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Hydroxychloroquine as a potential therapy for ANCA-associated vasculitis

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Abstract

Antimalarial agents have been used to treat various autoimmune rheumatic diseases for over a century. Hydroxychloroquine is a safe, effective and inexpensive antimalarial drug with additional antithrombotic, cardioprotective, antimicrobial, and anti-neoplastic benefits. It has been used extensively in various diseases, especially systemic lupus erythematosus and rheumatoid arthritis; however, it has not been used in anti-neutrophil cytoplasmic antibody associated vasculitides (AAVs). There exists a significant unmet need for safe and inexpensive treatments for non-severe AAV or those with low-grade "grumbling" disease activity who do not warrant significant escalation of therapy but who remain at risk of disease flares and damage accumulation. Hydroxychloroquine may be an option to help fill this void. Although the mechanisms of action of Hydroxychloroquine are not fully understood, it interacts with various inflammatory mediators involved in the pathogenesis of AAV. Based on these benefits, along with the unmet need in AAV, we present evidence to support the use of Hydroxychloroquine as a potential therapy for AAV.

Keywords: Hydroxychloroquine, antimalarials, vasculitis, anti-neutrophilic cytoplasmic antibodies, autoimmune, immunomodulatory

INTRODUCTION

The anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) are a group of small vessel vasculitides characterized by necrotizing inflammation of blood vessels and often positive autoantibodies to neutrophil proteins - leukocyte proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA)[1,2]. They comprise three distinct conditions - Granulomatosis with Polyangiitis (GPA, previously known as Wegener's Granulomatosis), Eosinophilic Granulomatosis with Polyangiitis (EGPA, previously known as Churg-Strauss syndrome), and Microscopic Polyangiitis (MPA)[2]. They usually present with severe life- or organ-threatening disease; however, less severe and limited disease also occurs[1]. Treatment usually involves remission induction (with high dose Glucocorticoids (GCs) + either Rituximab or Cyclophosphamide) followed by maintenance therapy (with either low dose GCs, Azathioprine, Methotrexate, Mycophenolate Mofetil, Rituximab or Avacopan)[3].

Despite significant advances in diagnoses and management, patients with AAV continue to have significant morbidity and mortality, reduced survival rates, poor quality of life, and increased socio-economic burden compared to the general population[4-7]. This is due to a combination of the disease itself, treatment adverse effects, poor physical health (mostly from fatigue), psychological factors (mainly anxiety), decreased social participation (due to lifestyle changes related to disease and social perceptions of vasculitis), and decreased employment (due to functional impairment)[8]. Despite adequate treatment, 20%-30% of patients have refractory disease[9], and relapse rates remain high (up to 50% at 5 years)[10].

There remain several unmet needs in AAV. Better and less toxic glucocorticoid-sparing therapies are required to reduce treatment-related adverse events. Avacopan is an exciting new therapy in the AAV armamentarium[11]. It is an orally administered small molecule complement C5a receptor blocker that inhibits neutrophil chemoattraction and activation (terminal C5a production is a component of AAV pathogenesis)[11]. There is also an unmet need for safe and inexpensive treatments for non-severe AAV or those with low-grade "grumbling" disease activity[12] who do not warrant significant escalation of therapy but who remain at risk of disease flares and damage accumulation. Trimethoprim/Sulfamethoxazole (TMP/SMX) is an option for this patient group; however, previous results have been variable[13-15].

Antimalarial agents have been used to treat various autoimmune rheumatic diseases for over a century. Hydroxychloroquine (HCQ) is a safe and effective antimalarial drug that was approved by the United States Food and Drug Administration in 1955 for the treatment of discoid lupus, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA). It has since become the cornerstone background therapy in SLE patients; however, it has not been used for AAV. HCQ has additional antithrombotic, cardioprotective, antimicrobial, and anti-neoplastic benefits, which would be immensely valuable in patients with AAV who are at risk of infections, malignancy, and thrombosis, owing to the disease itself and background immunosuppression.

Based on these benefits, along with the unmet need for safe and inexpensive treatments for non-severe/low-grade "grumbling" AAV, we suggest that HCQ may help fill this void. Through this review, we hope to present evidence to support the use of HCQ as a potential therapy for AAV.

HISTORY OF ANTIMALARIALS

The history of antimalarials is an interesting one, based on a mixture of facts and legends, and subsequently researched by several authors. The bark of the Cinchona tree (then called "fever tree") appears to have been first used by the Andean population to combat shivering, fevers (although it was not included in the Inca pharmacopeia)[16]. Since then, the medicine became known by several names such as Cortex peruanus,

Peruvian bark, or Jesuit's powder (since it was imported into Europe from Latin America by Jesuit missionaries). It is documented that in 1638, the Viceroy of Peru (Countess Cinchona) was treated by an Incan herbalist with the bark of a "fever tree" (subsequently named after her, the Cinchona tree), with dramatic improvement[17]. The Schedula Romana, published in 1649, is an early example of an efficient antimalarial recipe, assumed to have been designed by the Spanish cardinal Juan de Lugo, based on trial and error and recipes proposed by Roman apothecaries[16]. Oliver Cromwell, Lord Protector of England, died of a fever illness (later thought to be malaria) as he refused the Jesuit powder cure.

By the 18th and 19th centuries, Cinchona bark was in widespread use for treating intermittent fevers; however, it was not until 1820 that Quinine was discovered as the active ingredient[18]. It became one of the first drugs produced and sold by a global pharmaceutical industry, and factories in Europe, North America and later Asia dominated manufacturing. Initially, the raw material (the Cinchona bark) came from South America; however between 1890 and 1940, Cinchona plantations on Java (Netherlands East Indies) supplied 90% of the bark for the quinine pharmaceutical business (other sources were Latin America and British India)[19].

Subsequently Chloroquine (CQ) was synthesized in 1934 and used extensively as an antimalarial drug. However, due to its significant toxicity, a modification of Chloroquine (via hydroxylation) was required and led to the development of Hydroxychloroquine in 1945, which was less toxic[17,18].

The first documented use of antimalarials for rheumatic disease was in a postgraduate lecture by Joseph Frank Payne at St. Thomas' Hospital, London, where he described using Quinine for treating cutaneous lupus[20]. The subsequent discovered benefit of antimalarials for inflammatory arthritis and rashes was serendipitous - during the Second World War, millions of soldiers taking antimalarial prophylaxis noted significant improvement in their joint pains and rashes[17]. This led to the first trial (in 1951) showing the benefit of antimalarials (Mepacrine) in 18 SLE patients, many of whom had failed quinine[21]. Since then, antimalarials have been used for a wide variety of autoimmune rheumatic diseases. The first use of HCQ in rheumatic diseases was in 1956 for treating cutaneous and mild (benign) systemic lupus[22-24].

PHARMACOKINETICS AND PHARMACODYNAMICS

Hydroxychloroquine belongs to the antimalarial drug class 4-aminoquinolines[25]. It has a large volume of distribution and long half-life (around 50 days), which is responsible for its delayed onset of action and prolonged effect after drug discontinuation[26]. It is a weak base and hence accumulates in acidic compartments such as lysosomes and inflamed (acidic) tissues - this is thought to be crucial for its action[27]. HCQ also strongly binds to melanin and can deposit in melanin containing tissues such as the skin and eyes, which might explain its benefit in cutaneous disease and its adverse effects of retinopathy and skin pigmentation after prolonged use[27]. It is taken orally as Hydroxychloroquine Sulphate in doses between 100-400 mg, rapidly absorbed in the upper intestines, metabolized by the liver, and excreted by the kidneys with an oral bioavailability between 60%-90%[28]. It reaches peak concentration 2-4 h after an oral dose[28]. Caution must be taken regarding dosage in patients with kidney disease, as reduced creatinine clearance leads to increased bioavailability and subsequent toxicity of HCQ[28]. Despite the fact that HCQ crosses the placenta and previous concerns regarding drug-related pigmentation in foetal tissue, HCQ is considered safe to use in pregnancy and breastfeeding[29,30].

CURRENT INDICATIONS FOR HYDROXYCHLOROQUINE

Apart from malaria, HCQ has been used in the treatment of various rheumatic and non-rheumatic diseases [Table 1]; however, its use in systemic vasculitis has been very limited [Table 2]. The best-known use of HCQ has been in SLE and RA.

Table 1. Previous use of hydroxychloroquine in other diseases

Disease condition	Hydroxychloroquine use	Evidence base for hydroxychloroquine
1. Autoimmune rheumatic diseases:		
(a) Sjögren's syndrome (SS)	HCQ not routinely used Based on its benefits for SLE, a 12-month therapeutic trial is recommended in patients with mild systemic disease (rash, arthralgia/arthritis, fatigue). If there is no response at 12 months, advice is to stop[176,177]	• Previous studies - benefits on sicca symptoms (ocular/oral dryness), arthralgia, fatigue[178,179], immunoglobulin levels, and ESR*[180,181] • Subsequent trials and meta-analyses - no significant benefit[182-184]
(b) Antiphospholipid syndrome (APS)	HCQ may be used as an add-on therapy in patients with APS and recurrent thrombotic/pregnancy complications despite combination treatment with low-dose aspirin and prophylactic dose heparin[185]	• No RCT's** • Previous multicentre RCT of HCQ for primary thrombosis prevention in primary APS patients - terminated early due to poor recruitment[186] • Main evidence comes from SLE patients - HCQ shown to reduce the risk of both arterial and venous thrombosis[42,43] • Small European multicentre study - reduction in pregnancy losses from 81% to 19% ($P < 0.05$) in APS patients treated with HCQ during pregnancy[142] • Other studies, systematic reviews - similar benefits[187,188] • *In vitro* experiments - HCQ reduces antiphospholipid antibody mediated thrombosis in mouse models[189]
(c) Sarcoidosis	HCQ may be used as a second-line steroid sparing in patients with pulmonary and/or extrapulmonary sarcoidosis[190,191]	• No RCT's • Several trials and publications - benefit in pulmonary and extrapulmonary manifestations (musculoskeletal, cutaneous, osseous, and neurological)[192-195]
(d) Hand osteoarthritis	HCQ not used as treatment	• 2 RCT's - no significant benefit of HCQ in pain relief compared to placebo[196,197]
(e) Other autoimmune diseases	Variable success in: • Eosinophilic fasciitis[198] • Dermatomyositis (including clinically amyopathic and childhood-onset dermatomyositis)[199-201] • Kikuchi-Fujimoto disease[202,203] • Adult-onset Still's disease[204] • Juvenile idiopathic arthritis[204] • Chronic Chikungunya (viral) related arthritis[205] • Immune thrombocytopenia[206,207] • IgA nephropathy[208-210]	
2. Infections		
(a) Malaria	HCQ primarily developed as antimalarial drug. Due to widespread drug resistance, no longer recommended for the treatment of P. falciparum malaria	• WHO guideline[211] - CQ or HCQ are indicated for the treatment of uncomplicated malaria due to Plasmodium vivax, *P. malariae*, *P. ovale* and *P. knowlesi*
(b) COVID-19 infection	Although several trials showed no benefit in COVID-19 infection, HCQ was used in several countries as an inexpensive treatment with variable response	• HCQ initially thought to be a promising prospect based on previous benefits on SARS-COV1, 2 viruses[212] • Several RCT's including RECOVERY and REMAP-CAP trials - no benefit of HCQ *vs.* placebo[212,213] • Some trials, meta-analysis showing worse outcomes including cardiac complications and death[214-216]
(c) Other infections (variable benefit)	• Bacterial infections (e.g., Coxiella burnetti infections[217,218], Whipple's disease[219]) • Fungal infections (e.g., cryptococcal[220,221], Aspergillus[222], Paracoccidioides infections[223]) • Viral infections (HIV[224], Zika virus infection[225], Chikungunya virus infection[205], *etc.*)	
3. Graft versus host disease (GVHD)	HCQ not routinely used[226]; however may be beneficial as an adjuvant therapy	• Phase II clinical trials - some benefit of HCQ in treatment of chronic GVHD[227,228] • Phase III clinical trials - no benefit[229,230]
4. Malignancies	HCQ not routinely used	• HCQ shown to have possible benefit in chronic lymphocytic leukaemia (CLL)[231,232] and breast cancer[233,234]
5. Porphyria cutanea tarda (PCT)	Current recommended treatments for PCT are repeated phlebotomy or low dose HCQ (100 mg bd)[235]	• Previous trials, studies - HCQ in an effective therapy for PCT and is better than phlebotomy or desferrioxamine[236-238] • Large RCT's lacking
6. Other diseases	HCQ has shown some benefit in miscellaneous other diseases: • Polymorphous light eruptions[239]	

- Granuloma annulare[240]
- Lichen planus[241,242]
- Chronic ulcerative stomatitis[243]
- Hidradenitis suppurativa[244]
- Chronic urticaria[245,246]
- Multiple sclerosis[247]
- Alport syndrome[248]

There are several on-going clinical trials of HCQ in immunological, infectious, neurological, and neoplastic disorders[249] and the indications of use for this "wonder drug" are ever-growing

* ESR: erythrocyte sedimentation rate; ** RCT: randomized controlled trials.

Table 2. Previous use of hydroxychloroquine in systemic vasculitis

Disease condition	Hydroxychloroquine use	Evidence base for hydroxychloroquine
[A] Large vessel vasculitis:		
1. Giant cell arteritis (GCA)	HCQ not routinely used	• Previous retrospective study - steroid-sparing benefit of HCQ in GCA[250] • Subsequent double-blind RCT (only published in abstract form) - no benefit of adjunctive HCQ vs glucocorticoids (GC's) alone as a steroid sparing agent or on relapse rates[251] • No further RCT's done
2. Takayasu arteritis (TA)	HCQ not routinely used	• No RCT's • Longitudinal observational retrospective study by Rongyi *et al.* - HCQ enhanced anti-inflammatory effect, greater reduction in inflammatory markers (ESR, CRP), alleviated angiographic progression[252] • Case report - HCQ associated with improvement in arthralgia, reduced risk of relapse[253]
[B] Medium vessel vasculitis:		
3. Polyarteritis Nodosa (PAN)	HCQ has been used as an adjunctive therapy along with GC's[254]	• No RCT's • Case series - HCQ used as an adjunctive therapy along with GCs in cutaneous PAN[254]
4. Kawasaki disease (KD)	HCQ not routinely used	• No literature found • HCQ was empirically used in COVID-19 with multi-system inflammatory syndrome in children (MIS-C) which presents similar to Kawasaki disease; however, subsequent trials and meta-analyses found this not to be beneficial[255]
[C] Small vessel vasculitis:		
5. Urticarial vasculitis (UV)	HCQ is included in the recommendations for treatment of cutaneous UV[256]	• No RCT's • Several case reports and series - benefit of HCQ in hypo/normocomplementemic urticarial vasculitis with improvement in symptoms, anti-C1q antibody levels[257,258], associated retinal vasculitis[259], C1-esterase inhibitor concentration and activity[260] • Largest study from the French Vasculitis Study Group (n = 5) - HCQ was as effective as GC's for hypocomplementemic urticarial vasculitis[261]
6. IgA vasculitis (IgAV)/Henoch Schonlein purpura (HSP)	HCQ not routinely used	• No RCT's • Limited case reports - improvements in arthralgia, rash, fatigue, gastrointestinal symptoms, reduction in flare rates, steroid dose[253], reduction in proteinuria in IgA nephropathy patients[208-210]
7. Anti-GBM*** disease/Goodpasture syndrome	HCQ not routinely used	• No relevant literature found • Single case report - mentioned use of HCQ as maintenance therapy in a patient with Goodpasture syndrome and hemophagocytic lymphohistiocytosis (HLH). Patient relapsed after 2 months[262]
8. ANCA-associated vasculitis (AAV)	HCQ not routinely used	• No RCT's • Case reports - improvements in arthralgia, reduction in flare rates and dose of steroids with HCQ[253,263]

*** GBM: glomerular basement membrane.

Systemic lupus erythematosus

HCQ has become the recommended background therapy in all SLE patients and is a part of all major guidelines[31-34]. HCQ has been shown to improve disease activity in mild to moderate disease[35], improve long-term outcomes/survival[36], and reduce disease flares[37,38], steroid burden[38], damage accrual[39,40], hospitalisations[41], and mortality[36]. It has also been shown to improve cardiovascular risk (by lowering lipids, glucose, and atherosclerosis risk), reduce VTE risk[42,43], improve bone mineral density[44] and protect against osteonecrosis[45] and malignancies[46]. It was previously also suggested that HCQ use in antinuclear antibody (ANA) positive individuals may delay the progression to SLE[47] or onset of renal disease[48].

Apart from systemic lupus, HCQ has shown benefit in cutaneous lupus erythematous (including discoid lupus, lupus panniculitis and refractory disease)[49-51] and as an adjuvant therapy for lupus nephritis[52,53]. It is also considered safe for use in in pregnancy and improves disease activity[54,55], reduces risk of flare[55,56], protects against complications (preeclampsia[57,58], intrauterine growth restriction/prematurity[59]) and reduces the risk of developing neonatal lupus and congenital heart block in Ro positive patients[60].

Rheumatoid arthritis

HCQ is an established treatment for Rheumatoid arthritis (RA) and is a part of all major national and international guidelines[61-63]. It can be used as monotherapy in early mild disease (without poor prognostic factors) or palindromic rheumatism but is most commonly used as combination therapy with either Methotrexate and/or Sulfasalazine. Apart from improving disease activity[64,65], slowing the rate of disease progression[65], and enhancing Methotrexate exposure[66], HCQ also improves the lipid profile, blood sugar levels and cardiovascular profile in patients with RA, leading to an overall reduction in cardiovascular events[67,68]. HCQ has previously also been shown to have some benefit in rheumatoid vasculitis[69,70]. A recent large observational cohort study by Wu et al. showed that RA patients on HCQ also have a significantly lower (36%) incidence of chronic kidney disease compared to those not on HCQ (HR 0.64, 95%CI: 0.45-0.90, $P = 0.01$)[71].

PROPOSED MECHANISMS FOR HYDROXYCHLOROQUINE IN ANCA ASSOCIATED VASCULITIS

The exact mechanisms by which HCQ benefits in autoimmune rheumatic diseases are still not fully understood; however, HCQ interacts with various inflammatory mediators involved in the pathogenesis of ANCA-associated vasculitis and hence might be effective in treatment of this disease[72]:

1. HCQ is a weak diprotic base. At neutral pH (e.g., in serum), it remains uncharged and can easily diffuse across the lipid cell membrane of lysosomes. Once inside, the drug becomes protonated causing an increase in intracellular pH, which in turn causes disruption of proteins (cytokines, immune receptors) and impaired proteolysis, chemotaxis, and protein degradation (via endocytosis, phagocytosis, or autophagy). This in turn inhibits MHC (major histocompatibility complex) Class II auto-antigen processing and presentation to T-cells, production of lymphocytes and autoantibodies all of which play a role in AAV pathogenesis[27,73].

2. Recently it has been shown that the NLRP3 inflammasome may play an important role in the pathogenesis of several autoimmune and vascular disorders including vasculitis through inflammatory cytokines IL (interleukin)-1β and IL-18[74,75]. Inflammasome mediated IL-1β has also been shown to play a role in ANCA vasculitis associated renal involvement[76,77]. HCQ has been shown to inhibit NLRP3 inflammasome activation and IL-1β secretion without affecting inflammasome priming steps[78,79]. This might be an exciting and novel mechanism through which HCQ, and other drugs may be beneficial in AAV[75].

3. B-cell activating factor (BAFF) is a pro-survival factor for autoreactive memory B-cells[80]. BAFF has been shown to be elevated in patients with AAV[81,82]. HCQ has been shown to reduce BAFF levels in the serum of patients with Sjögren's syndrome, SLE and Rheumatoid arthritis[83-85], as well as in salivary and tear fluid in patients with Sjögren's syndrome[83].

4. There is increasing evidence to suggest that Toll-like receptors (TLR), especially TLR2, TLR4 and TLR9, are critically involved in the immune response in AAV[86-88]. These can be triggered by infections and microbial peptides (such as bacterial CpG oligodeoxynucleotide), leading to neutrophil activation[89] and ANCA formation[90-92]. TLR9 single nucleotide polymorphisms (SNPs) have been identified to be genetically associated with Granulomatosis with Polyangiitis (GPA), Microscopic Polyangiitis (MPA) and ANCA positive disease in genome wide disease association studies[93]. HCQ has been shown to inhibit TLR signalling and cell activation by altering the pH of endosomes, preventing TLR7 and TLR9 from binding to ligands and inhibiting the activity of nucleic acid sensor cyclic GMP-AMP synthase (cyclic guanosine monophosphate-adenosine monophosphate synthase)[27,94,95]. This in turn inhibits the production of pro-inflammatory cytokines. TLR inhibition has also been suggested as a potential target for several other autoimmune diseases[96,97] and this may apply to AAV as well.

5. Several cytokines are implicated in the pathogenesis of AAV, especially IL-6, IL-8, IL-10, IL-17 and TNF-α (tumour necrosis factor alpha)[98]. HCQ has been shown to inhibit the production of IL-6, IL-17 and TNF-α (possibly by inhibiting TLR pathways), in addition to other cytokines like IL-1, IL-2, IL-22, IFN-α (interferon alpha), and IFN-gamma[99,100].

6. T-cells play an important role in immunopathogenesis of AAV. Abnormalities in peripheral T-cell subset numbers and function have been varyingly identified in patients, consistent with the heterogeneity of disease phenotypes encompassed within AAV. Transcriptional changes in peripheral CD4 and CD8 T-cell subsets, including naïve and memory T-cell subsets are indicative of persistent activation and bear hallmarks of toll-like receptor activation and exposure to microbial infection[101-105]. Furthermore, increased levels of circulating $CD4^+CD25^+$ cells have been identified in AAV patients, including $CD4^+CD25^{lo}$ T-effector cells and $CD4^+CD25^{hi}$ T-regulatory (Treg) cells, which are vital cells in controlling the immune response to quell inflammation[102]. Despite the increased Treg levels, these cells were shown to be defective in their suppression of Teff proliferation *in vitro*[106]. In contrast, other studies noted reduced levels of circulating Tregs and increased follicular T-helper (TFH) cells in GPA patients, with no defect in suppressive function of Tregs[107]. The reasons for these differing observations remain unclear and may in part be due to clinical heterogeneity within AAV patients. Future studies exploring functional Treg subpopulations in blood and granulomatous tissue by deep sequencing technologies, will shed light on any functional defects in this population between different groups of AAV patients.

Interestingly, we have observed that HCQ treatment can inhibit the expression of the T-cell activation marker CD25 on unactivated CD4 T-cells from healthy donors, in plasma co-culture experiments using *ex-vivo* plasma from patients, or by addition of HCQ in *in-vitro* cultures. In contrast, HCQ has no effect on unactivated peripheral blood mononuclear cells (PBMCs) cultured with healthy control donor plasma [Figures 1 and 2][107]. This observation suggests that HCQ acts to suppress potential inflammatory mediators in patients. Mechanistically, HCQ suppresses T- and B-cell receptor mediated signalling by specifically inhibiting calcium signalling and subsequent NFAT (nuclear factor of activated T-cells) activation[108]. HCQ induces apoptosis in autoreactive memory T-cells[109], and reduces pro-inflammatory T-cell activation[110,111].

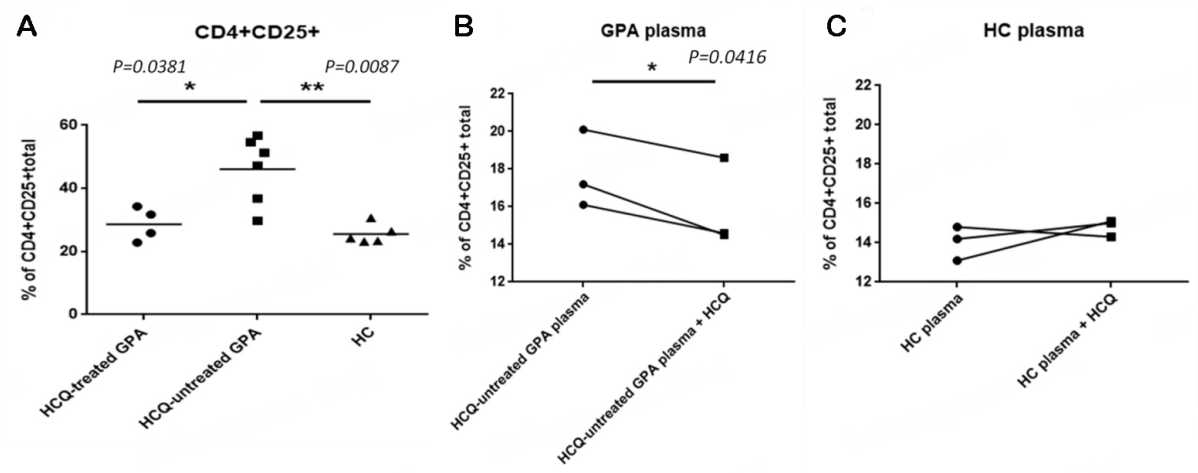

Figure 1. Effect of HCQ on CD25 activation on *ex-vivo* and *in-vitro* CD4 T cells from GPA patients. (A) Peripheral blood mononuclear cells (PBMCs) from healthy control (HC) donors were co-cultured with 10% plasma from HCQ-treated GPA patients (n = 4), HCQ-untreated GPA patients (n = 5) or HC plasma (n = 5) for 5 days. PBMCs from HC were co-cultured with (B) HCQ-untreated GPA plasma or (C) HC plasma samples with or without 3 μM HCQ treatment. Following co-culture for 5 days, PBMCs were stained with antibodies for CD4, CD8, CD19, and CD25 and evaluated by flow cytometry to identify lymphocyte subsets expressing CD25. Statistical significance was calculated by (A) Mann-Whitney test and (B) and (C) two-way ANOVA grouped analysis. *P < 0.05, **P < 0.01.

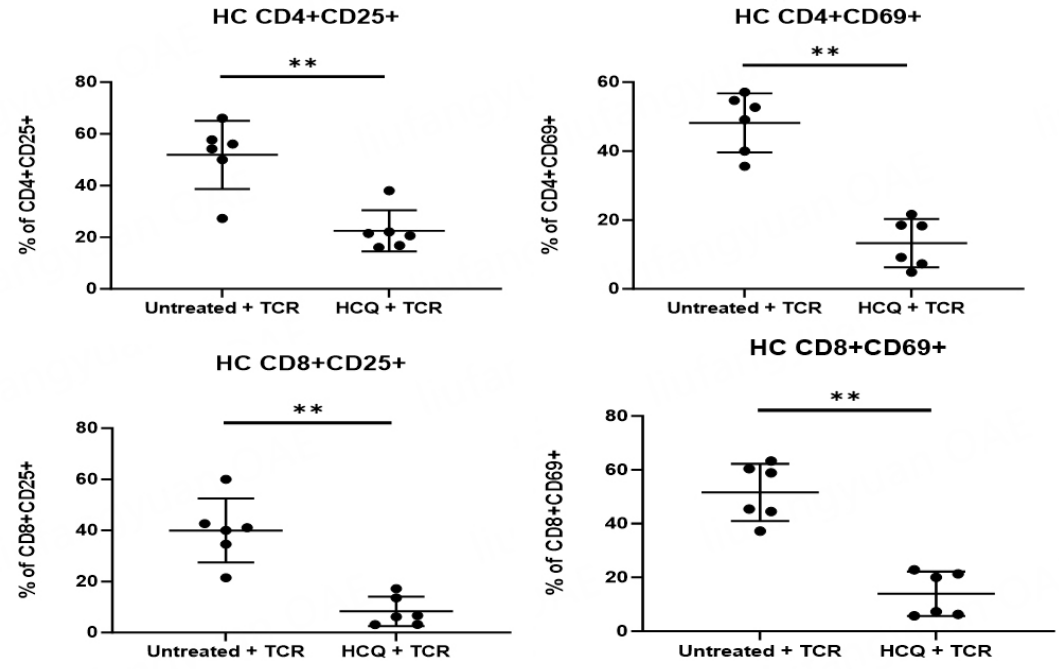

Figure 2. HCQ inhibits T cell activation *in vitro*. PBMC from 6 healthy control donors were incubated with 50 μM HCQ for 24 h, then left unstimulated or stimulated with plate-bound anti-CD3 (10 mg/mL UCHT1, Ancell) and anti-CD28 (1 mg/ml, ANC28.1/5D10, Ancell) for 18 h, and analysed by flow cytometry to identify $CD3^+CD4^+$ and $CD3^+CD8^+$ T cell subsets expressing activation markers CD69 and CD25. Statistical significance was assessed by the Mann-Whitney test. **P < 0.01.

7. High mobility group box 1 (HMGB1) is among the most important chromatin proteins in humans and is encoded by the HMGB1 gene. In the nucleus, it helps to organize DNA and regulate transcription; however, outside the nucleus, it is also a crucial cytokine that mediates response to infection, injury, inflammation,

and cancer[112]. HMGB1 is thought to play a role in a wide range of diseases including but not limited to cancers[113], inflammatory disorders[114-116], and vascular disorders[117] and might be a potential target for drug therapy[118]. Patients with AAV have higher levels of HMGB1[119,120] and this is associated with disease activity[120-122], presence of renal disease[119] and vascular inflammation[123]. Antimalarials such as CQ and HCQ have been shown to inhibit HMGB1 inflammatory signalling[124,125].

8. Matrix metalloproteinases (MMP) are a group of proteinases that degrade both matrix and non-matrix proteins in the extracellular space. They play an important role in wound healing, tissue repair and remodelling in response to injury[126]. Tissue inhibitors of matrix metalloproteinases (TIMP) are a family of proteins that function to inhibit MMPs[127]. Altered levels of MMP and TIMP have been implicated in several human diseases[128].

TIMP-1, TIMP-2, MMP-2, MMP-3, and MMP-7 levels are promising biomarkers in AAV and help to distinguish between active disease, remission, and renal disease. TIMP-1, MMP-3 and MMP-7 levels are elevated in active disease, whereas MMP-2 and TIMP-2 levels are elevated in remission. Elevated TIMP-1, MMP-3 and MMP-7 are associated with worsening renal function[129-132]. HCQ has been shown to modulate the levels of TIMP-1, MMP-2 and MMP-9[133,134] and this might be of use in AAV.

OTHER BENEFITS OF HYDROXYCHLOROQUINE

Hydroxychloroquine has been shown to have antithrombotic, cardioprotective, anti-infective and antineoplastic benefits. These benefits have been mainly described in patients with altered risk secondary to underlying autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus and antiphospholipid syndrome. Patients with AAV are at higher risk of comorbidities such as thromboembolism, cardiovascular events, infections, and malignancies due to active disease and side effects of systemic immunosuppression (especially steroids).

It remains unclear whether these benefits are as a direct result of HCQ itself, or an indirect consequence of the interacting mechanism outlined earlier, and whether these benefits may translate to a different autoimmune milieu as seen in AAV; however, this may provide an additional avenue of benefit of HCQ in AAV patients.

Antithrombotic effect

Patients with AAV have a 2-3 times higher risk of venous and arterial thromboembolism (VTE, ATE) compared to the general population[135,136]. This risk is higher earlier in the disease course (when disease activity is higher); however, the increased risk persists despite remission[137]. Apart from classical risk factors for VTE (e.g., older age, higher BMI, immobilization, major surgery, malignancy, *etc.*), AAV specific risk factors include higher disease activity, myeloperoxidase-ANCA (MPO-ANCA) positivity, and hypoalbuminemia[138,139]. Proteinase 3 ANCA (PR3-ANCA) positivity was previously thought to be a risk factor[139]; however, a recent meta-analysis showed an inverse relationship between PR3-ANCA positivity and VTE risk[138].

The earliest documented trials using HCQ for thromboprophylaxis were in the 1970's and 80's for reducing peri-operative VTE's in orthopaedic (joint replacement) and non-orthopaedic surgeries[140,141]. Since then, the antithrombotic benefits of HCQ have been well documented, especially in patients with SLE[42,43] and antiphospholipid syndrome (APS)[142,143].

The main mechanisms, although incompletely understood, are thought to be related to reduction in disease activity, platelet activation[144], atherosclerotic plaque formation[145], antiphospholipid antibody (aPL) levels[146], aPL related thrombus formation[145,147], aPL mediated disruption of the potent anticoagulant Annexin A5[148,149], and improvement in vascular endothelial function[150,151].

Antineoplastic effect

As with most other autoimmune diseases, patients with AAV have an increased risk of malignancy, particularly bladder cancer, leukaemia, and non-melanoma skin cancers[152-154]. This is thought to be due to impaired immunosurveillance, chronic immune stimulation and immunosuppressive medications (particularly Cyclophosphamide and Azathioprine). Reassuringly, with the increased use of Rituximab, the rates of cancer are declining[155].

The antineoplastic benefits of antimalarials were first observed when Chloroquine (CQ) was used for a malaria prophylaxis programme in Tanzania and was associated with a reduction in the incidence of Burkitt lymphoma[156]. Since then, CQ and HCQ have shown benefit in several malignancies (see Table 1). Antimalarials have also been shown to reduce the risk of malignancy in patients with SLE[46], Sjögren's Syndrome[157], and RA[158,159].

Improved cardiovascular risk

Patients with AAV have greater than three-fold risk of cardiovascular (CV) events compared to the general population[160,161]. This is thought to be due to a combination of endothelial dysfunction related to active vasculitis[162,163], accelerated atherosclerosis in systemic vasculitis[164,165], and comorbidities, i.e., diabetes mellitus, hypertension *etc.* related to steroids.

HCQ has previously been shown to have a beneficial effect in lowering blood sugars[166-168], improving lipid profiles[167,168], and reducing atherosclerosis/improving vascular elasticity[150,151] all of which improve CV risk profiles[169].

Reduced infection risk

Patients with AAV are at an increased risk of infections especially within the first year of diagnosis[170,171]. This is due to a combination of immune dysfunction due to the disease itself and concomitant immunosuppression. Infections are the most common cause of mortality within the first year[171].

HCQ is well known for its antimicrobial properties and is used in the treatment of various infections (see Table 1). HCQ has also been shown to reduce infection rates in other autoimmune diseases like SLE and RA[172,173].

SIDE EFFECTS OF HYDROXYCHLOROQUINE

Overall HCQ is considered safe and well tolerated; however, as with all medications, it may be associated with certain adverse effects. In contrast to other immunosuppressive medications, HCQ is not associated with an increased risk of infections or malignancy. The most common side effects are gastrointestinal (nausea, vomiting, diarrhoea, abdominal discomfort) and cutaneous (pruritis, rashes, urticaria). With chronic long-term use, patients may develop blue-grey hyperpigmentation (particularly over gums, palate, face and shins)[18,27].

The most well studied and worrying adverse effect of HCQ remains retinopathy (known as bull's eye maculopathy), which is often symptomatic in the early stages and may cause permanent visual loss. The

most common risk factors for HCQ-related retinopathy include long duration of treatment, cumulative dose, chronic kidney disease and pre-existing retinal disease. As a result, annual ophthalmic screening with OCT (optical coherence tomography) is mandatory for patients on HCQ based on local guidelines[18,27,174].

Apart from these, other rare but serious side effects may include cardiovascular (conduction defects, cardiomyopathy), dermatological (toxic epidermal necrolysis, Steven-Johnson syndrome, exacerbation of psoriasis), haematological (bone marrow toxicity, neutropenia), neuromuscular (myositis, toxic myopathy), neuropsychiatric (confusion, disorientation, hallucination) and others (ototoxicity, tinnitus, fulminant hepatic failure)[18,27].

HAVEN TRIAL

In order to study the hypothesis that HCQ has disease modifying activity in AAV, the HAVEN trial was launched in 2018. HAVEN (Hydroxychloroquine in ANCA Vasculitis Evaluation) is a United Kingdom (UK) multicentre, randomized, double-blind, placebo-controlled trial of HCQ in ANCA vasculitis. Seventy-six patients with AAV and a Birmingham Vasculitis Activity Score (BVAS) > 3 will be randomised 1:1 to HCQ or placebo over 52 weeks. The primary outcome measure is the ability of HCQ to control disease activity measured by the BVAS[175].

CONCLUSION

HCQ has the potential to be an effective, safe, well tolerated, and inexpensive disease modifying anti-rheumatic drug (DMARD) for AAV patients with low-grade "grumbling" disease activity. It already has efficacy in several other autoimmune diseases, especially SLE and RA. Apart from its potential mechanisms of action in AAV, HCQ has antithrombotic, cardioprotective, antimicrobial and antineoplastic effects, which would make it an excellent option in this disease. Similar to SLE, HCQ may have the potential to improve disease activity, reduce steroid use, reduce flares, and improve outcomes in patients with AAV. If the HAVEN trial is positive, this could lead to a change in the management of patients with AAV.

DECLARATIONS

Authors' contributions

Researched the literature and contributed to writing the manuscript: Jain S, John S
Generated the data for the figures under the supervision of John S: Kim S
Conceived the design, supervised the whole project, and critically reviewed the finalized manuscript: D'Cruz D, John S, Sangle SR
All authors approve the final version and take full responsibility for all its parts.

Availability of data and materials

Not applicable.

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Conflicts of interest

D'Cruz D has provided consultancy services to CSL Vifor and GSK. All other authors declare that there are no conflicts of interest.

Ethical approval and consent for participation

This study involving patients [Figures 1 and 2] was approved by the U.K. Research Ethics Committee (REC no. 11/LO/1433). All patients and healthy individuals who donated blood provided fully informed written consent.

Consent for publication

Not applicable.

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