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# Hydroxychloroquine: New Perspectives for an Indispensable Old Drug

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**Abstract**

Review Article

Hydroxychloroquine (HCQ) is a multi-functional drug owing to its lysosomotropic, immunomodulatory, anti-inflammatory, anti-infective, antithrombotic, antitumoral (pronounced effects on autophagy and apoptosis processes) and beneficial metabolic properties (improved lipid profiles, decreased insulin resistance). We know that chronic low-dose HCQ therapy has been successfully used in a variety of chronic diseases such as rheumatological and dermatological disorders. Additionally, with all these effects mentioned above and showing synergism, HCQ can also be useful mostly as an adjuvant in the management of many chronic metabolic disorders, serious life-threatening conditions such as cardiovascular, neurological, oncological and infectious diseases, as well as their accompanying morbidities. More recently, this former drug, whose effectiveness has been shown in the global coronavirus disease 2019 (COVID-19) pandemic, has entered the spotlight again. Ongoing clinical trials testing HCQ in new indications and challenging diseases are still receiving great attention. In this article, the mechanisms of action, current clinical uses and new indications of HCQ therapy have been overviewed with a comprehensive literature review.

## INTRODUCTION

The process of finding new therapeutic indications for existing drugs currently in use for other diseases has been gaining increasing attention over the past few years. This process is defined as "reuse of old drugs" or "repurposing of drugs", and has often been shown to be successful [1]. As it is known today, hydroxychloroquine (HCQ), which was first indicated to prevent or cure malaria, have also been used successfully in the treatment of some autoimmune, rheumatological, dermatological, immunological and infectious diseases [2-9]. In addition, there are still ongoing in vitro or in vivo investigations through some clinical, animal and/or laboratory studies to evaluate their effectiveness in some clinical situations particularly in the fields of oncology and neurology [3,4,8]. More recently, this old drug has entered the spotlight again due to the fact that it has been shown to be effective promisingly in the global coronavirus disease 2019 (COVID-19) pandemic [10-19].

In this article, case reports, clinical trials, cohort studies, systematic reviews and meta-analyses associated with current and new indications of HCQ published up until now were comprehensively evaluated. The Medline literature database was searched through PubMed using the key words, individually and in combination: 'hydroxychloroquine', 'antimalarial', 'indications', 'clinical use', 'therapeutic use', 'new perspectives', 'drug repurposing', 'medicine', 'rheumatology', 'dermatology', 'neurology', 'oncology', 'cardiology', 'infection' and 'novel coronavirus disease'. Only articles available in original or translated English were reviewed.

### *History of HCQ*

Antimalarials have existed for more than 300 years. In ancient times, the native Quechua people of South America were known to crush the bark of the cinchona tree and add sweetened water to produce tonic water for the treatment of many ailments, including fever. The name of the bark 'Quina quina' derives from the Inca language. This quinine-containing extract brought to Europe by Jesuit missionaries to treat malaria in the 1600s and was the first recorded drug to treat an infectious disease. This growing interest in the beneficial medicinal properties of the chinchona bark caused the British and Dutch to invest money in the plantations of these trees, and the use of quinine was the basis of antimalarial therapy [20]. In July 1934, Hans Andersag modified the quinacrine to replace the acridine ring with a quinoline ring and gave the world a gift of "chloroquine" (CQ) [21]. The observation of the effectiveness of CQ analogs in autoimmune diseases has been accidental like

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many important discoveries in medicine, and has been noticed during World War II by antimalarial prophylaxis with quinacrine and CQ improving skin rashes and inflammatory arthritis in soldiers [20]. However, this was not an entirely new discovery, as a British doctor Joseph Frank Payne, who was a doctor at St Thomas' Hospital in London, suggested using quinine as a possible treatment for systemic lupus erythematosus (SLE) in 1894, considering a vascular etiology [22]. In the postwar period, in 1955, "HCQ" was produced in America by the addition of a β-hydroxy chain to the CQ molecule as a more reliable and less toxic alternative agent than CQ [23]. Currently, HCQ (Plaquenil® tablets contain 200 mg HCQ sulfate) is still the most commonly used so-called antimalarial agent in the treatment of non-malarial and chronic autoimmune diseases [2-4].

### *Mechanisms of therapeutic action of HCQ*

HCQ shows its therapeutic effects in general by anti-inflammatory, immunomodulatory, anti-infective, antithrombotic and metabolic properties [5,23-28]. It is well known that this aminoquinoline accumulates in lysosomes, phagolysosomes and other intracellular acidic compartments and concentrates approximately 1000 times higher. HCQ has a powerful immunomodulatory feature that affects multiple consecutive steps in the process of immune recognition, response and downstream generation of inflammation. Since it has a weak diprotic base structure, it increases the pH of intracellular compartments, and so prevents the functions of phagocytosis and antigen presentation to T cells [23,24]. In vitro investigations have shown that HCQ at concentrations routinely achieved with chronic low-dose therapy potently inhibits the nuclear factor of kappa B pathways in the macrophages and T helper type 1 lymphocytes. It has antiproliferative effect on T cells. Thus, it reduces the production of various pro-inflammatory cytokines including interferon-gamma, tumor necrosis factor-alpha, interleukin (IL) -1, IL-2 and IL-6, which play a key role in adaptive immune response. HCQ also reduces natural immune activation by blocking the interaction of the Toll-like receptor (TLR) family, especially TLR3, TLR7 and TLR9 with nucleic acid ligands [3,5].

With the above-mentioned lysosomotropic, immunomodulatory and anti-inflammatory properties, chronic low-dose HCQ therapy can be used in a variety of chronic diseases with altered immune recognition and/or responses such as rheumatological diseases, cardiovascular disorders, chronic kidney disease, dermatological diseases and infectious diseases [2-9]. Anti-infective, antithrombotic and antitumoral properties of this drug have also been described [8,9,25,26]. In addition, beneficial metabolic properties of HCQ including improved lipid profiles, decreased insulin resistance and reduced incidence of diabetes mellitus have been identified [27-29]. The use of HCQ has been associated with a lower risk of hyperlipidaemia as decreased levels of serum low-density lipoprotein-cholesterol, total cholesterol and triglyceride concentrations, irrespective of concomitant steroid use in rheumatoid arthritis and SLE patients [30]. This favourable effect may be due to HCQ upregulating LDL receptors and increasing lipid excretion [31]. Similarly, over the past 2-3 years, it has become clear that high doses of these compounds have pronounced effects on autophagy and apoptosis processes, leading to the applications of HCQ in oncology [3,4,8]. With all these effects showing synergism, HCQ can be useful alone or as an adjuvant in the treatment of many chronic metabolic diseases, serious life-threatening conditions, and serious infectious diseases, as well as their accompanying morbidities. Recently, some clinical trials support that the repurposing of HCQ has included some neurological and oncological diseases, some major adverse cardiovascular events, and promisingly COVID-19 pandemic [3,4,10-19]. Ongoing clinical trials testing HCQ in new indications and challenging diseases are still receiving great attention.

## *Clinical Use of HCQ*

### *Use of HCQ in Infectious Diseases*

CQ and HCQ, mainly used for malaria treatment and prophylaxis, have not only antiparasitic but also antiviral, antibacterial and antifungal effects. HCQ is thought to inhibit the growth of intracellular pathogens, leading to alkalization of lysosomes and other intracellular acidic compartments. Alkalization leads to the expansion and vacuolization of lysosomes, followed by inhibition of their functions. This process can reduce post-transcriptional modification of proteins, release of enzymes, recycling of receptors, activation of cell signaling pathways and repair of cell membranes [3,9,24]. CQ analogs used in the treatment of uncomplicated malaria due to Plasmodium vivax, P. malariae, P. ovale and P. knowlesi directly interfere with the heme polymerization process of plasmodium and indirectly contrast the hemoglobin digestive

tract of the parasite [3,23].

The primary option for the treatment of Q fever (a bacterial zoonosis caused by Coxiella burnetii) and Whipple's disease (a chronic bacterial infection caused by Tropheryma whippeli) is a combination of HCQ and doxycycline. Recurrences have been reported even after long-term treatment when doxycycline is used alone or combined with other antibiotics. Replication of bacteria that play a role in the pathogenesis of these infections in low pH phagolysosomes and this reduces the effectiveness of antibiotics have been associated with relapses. Due to the vacuole alkalization induced by HCQ, doxycycline shows antibacterial activity and successful results are obtained [32-34].

Although HCQ does not represent primary care in fungal diseases, antifungal activity of aminquinolines has also been reported. It has been hypothesized that suppression of fungal growth with CQ derivatives may be due to pH deprivation of iron or alkalization of intracellular vacuoles [9,24,35]. HCQ has been shown to have in vitro antifungal activity, mainly against intracellular fungi such as Histoplasma capsulatum and Cryptococcus neoformans [9]. Yeo et al. reported that Pneumocystis pneumonia was more common in patients with SLE who had moderate to severe renal disease, mycophenolate mofetil/mycophenolic acid use, and high doses of glucocorticoid/cyclophosphamide use; however, they have shown that this risk is reduced by the antifungal effect of high doses of HCQ [36].

The antiviral effects of HCQ have also been extensively studied. HCQ is a cellular autophagy modulator that interfere with late stages of replication of enveloped viruses such as retroviruses, flaviviruses, and coronaviruses. Several studies have demonstrated that it offers considerable broad-spectrum antiviral effects via decreasing the pH and interfering with the fusion process of these viruses [12,37]. The serious clinical consequences of viral diseases arise not only from direct viral infection and the destruction of sensitive cells, but also from the effects of the immune response mediated by the release of pro-inflammatory cytokines, chemokines and other mediators [9,38]. As previously known, a number of case-control studies have supported the associations between human TLR polymorphisms and susceptibility to viral infections (e.g., Crimean-Congo hemorrhagic fever virus, human immunodeficiency virus, respiratory syncytial virus, hepatitis C virus, herpes simplex virus) [39,40]. It has also been demonstrated that there is a relationship between some TLRs (such as TLR8,TLR9) polimorphism with increased mortality in severe viral infections [40]. The therapeutic mechanism of HCQ in these infections may be related to its TLR antagonist activity [3,9].

The anti-human immunodeficiency virus (HIV) feature of CQ analogs appears to be associated with the inhibition of post-translational modification of glycoprotein 120 primarily in T cells and monocytes, thereby altering its immunogenic properties. Another important factor modulated by CQ analogs is immune activation seen in HIV infection [40,41]. It has been suggested by Piconi et al. that HCQ reduces lipopolysaccharide/TLR-mediated immune activation and may be a useful immunomodulant in HIV-infected patients. It has been reported that HCQ-induced immune modulation was associated with increased percentages of circulating CD4(+) T cells and this compound had a notable impact on immune activation as shown by significant modifications of the following parameters: reduced plasma lipopolysaccharide; decreased TLR4-expressing CD14(+) cells, TLR4-mediated signal transduction, and mRNA synthesis; reduced percentages of activated CD4(+) (CD4(+)/Ki67(+)) and CD14(+) (CD14 (+)/CD69(+)) cells; increased T-regulatory cells (Tregs), naive Tregs, and TLR4-expressing Tregs; augmented plasmacytoid dendritic cells and reduced IFNα-secreting plasmacytoid dendritic cells; and reduced IL-6 and tumor necrosis factor-alpha production [43]. In addition, the selective apoptosis of the memory T cell compartment (CD45RA-CD45RO+) has been shown to be induced by HCQ, which can significantly reduce the HIV viral reservoir [44]. This activity of CQ analogs appears synergistic with that of other antiretroviral drugs. However, unlike early studies, the benefit of HCQ in changing the course of HIV disease has not been demonstrated in patients with high CD4 cell counts that have not yet begun antiretroviral therapy in a randomized, double-blind, placebo-controlled clinical trial [40]. Recent reviews have placed particular emphasis on HCQ start time and dosage selection to maximize effectiveness in HIV-infected patients [38,41].

HCQ was also reported to interfere in mosquito-born flavivirus infections at different stages such as Japanese encephalitis virus internalization, yellow fever virus replication, and Dengue virüs (DENV) maturation. DENV-infected people show self-limited febrile dengue fever and dengue hemorrhagic fever. Rarely, life-threatening dengue shock syndrome occurs after a second DENV infection. Wang

et al. have found HCQ greatly inhibited viral replication in cells treated with HCQ before but not after DENV-2 infection. Therefore, it has been reported that the cellular environment change with HCQ treatment may be crucial to restrict DENV-2 infection, whereas the late stage of interference in virion maturation might not be the major mechanism of HCQ anti-DENV-2 activity. As a result, it was thought that HCQ preventive therapy may be an appropriate strategy to reduce the severity and spread of the DENV outbreak in the region of the dengue pandemic or during the season [45]. The Zika virus (a member of the Flaviviridae family that infects humans with mosquitoes and ticks) has been shown to infect human neural precursors and is associated with serious microcephaly if the infection is obtained during pregnancy [46]. Given the absence of any established treatment against the Zika virus during pregnancy and the safety of HCQ during pregnancy, it has recently been proposed to use this drug for the treatment and prophylaxis of Zika virus infection in humans [47,48]. In the infection of the Chikungunya virus (an alphavirus transmitted by Aedes aegypti and A. albopictus mosquitoes), the early phase characterized by the acute (fever, severe polyarthritis, rash) and following the post-acute phase (peripheral vascular disorders, neuropathy, neuropsychiatric disorders) gets better generally in three months. However, after the third month, the disease can enter a chronic stage with rheumatic, musculoskeletal and other symptoms. HCQ therapy has been shown to be effective when combined with methotrexate and sulfasalazine in patients with chronic persistent chikungunya arthritis [49].

More recently, COVID-19, caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), outbreak in December 2019, starting in Wuhan, China and spreading quickly to almost all of the countries worldwide. The WHO declared the epidemic of COVID-19 as a pandemic on March 12th 2020 [19]. The disease is mild in approximately 80% of infected patients. The transition from first symptoms to acute respiratory distress syndrome occurs in many severe COVID-19 cases and the median time between these periods is approximately 8 days (6–12 days). This process is likely to result from uncontrolled cytokine release. As a result of cytokine release syndrome or 'cytokine storm', which is characterized by excessive proliferation of immune cells and cytokines, multi-organ system failure and fatal damage to vital organs such as lungs, kidneys and heart [11-17].It was observed that the overall case-fatality ratio, which was 2.3% on average, increased to 8% in patients aged 70-79 years and to 14.8% in patients older than 80 years. However, it is believed that the number of asymptomatic carriers in the community is not actually low, and possibly mortality rates are reported lower than they are [19].

Although more clinical data are available on the anti-coronaviral activity of CQ than HCQ, both agents show theoretically similar antiviral activities [15,37]. Inhibition of SARS -CoV2 spread in CQ-treated cells before or after infection has demonstrated both prophylactic and therapeutic advantages of CQ in the fight against SARS-CoV2 [10]. Given that HCQ shows molecular mechanisms similar to CQ, HCQ is likely to perform similarly in terms of early prevention and progression of the disease. Zhou et al. have suggested that HCQ is a better therapeutic approach than CQ for the treatment of SARS-CoV-2 infection. The clinical safety profile of HCQ is better than CQ because it can be used at higher doses for a short time and less drug interactions [11]. Yao et al.'s. has recommended a treatment protocol (HCQ sulfate 400 mg given twice daily for 1 day, followed by 200 mg twice daily for 4 more days) to treat SARS-CoV-2 infection [17]. Gautret et al. has shown that the effect of HCQ treatment on viral load reduction/ disappearance in patients with COVID-19 increased with azithromycin [18].

HCQ alters the glycosylation of the angiotensin-converting enzyme 2 cellular receptors of coronaviruses. It can also increase the pH of the endosomes and lysosomes, thereby preventing the fusion process and subsequent replication of the virus with the host cells. When HCQ enters antigen-presenting cells, it prevents antigen processing and MHC class II-mediated autoantigen presentation to T cells. As a result of disruption of the interaction of DNA/RNA with TLRs and the nucleic acid sensor cGAS, the transcription of pro-inflammatory genes cannot be stimulated with HCQ treatment. It will also alleviate the serious progression of COVID-19 by reducing CD154 expression in T cells and inhibiting cytokine storm. Consequently, the application of CQ/HCQ not only prevents invasion and replication of the coronavirus, but also reduces the possibility of cytokine storm [11].

### *Use of HCQ in Rheumatologic Diseases*

The incidental discovery of the clinical benefits of antimalarial agents in rheumatological diseases has led to many in vitro and in vivo studies of the potential mechanisms of HCQ in autoimmunity and inflammation. HCQ use has become a part of current treatment guidelines for SLE, rheumatoid arthritis, primary Sjögren syndrome and antiphospholipid syndrome [4,5].

We know that the use of CQ derivatives in patients affected by SLE is not new, and HCQ has been historically used primarily in patients with cutaneous lupus. Successful results for HCQ have been confirmed in patients affected by discoid SLE as a result of systematic reviews of the Cochrane database [3,4,7]. It should be borne in mind that a significant proportion of patients may not initially respond to HCQ, but clinical response can be obtained in most by gradually increasing the dosage [50]. The use of HCQ has been shown to prevent lupus flares, increase long-term survival, and protect against irreversible organ damage, thrombosis and loss of bone mass. The use of HCQ in lupus is associated with reduced incidence of new onset organ damage, including serious complications such as nephritis and cerebritis. It has been shown that the risk for thrombotic events, cardiovascular events, and cardiovascular mortality are reduced with HCQ. Current or past use of HCQ has also been associated with higher spinal bone mineral density in female patients with SLE [51-53]. In another study, Mena-Vázquez et al. has observed that HCQ could delay the development of polyautoimmunity (co-occurrence of SLE and another autoimmune disease, such as autoimmune thyroiditis, rheumatoid arthritis, scleroderma, inflammatory myopathy and mixed connective tissue disease) in SLE patients [54].

Similar to SLE, positive results of HCQ therapy have been shown in the long-term management of rheumatoid arthritis in relation to its pleiotropic effects on inflammation and bone metabolism [4,55]. It is used as a sole agent in patients with milder severity of disease or as an adjunct to disease-modifying agents in those with severe systemic manifestations. "Triple drug therapy" containing methotrexate, sulfasalazine and HCQ is considered cheaper than new biological treatments [4,5].

HCQ is also used in the treatment of Sjogren's syndrome, which affects the exocrine sweat glands and many extraglandular organs, but its effectiveness is still controversial [5]. In a recent review, the effectiveness of HCQ in primary Sjogren's syndrome was not different from placebo in the treatment of xerostomy and xerophthalmia, and was even lower in fatigue treatment than placebo; however, it was more effective in relieving pain [56].

Previous studies have demonstrated a lower prevalence of antiphospholipid syndrome-related morbidity, a lower incidence of pregnancy complications and a higher rate of live births in women with antiphospholipid syndrome treated with HCQ [5,57]. In some studies, HCQ has been reported to effectively prevent recurrence of catastrophic antiphospholipid syndrome in pregnancy. The therapeutic role of HCQ in two patients with catastrophic antiphospholipid syndrome has also been observed [58].

### *Use of HCQ in Dermatological Diseses*

With the effects of immune-modulatory, anti-inflammatory, inhibition of mast cell infiltration, angiogenesis suppression and protecting cells against UVB exposure, HCQ has been used in many dermatological diseases but variable results have been reported [7,59,60]. In cutaneous lupus erythematosus (which provides the most clinical benefit from HCQ), responses vary by disease subtype but it is effective in more than half of the cases [7]. In a meta-analysis of 1,284 courses of HCQ among 16 studies, the total response rate was found to be 61% (highest in acute cutaneous lupus, lowest in chilblain lupus) [61].

Recently, 84 patients with morphea who had been using HCQ as monotherapy for six months were evaluated by Kumar et al. and a full recovery of 43% was determined. Among the morphea types, plaque morphea has been observed to respond better to HCQ than generalized, linear and deep subtypes [62,63]. In many case reports, 70-80% effectiveness of HCQ has been demonstrated in the treatment of cutaneous, oral and genital lichen planus [64,65]. Response rates ranging from 41% to 83% have been reported in patients with lichen planopilaris/frontal fibrosing alopecia[66]. Approximately half of the patients with granuloma annulare (55.6%) responded to HCQ, and it has been recommended as a third-line treatment especially in those with widespread lesions [67]. In an open clinical study involving patients with cutaneous sarcoidosis, approximately 70% complete response was received with HCQ, and it has been proposed as an adjuvant therapy for systemic or severe cutaneous sarcoidosis[68]. HCQ has also been reported to be effective in panniculitis patients such as chronic erythema nodosum, lupus panniculitis and lipoatrophic

panniculitis [69]. It is a recognised therapeutic option for the treatment of chronic idiopathic urticaria and can also be considered as a steroid-sparing agent in patients with urticarial vasculitis [70,71]. In patients with polymorphous light eruption, HCQ can be used to prevent a flare in those with inadequately controlled with standard therapy [72].

In small clinical studies, positive benefits of HCQ in porphyria cutanea tarda have also been demonstrated. Porphyria cutanea tarda, the most common human porphyria, represents a disease caused by an iron-related disorder due to the reduced activity of hepatic enzyme uroporphyrinogen decarboxylase. This disease is effectively and readily treatable with the use of either repeated phlebotomy or use of HCQ 100–200 mg twice weekly; however, low-dose HCQ is cheaper and as effective as venesection. HCQ can interact with large amounts of porphyrin stored in acidic hepatocyte organelles, causing them to be released into the plasma. It should not be used in patients with severe liver damage or advanced kidney failure, since excess porphyrin released into the plasma cannot be effectively dialyzed [73,74].

HCQ treatment has been tried in many dermatological disorders such as systemic sclerosis, dermatomyositis and alopecia areata, but the results have not been consistently positive in these cases [7,59,75]. Additionally, anecdotal reports exist for the use of HCQ in many other diseases including reticular erythematous mucinosis, annular elastolytic giant cell granuloma, actinic reticuloid, lipodermatosclerosis, chronic ulcerative stomatitis, eosinophilic annular erythema, Schnitzler syndrome and actinic prurigo [7].

### *Use of HCQ in Cardiovascular Diseases*

We know that patients with inflammatory rheumatic disease are at higher risk of developing cardiovascular complications compared to the general population. This increased risk is caused by the underlying disease, medications (such as COX2 inhibitors, high-dose glucocorticoids) and the presence of comorbidities such as arterial hypertension, hyperlipidemia, chronic kidney failure, and diabetes mellitus [25-31]. HCQ treatment in this group of patients provides a protective effect in terms of cardiovascular events in the long term by lowering fasting glucose levels and reducing hyperlipidemia [27].

Recently, Liu et al.'s systematic review involved 19 observational studies with 19,679 CQ/HCQ treated patients and controls has found that CQ/HCQ was associated with an approximately 30% reduction in the risk of cardiovascular disorders [30]. In a variety of animal studies including the models of metabolic syndrome, diabetes mellitus, hyperlipidemia, cardiovascular disorders and chronic kidney disease, it has been demonstrated the direct anti-atherosclerosis and vasculoprotective actions of HCQ [76,77]. HCQ also leads to improvement in endothelial function and vascular stiffness (as judged by recovery in flow-mediated dilation and reduction in aortic pulse wave velocity), and decrease incidence of new-onset hypertension [3]. HCQ can protect the annexin V anticoagulant shield against degradation on phospholipid double layers by antiphospholipid antibodies and directly reduce the binding of antiphospholipid antibody-b2-glycoprotein I complexes to phospholipid double layers [78]. In addition, HCQ inhibits platelet aggregation in a dose-dependent manner and reduces the release of arachidonic acid through activated platelets [3,25]. As a result, it is believed that HCQ has a vascular protective effect and prevents the development of thrombotic complications although it is not an anticoagulant, thus it appears to be a suitable option in patients with primary antiphospholipid syndrome and secondary coagulopathy associated with systemic inflammation [30,57].

### *Use of HCQ in Neurological Diseases*

It has been mentioned that HCQ can be effective in patients with progressive neurosarcoidosis who need long-term treatment and who are intolerant/unresponsive to corticosteroids [79]. Chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids (CLIPPERS) is another disease treated empirically with HCQ but with equivocal results [80,81]. Firstly, a patient with CLIPPERS who demonstrated radiological progression despite clinical remission with the use of HCQ has been reported [80], but subsequently remission has been achieved in another patient [81].

Based on the observation that inflammation plays a crucial role in the pathogenesis of Alzheimer's disease, Van Gool et al. has used HCQ treatment in 168 patients for 18 months; however, they have demonstrated that HCQ did not cause improvement in minimal or mild Alzheimer's disease [82]. Contradictory results have also been mentioned recently in terms of HCQ and Alzheimer's disease development by Lee et al. [83], but later studies have shown no definitive evidence related to HCQ use and Alzheimer's disease risk [84,85]. Nevertheless, it has been reported that randomized-controlled

studies with larger case series should be performed in patients with Alzheimer's disease [84,85].

The possible role of HCQ in the treatment of multiple sclerosis (MS) has also recently been reported. HCQ has been shown to reduce human microglia activation and reduce disease activity in MS animal models. Koch et al. has demonstrated that pretreatment with HCQ in experimental autoimmune encephalomyelitis delayed disease onset depending on the dose and that the pretreated animals had less active macrophage / microglia in their spinal cords than untreated animals [86]. There are ongoing studies testing the effect of HCQ in slowing the progression of clinical disability in progressive MS. Mandoj et al. has shown that high total and LDL cholesterol levels in MS patients are significantly associated with anti-annexin V positivity, thereby correlating between neurodegenerative and thrombogenic mechanisms in MS [87]. Rand et al. has found that HCQ protects the annexin V anticoagulant shield on phospholipid bilayers from disruption by antiphospholipid antibodies; therefore, it has been suggested that annexin V may be a possible new therapeutic target and the use of HCQ seems very promising in MS [3,78].

### *Use of HCQ in Oncological Diseases*

HCQ can be used in combination with radiotherapy or various chemotherapeutic agents to enhance antineoplastic effects [8,88-90]. The antitumoral effect of HCQ was determined incidentally, similar to rheumatological disorders, with a decrease in the incidence of Burkitt lymphoma among patients using prophylactic CQ against malaria in Tanzania approximately five decades ago [91]. Since then, CQ and HCQ have been tested in many tumors, including gliomas, breast cancer, head and neck cancers, metastatic cancer, melanoma, multiple myeloma, lymphoma, and leukemia [8,90,92-95]. Ongoing research is increasing to support the important adjuvant role of HCQ in the treatment of neoplasms. Although it is not clear yet whether there are differences in terms of antineoplastic therapeutic efficacy between CQ and HCQ, the clinical trials suggest that CQ might be more efficacious than HCQ. However, no comparative clinical trial has been set up to confirm this hypothesis. Consequently, CQ/HCQ acts as an adjuvant anti-cancer agent with direct anti-tumoural effects (autophagy inhibition, inhibition of the TLR9/nuclear factor kappa B signalling pathway, inhibition of CXCL12/CXCR4 signalling, interference with the p53 pathway), modulation of tumour micro-environment (immunomodulation, normalisation of the tumour vasculature, disruption of the CAF-cancer cell interplay) and synergism with approved anti-cancer drugs [90].

By blocking fusion of autophagosomes with lysosomes, HCQ can inhibit autophagy and facilitate the radiosensitization of tumors. Autophagy is a known survival mechanism in many tumor types. Many studies have proven that antitumoral effects can be improved by combining various anticancer drugs with pharmacological or genetic autophagy inhibitors. Currently, CQ and HCQ are the only available autophagy inhibitors in clinical [8,89]. In the systematic review and meta-analysis of Xu et al., it has shown that adding CQ and HCQ as autophagy inhibitors to the treatment of cancer patients may contribute to higher overall response rate, 1- year overall survival rate and 6-month progression-free survival rate. They have found HCQ-based therapy can better benefit than CQ-based therapy in terms of 1-year overall survival rate and 6- month progression-free survival rate. When evaluated in terms of cancer types, the use of autophagy inhibitor has associated with the best overall response rate in non-Hodgkin lymphoma patients, and the best 1- year overall survival and 6-month progression-free survival rates in patients with gliob-lastoma; however, in patients with nonsmall-cell lung cancer or breast cancer overall response and 6-month progression-free survival rates have not significantly improved [8].

In summary, the effectiveness of HCQ has been increasingly recognized in nearly all major fields of medicine, including rheumatology, immunology, haematology, oncology, dermatology, cardiology and severe infectious diseases. This old drug still maintains its place in our clinical practice as monotherapy and/or adjuvant therapy in many medical conditions ranging from chronic metabolic diseases to serious life-threatening infections due to its multi-functional beneficial therapeutic effects. In this context, successful results can be achieved with the appropriate dose and duration of HCQ treatment adjusted according to the clinical indication and side effect profile.

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