systems

Eyes

Chloroquine and its congeners can cause two typical adverse effects in the eyes: keratopathy and retinopathy. Both of these effects are associated with the administration of the drugs over long periods of time. Chloroquine-induced keratopathy is limited to the corneal epithelium, where high concentrations of the drug are usually used. The retinopathy encountered with the prolonged use of chloroquine analogues is a much more serious clinical problem and can lead to irreversible damage to the retina and loss of vision. The hallmark feature of hydroxychloroquine toxicity is bilateral pigmentary retinopathy.[98,99] At an early stage in hydroxychloroquine-induced retinal disease, patients may often be asymptomatic despite having subtle paracentral scotomas. Later in the disease, patients may develop a 'bull's eye' maculopathy, characterized by a ring of retinal pigment epithelium (RPE) in the macular area closer to the fovea, which is often accompanied by paracentral and central scotomas.[100,101] End-stage hydroxychloroquine toxicity leads to widespread RPE and retinal atrophy with a loss of central, peripheral and night vision.[102] The incidence of retinopathy associated with the use of chloroquine analogues is low, as long as the dose does not exceed the therapeutic doses and the medication is used for <10 years in patients with normal renal function. Quinacrine does not cause retinopathy. Other adverse effects on the eyes include rhegmatogenous retinal detachment and bitemporal hemianopsia in association with chloroquine retinopathy. Diplopia and impaired accommodation are also observed even at lower doses in some patients. The best current opinion seems to be to avoid retinopathy by using doses of hydroxychloroquine not exceeding 6.5 mg/kg/day, with periodic checking of renal and hepatic function.[103]

Ears

Besides their well-known retinal toxicity, chloroquine analogues are suspected to be associated with ototoxicity. There are reports suggesting sensorineural hearing loss, tinnitus, a sense of imbalance and cochleovestibular manifestations.[104,105] The reversibility of chloroquine ototoxicity is debatable, but there is a suggestion that such complications can be corrected if the medication is stopped and appropriate therapy, with steroids and plasma expanders, is instituted.

Cardiovascular system

Cardiac side effects of chloroquine analogues are rarely reported, but in some cases can be severe and irreversible. Conduction disturbances (bundle-branch block, atrioventricular block), cardiomyopathy (often with hypertrophy and congestive heart failure) are the major toxic effects.[106–109] A case report suggested that chloroquine cardiotoxicity manifested suddenly as atrioventricular block with QT(c) interval prolongation and short torsade de pointes.[109] Symptoms like syncope and Stokes–Adams attacks and signs of cardiac failure can also occur. Acute intoxication can cause fatal cardiovascular collapse and/or respiratory failure.

Gastrointestinal system

Gastrointestinal discomfort is the most common reaction in patients receiving chloroquine analogues, although the discomfort is usually mild and can be managed by dose reduction. The

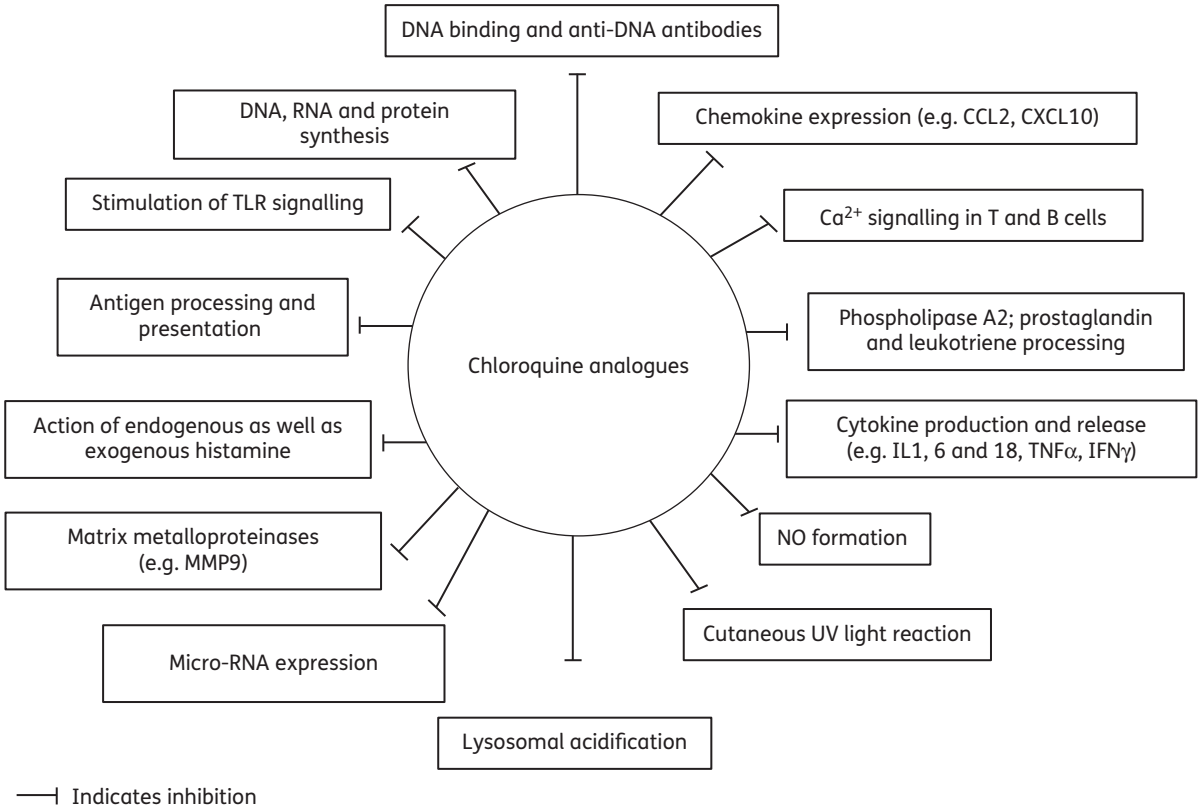


Figure 3. Major anti-inflammatory and immunomodulatory effects of chloroquine analogues.

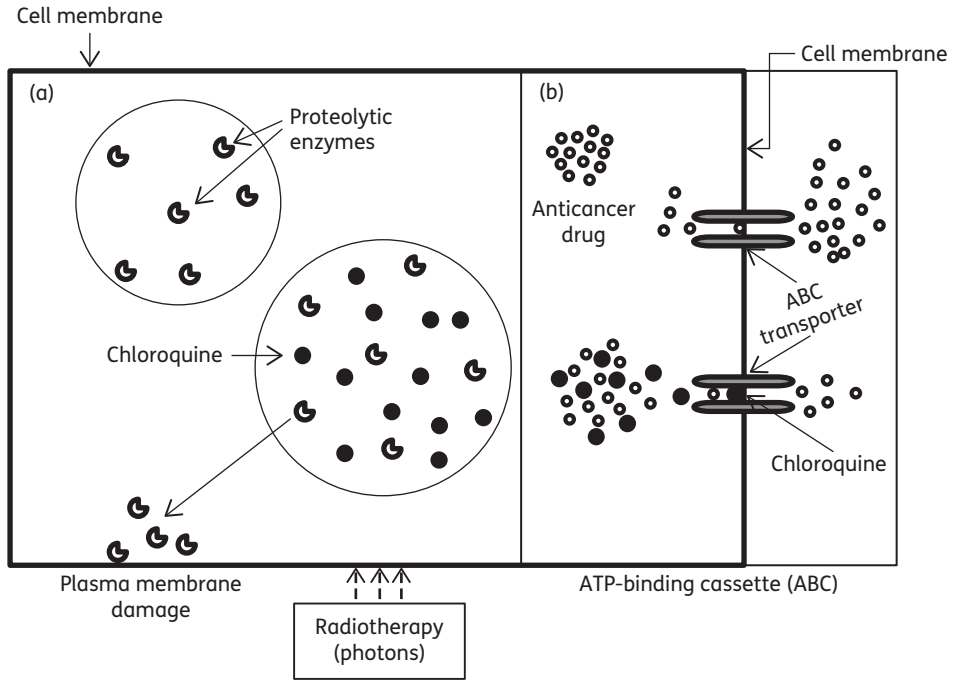


Figure 4. Mechanisms of anticancer actions of chloroquine analogues. (a) Radiosensitizing effect: membrane-damaging proteolytic enzymes are released and lysosomal permeability is increased as a result of radiation and the effect of chloroquine. (b) Chemosensitizing effect: anticancer drug extrusion is prevented via blockade of ABC transporters with chloroquine, and intracellular drug availability is increased and cells damaged.

Table 4. Major adverse effects of chloroquine analogues

Organ/system	Major adverse effects	Duration of therapy/overdose	Reversibility	Estimated frequency
Sensory systems				
eyes	keratopathy	higher dose and/or prolonged periods	irreversible	low incidence
	retinopathy		irreversible	
ears	ototoxicity	higher dose and/ or prolonged periods	reversible	rare
tongue and nose	disturbances of taste and smell	long time	reversible	report on only one patient
Gastrointestinal system	gastrointestinal discomfort	therapeutic doses	reversible	most common
Cutaneous system	pruritus	short time	reversible	most common, 1% of patients
	photosensitivity	short time	reversible	
Nervous system	neuromyopathy	higher doses	reversible	several reports suggested more common
	seizures, psychosis	prolonged periods		
Musculoskeletal system	myopathy	5–7 months	reversible	occasionally
Cardiovascular system	conduction disorders cardiomyopathy	long term/high dose	irreversible	occasionally
Haematological system	haemolysis	long term/high dose	irreversible	less common
	leucopenia		reversible	less common
	aplastic anaemia		reversible	(1/50 000)
Respiratory system	alveolitis	overdose	reversible	rare
Urinary tract system	impaired renal function	long time	reversible	occasional
Immunological system	allergic contact dermatitis	short time	reversible	report on a patient
Metabolism	hypoglycaemia	long time	reversible	several reports less common
Other	hearing loss	long time	irreversible	very rare

Long time, >1 year; short time, <1 year; reversible, relief of symptoms on withdrawal of treatment; irreversible, permanent loss.

other common gastrointestinal events are nausea, vomiting and diarrhoea. Overdoses of the analogues can cause vomiting. Stomatitis with buccal ulceration has occasionally been reported with the analogues. Less frequent gastrointestinal effects include anorexia, abdominal distress, abnormal liver function and transaminitis.[110] Hepatotoxicity, which is uncommon with either chloroquine or proguanil, has been reported after the use of a fixed-dose combination of chloroquine and proguanil.[111]

Cutaneous system

The most common dermatological adverse event associated with chloroquine analogues is pruritus.[112] It is much more common in darker-skinned people, in whom chloroquine binds to melanin in the skin. Recent case reports have suggested that pigmentary changes of the skin and mucous membranes develop during the course of chloroquine therapy for connective tissue disorders. Chloroquine analogues induce hyperpigmentation and longitudinal melanonychia in older patients. Quinacrine causes darker pigmentation than chloroquine and hydroxychloroquine.[113,114] Prolonged use of chloroquine may occasionally cause lichenoid skin eruptions, bullous skin eruption, skin lesions (including epidermal necrolysis) and bleaching of the hair.[115] Chloroquine can turn the nail bed blue–brown and the nail itself may develop longitudinal stripes. Photosensitivity and photoallergic dermatitis have been seen, particularly during prolonged therapy with high doses of the analogues. A near fatal case of Stevens–Johnson syndrome has been reported after treatment with an analogue.[116] Chloroquine therapy can also cause vitiligo,[117] pemphigus[118] and severe cutaneous necrotizing vasculitis.[119]

Nervous system

Chloroquine analogues can cause a marked neuromyopathy, characterized by slowly progressive weakness, particularly with long-term use or standard doses in elderly people. Chloroquine can also cause seizures in patients with epilepsy and SLE.[120] Convulsion has also been reported in patients in whom chloroquine is part of a prophylactic regimen; the condition is reversible if the analogues are withdrawn. The many mental changes attributed to chloroquine analogues include agitation, aggressiveness, confusion, personality changes, loss of memory, psychotic symptoms and depression.[121,122] Hallucinations have also been reported after hydroxychloroquine treatment for erosive oral lichen planus.[51]

Musculoskeletal system

Chloroquine analogues occasionally cause a myopathy associated with muscle weakness, and reduced or absent tendon reflexes. Severe vacuolar myopathy has also been reported with hydroxychloroquine.[123]

Haematological system

Haematological side effects of chloroquine analogues are uncommon. Rare instances of haemolysis and blood dyscrasias have been reported. Haemolysis in patients with glucose-6-phosphate dehydrogenase deficiency, aplastic anaemia and leucopenia have been recorded with chloroquine analogues, particularly quinacrine at higher doses. Leucopenia,

Table 5. Selected examples of clinical trials of chloroquine analogues

Status	Condition	Phase	Intervention	Trial ID
Recruiting	SLE	II	mycophenolate mofetil; HCQ or CQ; prednisone	NCT01946880
Recruiting	RA	III	tocilizumab; DMARD (HCQ or CQ)	NCT01941940
Recruiting	RA	III	DMARD (HCQ or CQ); tocilizumab	NCT01941095
Completed, has results	RA, insulin resistance	III	HCQ	NCT01132118
Completed	osteoarthritis	IV	GS and CS; (GS and CS)+PS or/and CQ	NCT00805519
Recruiting	colorectal adenocarcinoma	I/II	QC; capecitabine	NCT01844076
Recruiting	metastatic renal cell carcinoma	I/II	HCQ; IL2	NCT01550367
Recruiting	pancreatic cancer	II	capecitabine; HCQ; proton or photon radiation therapy	NCT01494155
Recruiting	sarcoma	II	sirolimus and HCQ	NCT01842594
Active, not recruiting	prostate cancer	II	HCQ	NCT00726596
Recruiting	renal cell carcinoma	I	HCQ	NCT01144169
Active, not recruiting	pancreatic cancer	II	HCQ	NCT01273805
Active, not recruiting	pancreatic cancer	I/II	HCQ; gemcitabine	NCT01128296
Recruiting	brain metastasis		CQ	NCT01727531
Recruiting	pancreatic cancer	I	CQ; gemcitabine	NCT01777477
Recruiting	intraductal carcinoma	I/II	CQ	NCT01023477
Recruiting	advanced solid tumours	I	CQ; carboplatin; gemcitabine	NCT02071537
Recruiting	small cell lung cancer	I	CQ	NCT01575782
Recruiting	small cell lung cancer	I	CQ	NCT00969306
Recruiting	advanced cancers	I	HCQ; sirolimus; vorinostat	NCT01266057
Recruiting	HIV		CQ	NCT01650558
Recruiting	malaria	III	sulfadoxine/pyrimethamine; CQ	NCT01443130
Recruiting	hepatitis C virus	IV	CQ	NCT02058173
Unknown	influenza	II	CQ	NCT01078779
Completed	HIV		CQ	NCT02004314
Active, not recruiting	metabolic syndrome X, overweight, hypertension, dyslipidaemias, pre-diabetic state	II	CQ	NCT00455325
Completed	primary SS	III	HCQ	NCT00632866
Active, not recruiting	pre-diabetes	IV	HCQ; sugar pill	NCT01326533
Recruiting	LAM	I	sirolimus and HCQ	NCT01687179
Completed	autoimmune diseases, SS, dry eye	III	HCQ	NCT01601028
Recruiting	type 2 DM		HCQ	NCT02026232
Completed	Hashimoto's thyroiditis		HCQ	NCT01760421
Completed	pulmonary sarcoidosis	III	PS; HCQ+PS	NCT02200146
Active, not recruiting	Hashimoto's thyroiditis		HCQ	NCT02126683
Recruiting	congenital heart block, neonatal lupus, autoantibody-associated heart block	II	HCQ	NCT01379573
Recruiting	NSCLC; advanced NSCLC, recurrent NSCLC	II	paclitaxel; carboplatin; HCQ; bevacizumab	NCT01649947
Recruiting	malignant solid tumour	I	HCQ; vorinostat	NCT01023737
Recruiting	DM type 2 with hyperglycaemia	II	HCQ; pioglitazone	NCT02303405
Recruiting	Crohn's disease	II	ciprofloxacin; doxycycline; HCQ; budesonide	NCT01783106
Recruiting	PCT	II	HCQ; phlebotomy	NCT01573754
Unknown	HIV infections	II	HCQ	NCT01067417
Recruiting	APS	III	HCQ	NCT01784523
Completed, has results	CJD	II	QC	NCT00183092
Unknown	prion disease		QC	NCT00104663

CQ, chloroquine; CS, chondroitin sulphate; DM, diabetes mellitus; DMARD, disease-modifying antirheumatic drug; GS, glucosamine; HCQ, hydroxychloroquine; NSCLC, non-small cell lung cancer; PS, prednisolone; QC, quinacrine; SS, Sjögren's syndrome.

agranulocytosis and the occasional case of thrombocytopenia have been noted.[124] There is some evidence that myelosuppression is dose-dependent. Liver function and blood tests, particularly complete blood counts, should be performed monthly at the start of therapy and at least every 4–6 months throughout treatment.

Metabolism

Therapeutic doses of chloroquine analogues can cause hypoglycaemia.[125] Convulsion is more common in hypoglycaemic children. Although hydroxychloroquine has been used to treat PCT, there are some reports that it can also worsen porphyria in SLE patients.[126]

Others

Chloroquine-induced impaired renal function has occasionally been reported. Allergic contact dermatitis followed by severe asthma has occurred in a patient (60 years old) with hydroxychloroquine exposure.[127] It is reported that induction or exacerbation of psoriasis is observed in lichen planopilaris (a 40-year-old female) and psoriatic arthritic (a 37-year-old primigravida) patients treated with hydroxychloroquine.[128] An acute gluteal abscess after an injection of chloroquine has also been reported.[129] Acute generalized exanthematous pustulosis has been reported in Canada as an adverse reaction to hydroxychloroquine.[130]

Pregnancy and infants of treated women

Chloroquine inactivates DNA and crosses the placenta in animals. Caution is generally advised with respect to the use of chloroquine analogues during pregnancy, but there are no reports available to date of complications in mothers or their newborn infants after treatment with chloroquine during pregnancy and lactation.[11,24,88] During lactation, the amount of hydroxychloroquine transferred to the infant seems to be negligible and does not confer a risk of toxicity to the infant.[24]

Conclusion

Chloroquine analogues have been credited with saving the lives of thousands of patients with malaria. Since the first use of chloroquine analogues nearly a century ago, their effectiveness has been increasingly recognized in nearly all major branches of medicine, including immunology, oncology, haematology, dermatology, cardiology and severe infectious diseases such as AIDS and SARS. Although these drugs are not FDA approved for several therapies, rheumatologists, dermatologists and other professionals have recognized their effectiveness for various pathologies in their specialities. To date, chloroquine analogues have established roles in the treatment of SLE, RA, osteoarthritis, cancers and various skin diseases (e.g. lichen planus and Sjögren's syndrome). There are also currently many clinical trials studying the effects of chloroquine analogues in various diseases, such as malignant neoplasms of the lung, breast, prostate, pancreas and colon, melanoma, renal cell carcinoma, multiple myeloma, influenza, HIV infection, and the metabolic syndrome (Table 5). To investigate the roles of these analogues in a wide variety of diseases, a number of molecular modifications, such as the prodrug[131] and metabolomic approaches,[132] have been used with the aims of improving their pharmacokinetic and pharmacodynamic properties, reducing undesirable side effects, costs and drug sensitivities. However, the exact mechanisms of action of the analogues and their effectiveness in these diseases remain to be demonstrated. Despite their benefits and their current use in >70 countries, chloroquine analogues remain unavailable for clinical use to treat patients in Japan due to a series of lawsuits as a result of the retinal toxicity of chloroquine in the 1970s. However, because of their use as the standard of care worldwide, clinical trials of hydroxychloroquine for SLE in Japan have recently been started.[133,134] Our understanding of the history of chloroquine analogues suggests that the appropriate use of an efficacious therapy will soon lead to an era of improvement of patient care, survival and quality of life for many patients.

Acknowledgements

I would like to acknowledge M. Shinohara and H. Takayanagi (Department of Cell Signaling, Tokyo Medical and Dental University) for technical assistance with the literature search.

Transparency declarations

None to declare.

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