Will Auranofin Become a Golden New Treatment Against COVID-19?

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Auranofin is an FDA-approved disease-modifying anti-rheumatic drug that has been used for decades for treatment of rheumatoid arthritis. This gold(I) compound has anti-inflammatory properties because it reduces IL-6 expression via inhibition of the NF-κB-IL-6-STAT3 signaling pathway. Also, by inhibiting redox enzymes such as thioredoxin reductase, auranofin increases cellular oxidative stress and promotes apoptosis. Auranofin also possesses antiviral properties. Recently, it was reported that auranofin reduced by 95% SARS-CoV-2 RNA in infected human cells in vitro and decreased SARS-CoV-2-induced cytokine expression, including IL-6. During SARS-CoV-2 infection, a cytokine storm involving IL-6 increases severity of illness and worsens prognosis. Therefore, auranofin could, in our point of view, reduce pathology due to SARS-CoV-2-induced IL-6. COVID-19 is a rapidly-evolving respiratory disease now distributed worldwide. Strikingly high numbers of new COVID-19 cases are reported daily. We have begun a race to vaccinate people, but due to the complex logistics of this effort, the virus will continue to spread before all humans can be immunized, and new variants that may be less well contained by current vaccines are of concern. The COVID-19 pandemic has overwhelmed health care systems and new treatments to reduce mortality are urgently needed. We encourage to further evaluate the potential of auranofin in the treatment of COVID-19 in vitro and in animal models of SARS-CoV-2 infection and, if preliminary data are promising, in clinical trials with COVID-19 patients. In our opinion, auranofin has the potential to become a valuable addition to available therapies in this pandemic.

Keywords: auranofin, gold, COVID-19, coronavirus, SARS-CoV-2, thioredoxin, cytokine storm, IL-6

INTRODUCTION

COVID-19, caused by the coronavirus SARS-CoV-2, has infected, as of March 7th 2021, more than 116 millions of people and caused more than 2.5 million deaths worldwide due to sustained human-to-human transmission (1). The number of new COVID-19 cases has recently skyrocketed in many countries, particularly in the Americas and Europe (1). Many countries implemented regional or nationwide lockdown policies, curfews or stay-at-home orders to attempt to contain the spread of the virus. While vaccines targeting SARS-CoV-2 are now available, the appearance of more
contagious new SARS-CoV-2 variants is a serious threat (2). If vaccine efficacy is reduced against emerging variants, all ground gained during the past year could be lost and SARS-CoV-2 could resurge again (2). We must also take into consideration the reluctance of some people to be vaccinated, and the time needed to immunize whole communities, worldwide. All these factors will leave part of the population non-immune, at least for some time. Moreover, immunity from natural infection or vaccination may not last for years and humanity would not be protected from a resurgence of SARS-CoV-2 or a SARS-like coronavirus in the future. For these reasons, there is an urgent need to find new treatments to significantly reduce COVID-19-related pathogenicity and mortality. That being said, discovering and developing a new drug as well as testing its safety is an expensive and time-consuming endeavor. On the contrary, repurposing an approved drug is more affordable and time efficient, which constitute important factors during a pandemic. However, repurposed drugs should be thoroughly evaluated in vitro, in animal models and clinical trials before being recommended in the treatment of COVID-19 patients. So far, clinical management of COVID-19 patients was based on supportive care while several repurposed drugs are being tested in clinical trials (3).

CLINICAL TRIALS AND IMMUNOMODULATORY TREATMENTS

Clinical trials tested, among others, antiviral (e.g. remdesivir), anti-parasitic (e.g. ivermectin), anti-inflammatory (e.g. glucocorticoids) and antibody (e.g. tocilizumab) therapies to treat COVID-19 (4). In a randomized trial, remdesivir, a promising antiviral drug, shortened the time to recovery compared to placebo in COVID-19 patients (5). However, treatment with an antiviral drug alone is not sufficient to prevent mortality in all patients (5, 6). It was suggested that remdesivir therapy could show improved efficacy if administered before the inflammatory phase of COVID-19; this hypothesis would benefit further investigation (4, 6, 7). Recently, ivermectin, a macrocyclic lactone with broad-spectrum antiparasitic action, was considered promising in the treatment of COVID-19 due to its anti-SARS-CoV-2 effects in vitro (8). Ivermectin has known antiviral properties against several viruses such as yellow fever virus, dengue virus and chikungunya virus (9, 10). But, simulations based on pharmacokinetic studies performed in healthy volunteers predicted that ivermectin is unlikely to reach concentrations in lungs needed for anti-SARS-CoV-2 action, even if 10 times the approved dose is administered orally (11). At the latter dose, the maximum plasma concentrations would be one order of magnitude lower than the in vitro IC₅₀ of ivermectin against SARS-CoV-2 (8, 12). Clinical trials are being performed and results will determine the potential of ivermectin in the treatment of COVID-19.

The COVID-19-associated inflammatory cytokine storm is at least partly responsible for increased severity of illness and mortality (13). COVID-19 patients could then benefit from the anti-inflammatory activity of corticosteroids during the inflammatory phase of the disease (3, 7). Preliminary studies showed that low-dose corticosteroids do not delay viral clearance (14) and that corticosteroids can improve survival of critically ill patients with COVID-19 (3, 7). Particularly, the Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial of dexamethasone showed that this glucocorticoid reduced mortality in hospitalized patients receiving respiratory support (invasive mechanical ventilation or oxygen alone) (15). Based on data from this clinical trial, the Panel of the US National Institutes of Health (NIH) now recommends administration of dexamethasone to COVID-19 patients requiring supplemental oxygen (16).

Due to the major role of IL-6 in the COVID-19-associated inflammatory cytokine storm (13, 17), IL-6 inhibitors are attractive therapeutic options and several clinical studies evaluated or are ongoing to evaluate their potential to improve outcome during COVID-19 (18–21). A comprehensive review of ended and ongoing clinical trials on IL-6 inhibitors was already published (21). Tocilizumab is a monoclonal antibody against IL-6 receptor used in the treatment of rheumatoid arthritis and is, by far, the IL-6 inhibitor most studied as a potential therapy against COVID-19 (21). The promising role of tocilizumab was first revealed in anecdotal reports and small scale clinical studies. As examples, some reports showed that tocilizumab significantly reduced COVID-19-associated inflammatory response and prevented rapid clinical deterioration of COVID-19 patients with severe pneumonitis (18). Some clinical trials reported that treatment with tocilizumab leads to improvement of oxygenation and reduction of risk of mechanical ventilation and mortality (21). In the EMPACTA clinical trial, hospitalized COVID-19 patients not receiving mechanical ventilation, tocilizumab reduced the likelihood of progression to mechanical ventilation or death (22). However, in the EMPACTA clinical trial, mortality by day 28 was similar in patients treated with tocilizumab compared to patients treated with placebo (22). Other studies also showed no clinical improvement or no reduction in mortality (e.g. randomized controlled COVACTA trial) (21). Sarilumab is another anti-IL-6 receptor antibody used in the treatment of rheumatoid arthritis (18). Like tocilizumab, sarilumab is sometimes, but not always, associated with clinical improvement in COVID-19 patients (21). In contrast with tocilizumab and sarilumab, siltuximab is a human–murine chimeric monoclonal antibody that binds soluble forms of human IL-6 (19). Several clinical trials using tocilizumab, sarilumab or siltuximab are ongoing; their results will provide an important insight on the role of these IL-6 inhibitors in the treatment of COVID-19.

While IL-6 inhibitors are promising in the treatment of COVID-19, lack of efficacy of IL-6 inhibitors in some clinical trials advice against their use alone in COVID-19 patients. Therefore, combination therapies with other immunomodulatory molecules are of immediate interest (20, 21). As an example, in the ongoing COV-AID clinical trial, COVID-19 patients received in addition to standard care either tocilizumab, siltuximab or anakinra (anti-IL-1 binding the IL-1
receptor) or a combination of tocilizumab with anakinra or siltuximab with anakinra (20). Results of these clinical trials will define the impact of combination therapies in the outcome of COVID-19. Moreover, the combination of tocilizumab with corticosteroids already showed significant clinical improvement. The two largest randomized controlled trials on tocilizumab, named 'Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community- Acquired Pneumonia (REMAP-CAP)' and 'Randomized Evaluation of COVID-19 Therapy (RECOVERY)', reported a reduced mortality following tocilizumab treatment in COVID-19 patients. The REMAP-CAP trial was a large international clinical trial enrolling critically ill hospitalized COVID-19 patients within 24 hours of intensive care unit level care (23). Clinical improvement in the REMAP-CAP trial was measured by median number of respiratory or cardiovascular organ support–free days, this number was 10 for COVID-19 patients treated with tocilizumab with standard of care (SOC) compared to 0 for control COVID-19 patients receiving only SOC (23). Also, tocilizumab treatment with SOC reduced mortality in the REMAP-CAP trial compared to SOC alone (23). During the time the REMAP-CAP trial was conducted, glucocorticoids were recommended in the SOC following the publication of the data of other clinical trials such as the REMAP-CAP on hydrocortisone (24) and the RECOVERY trial on dexamethasone (15). Therefore, the combination of tocilizumab with glucocorticoids as part of the SOC probably improved the results of the REMAP-CAP trial (23). In the RECOVERY trial on tocilizumab, hospitalized patients with severe or critical COVID-19 with hypoxia and high C-reactive protein levels were enrolled; 82% of enrolled COVID-19 patients also received systemic corticosteroids (25). Tocilizumab decreased mortality and increased chances to be discharged from hospital within 28 days (25). Also, among patients not under invasive mechanical ventilation at baseline, tocilizumab reduced the risk of invasive mechanical ventilation and death (25). The RECOVERY Collaborative Group therefore concluded that the benefits of tocilizumab were additional to the benefits of systemic corticosteroids (25). Consequently, the Panel of the NIH provides the following recommendations on their website (26): “The Panel recommends using tocilizumab in combination with dexamethasone in certain hospitalized patients who are exhibiting rapid respiratory decompensation due to COVID-19”; however “The Panel recommends against the use of anti-IL-6 monoclonal antibody therapy (i.e., siltuximab) for the treatment of COVID-19, except in a clinical trial” (26).

Supportive therapies for clinical management of severe COVID-19 are administered in hospital settings with close monitoring of patients. Effectively, COVID-19 is a rapidly-evolving disease and severe cases need to be hospitalized and treated under meticulous monitoring. However, an oral treatment that can be prescribed to patients with mild to moderate symptoms would help them recover at home while reducing viral replication and environmental contamination, therefore diminishing spread of the virus in the household. For patients, such therapy would reduce their risk of developing severe clinical symptoms and requiring hospitalization. For communities, such treatment would lower pressure on the healthcare system. The threat of a marked surge of COVID-19 cases that would overwhelm the healthcare system is a sword of Damocles hanging over us. If the pandemic worsens and hospitals overflow, prioritization of access to intensive care would need to be established. In that catastrophic scenario, an oral treatment could offer an alternative therapy for patients not requiring intensive care such as mechanical ventilation.

An oral drug with anti-inflammatory and anti-SARS-CoV-2 properties, that is already FDA-approved with a known toxicity profile would be a promising candidate in the treatment of COVID-19. Auranofin is FDA-approved and well tolerated in humans, based on decades of use for treatment of rheumatoid arthritis (RA) (27). Auranofin can be administered orally and its pharmacokinetics, pharmacodynamics and adverse effects have been described (28, 29). RA is an autoimmune disease causing inflammation, pain and swelling in articulations (30). While the mechanism by which auranofin reduces inflammation during RA is not fully understood, it is reported that auranofin can decrease expression of some pro-inflammatory cytokines (30). In fact, peripheral blood monocytes of RA patients treated with auranofin have lower basal and lipopolysaccharide-stimulated IL-6 productions compared to untreated RA patients (31). Also, expression of macrophage-derived IL-6, which is abundant in rheumatoid synovium, is also reduced by auranofin treatment in vitro (30). By its action on the redox milieu, via inhibition of redox enzymes such as thioredoxin reductase, auranofin also possesses antinecancer, antiparasitic, antibacterial and antiviral properties which were reviewed elsewhere (32). In human cells infected with SARS-CoV-2, auranofin inhibited viral replication and markedly decrease expression of proteins of the inflammatory response, including IL-6 (33). If further investigations in vitro and in animal models of SARS-CoV-2 infection validate the anti-COVID-19 effects of auranofin, this gold compound would become a promising candidate in the treatment of COVID-19.

MECHANISMS OF ACTION OF AURANOFIN

To understand the potential of auranofin for the treatment of COVID-19, knowledge of its mechanisms of action is necessary. Manipulating the redox milieu by inhibiting redox enzymes is the main mechanism of action of auranofin (27). Auranofin is a potent inhibitor of thioredoxin reductase, an enzyme that minimizes oxidative stress and promotes cell survival (27). Disruption of redox homeostasis by auranofin can therefore lead to redox-sensitive apoptosis (27). Thioredoxin reductase also regulates the transactivation of NF-κB, a transcription factor involved in inflammation and cell survival (34). Inhibition of thioredoxin reductase prevents NF-κB DNA binding and NF-κB-dependent gene expression (34). From previous studies on SARS-CoV, we predict that high IL-6 expression during COVID-19 cytokine storm is mediated by NF-κB (35). In fact, the SARS-
CoV viral spike protein and nucleocapsid protein promote NF-κB-dependent IL-6 expression (13, 35). Strong NF-κB activation induces SARS-CoV-mediated lung inflammatory immunopathology and inhibition of NF-κB decreases mortality in SARS-CoV infected mice (13). Auranoﬁn also inhibits homodimerization of TLR4 and TLR4-mediated activation of NF-κB (36). In silico studies recently showed that toll-like receptor (TLR) 4 could have a crucial role in SARS-CoV-2-induced inflammatory responses and that TLR4-antagonists are promising therapeutic candidates (37). Moreover, other TLRs are targeted by auranoﬁn, including TLR3 (38), which is involved during SARS-CoV-2 infection (13). Auranoﬁn also prevents activation of 1κB kinase (IKK) which initiates phosphorylation of 1κB and promotes NF-κB activity (39). In addition, auranoﬁn suppresses the degradation of inhibitory 1κB proteins associated with NF-κB (39). Equally important, auranoﬁn inhibits IL-6-induced phosphorylation of Janus kinase 1 (JAK1) and signal transducer and activator of transcription 3 (STAT3) (40). Moreover, due to its inhibitory effect on STAT3 translocation to the nucleus, auranoﬁn blocks expression of STAT3-regulated genes (40). Thus, auranoﬁn inhibits several steps in the NF-κB-IL-6-STAT3 signaling pathway (Figure 1).

Because auranoﬁn acts upstream and downstream of NF-κB-dependent IL-6 expression, both IL-6 secretion and STAT3-derived IL-6 action are inhibited (39, 40). As an important proinflammatory cytokine, IL-6 is a potent inducer of inflammatory sequelae, including from COVID-19 (41, 42). The SARS-CoV-2-associated inflammatory cytokine storm, comprising cytokines including IL-6, leads to severe illness and multiple organ dysfunction (13, 17, 43). NF-κB-derived high IL-6 levels are biomarkers of COVID-19 severity and predict mortality; IL-6 is thus a potential target for immunotherapy (13, 17, 41–44). Pulmonary ﬁbrosis in severe COVID-19 cases or as sequelae after COVID-19 are associated with the cytokine storm (45). Because IL-6 is linked to pulmonary ﬁbrosis, for example, during idiopathic pulmonary ﬁbrosis (46), high IL-6 levels during COVID-19 could promote pulmonary ﬁbrosis; this hypothesis would need further investigation. Since auranoﬁn inhibits ﬁbrosis in human hepatic stellate cells (47), auranoﬁn could hypothetically have the same inhibitory effect on SARS-CoV-2-associated pulmonary ﬁbrosis; this hypothesis will need to be further investigated.

**ANTIVIRAL PROPERTIES OF AURANOFIN**

Antiviral actions of auranoﬁn have been an active ﬁeld of research. For example, auranoﬁn has anti-HIV properties. Protein-protein interactions, involving the viral glycoprotein gp120, are essential for HIV to enter human cells (48). Thioredoxin, or another redox enzyme, reduces gp120 for proper conformation for protein interaction and viral entry (48). Auranoﬁn, by inhibiting thioredoxin reductase, was shown to inhibit HIV infection of cultured cells (48). SARS-CoV-2 also relies on protein-protein interactions, particularly between the viral spike protein and host angiotensin converting enzyme 2 (ACE2), for entry into host cells (35, 49). This interaction was illustrated by the crystal structure of the
receptor-binding domain of the spike protein bound to the host ACE2 (50, 51). Cysteines are present in spike and ACE2 proteins and are involved in redox-active disulﬁde bonds (52). Conformation of both the spike and the ACE2 proteins can thus be subject to the redox milieu (52). A thioredoxin-dependent redox model of spike-ACE2 interaction was proposed and the authors hypothesized that oxidized ACE2 with intact disulﬁde bonds is necessary for SARS-CoV-2 entry, and that the thioredoxin system, including thioredoxin reductase, would block viral entry via reduction of ACE2 (52). However, our understanding of the spike-ACE2 interface is not complete because, in fact, auranoﬁn (an inhibitor of thioredoxin reductase) inhibits the spike-ACE2 interaction at IC50 value of 22.2 µM (49, 53, 54). Studies using humanized ACE2 transgenic mice would probably determine the potential of auranoﬁn to block SARS-CoV-2 entry in host cells. Auranoﬁn also inhibits SARS-CoV and SARS-CoV-2 papain-like proteases, (which are important for viral replication) at IC50 values of 25.5 µM and 0.75 µM, respectively (49).

Rothan et al. reported that, at 24 hours and at 48 hours after infection, auranoﬁn (4 µM) reduced SARS-CoV-2 RNA by 70% and 85%, respectively, in human cell culture supernatants and by 85% and 95%, respectively, in human cell lysates (33). The EC50 at 48 hours after infection was approximately 1.4 µM (33). In cell culture supernatants at 48 hours after infection, auranoﬁn also signiﬁcantly reduced viral infectivity as determined by plaque assay (33). To explain its inhibitory effect on viral replication, it was hypothesized that auranoﬁn could affect SARS-CoV-2 protein synthesis partially due to its action on the redox milieu via inhibition of thioredoxin reductase (33). We recommend to further investigate in vitro the potential direct antiviral action of auranoﬁn during SARS-CoV-2 infection to better understand its mechanism of action. Then, we encourage pre-clinical studies in animal models of COVID-19 to validate in vivo the direct antiviral property of auranoﬁn. Also, pre-clinical studies will determine if treatment with auranoﬁn for more than 48 hours will be safe for SARS-CoV-2-infected animals. Rothan et al. reported that, not only did auranoﬁn inhibit SARS-CoV-2 replication, it also signiﬁcantly reduced the expression of key proteins of the inﬂammatory response (IL-6, NF-kB, TNFα, ILβ) 48 hours after infection of human cells with SARS-CoV-2 (33). The effect of auranoﬁn on IL-6 is particularly marked and can probably be explained by inhibition of the NF-κB pathway. In fact, SARS-CoV-2 infection increased by 200-fold the mRNA expression of IL-6 in human cells compared to mock-infected cells, while auranoﬁn-treated SARS-CoV-2-infected cells had only a 2-fold increase in IL-6 expression compared to mock-infected cells (33). High levels of IL-6 in the COVID-19-associated cytokine storm are linked with disease severity and mortality (17, 41). Therefore, to our point of view, the major advantage of auranoﬁn treatment is its signiﬁcant reduction of IL-6 expression in SARS-CoV-2-infected cells. Animal models, particularly with humanized ACE2 transgenic mice, could determine if auranoﬁn has the potential to reduce SARS-CoV-2-associated IL-6-derived pathogenicity and decrease COVID-19 immunopathology.

Auranoﬁn also inhibits replication of other viruses, including Zika virus and Venezuelan equine encephalitis virus, as well as chikungunya virus both in vitro and in a murine model of infection (55). Apoptosis of auranoﬁn-treated virus-infected cells was proven by the presence of viral genomes and misfolded/incompletely assembled particles in supernatants (55). Selective apoptosis triggered by auranoﬁn is illustrated by its action on the HIV viral reservoir. HIV eradication from the body is compromised by the viral reservoir in long-lived central memory and transitional memory CD4+ T cells harboring the retroviral genome (56). In vitro, auranoﬁn induces differentiation and death of these CD4+ T cell subpopulations constituting the viral reservoir in humans (56). Also, in SIVmac251-infected rhesus macaques, auranoﬁn reduced long-lived central memory and transitional memory CD4+ T cells and decreased cell-associated viral DNA (56). The anti-HIV reservoir effects of auranoﬁn were attributed to the lower antioxidant defenses of central memory and transitional memory CD4+ T cells (57). The inhibitory effect of auranoﬁn on the redox enzyme thioredoxin reductase increases cellular oxidative stress and promote redox-sensitive apoptosis (27); auranoﬁn therefore has the ability to select those cell populations for apoptosis (57). In addition, human cells also possess mitochondrial thioredoxin reductase and auranoﬁn can, at very low micromolar concentrations, inhibit mitochondrial thioredoxin reductase (58). Consequently, auranoﬁn can induce mitochondrial membrane permeability transition, loss of mitochondrial membrane potential, release of cytochrome c and apoptosis (58).

SAFE USE OF AURANOFIN IN HUMANS

Auranoﬁn has been generally replaced in the treatment of rheumatoid arthritis by more targeted therapies, in part due to its side effects (32). Auranoﬁn is not a harmless drug and treated patients should be monitored (28). Effectively, as a metal-based drug, toxicity issues need to be considered (54). Loose stool and diarrhea commonly occur following oral treatment with auranoﬁn, while rash and proteinuria are less common side effects (28). Fortunately, thrombocytopenia and bone marrow suppression only happen rarely; but long term therapies should be monitored for their potential impact on immune functions (28). Also, auranoﬁn is not recommended in pregnant women and a highly effective birth control should be used (59). In a recent Phase I clinical trial investigating the potential of auranoﬁn for short term therapies, this gold compound was safe and well tolerated (59). Adverse effects were frequent (headache was the most frequently reported), but all adverse effects were mild and resolved without treatment (59). In most cases, anti-COVID-19 treatment with auranoﬁn should be short term (54). The advantages of auranoﬁn treatment in SARS-CoV-2 infections will prevail over potential toxicity for most patients, but we recommend administering auranoﬁn under close medical supervision until safety data are available for COVID-19 patients. Auranoﬁn is FDA-approved and safe in humans and...
the fear of side effects, which are mostly mild, should not stop its evaluation in clinical trials for the treatment of COVID-19. In fact, auranoﬁn is now in several Phase II clinical trials for its antiviral and antiparasitic properties (27, 33, 59). Auranoﬁn could be tested by adding it to currently accepted therapies with minimal additional risk for COVID-19 patients. If clinical trials prove its safety in COVID-19 patients, auranoﬁn could then be administered outside of hospital settings.

**DISCUSSION**

An orally-administered FDA-approved drug with anti-inflammatory and anti-SARS-CoV-2 properties with an acceptable toxicity proﬁle would offer signiﬁcant beneﬁts for the control of the COVID-19 pandemic. Auranoﬁn, an antirheumatic drug, has anti-inﬂammatory properties via its modulation of the NF-κB-IL-6-STAT3 signaling pathway by inhibiting several components upstream and downstream of IL-6 expression (34, 36, 39, 40). Because NF-κB and NF-κB-dependent IL-6 are major actors in the COVID-19-associated cytokine storm and high IL-6 levels worsen prognosis and predicts mortality (13, 17, 35, 41), drugs that can block this pathway are of immediate interest (18–21, 42–44). Auranoﬁn is a potent inhibitor of SARS-CoV-2-induced cytokine expression (including IL-6) in human cells in vitro (33). Moreover, unlike monoclonal antibodies speciﬁcally targeting IL-6 or its receptor or glucocorticoids such as dexamethasone, auranoﬁn also has a potential direct antiviral action that could be of additional beneﬁt. Effectively, if further investigations in vitro and in COVID-19 animal models (such as in humanized ACE2 transgenic mice) validate the potential direct anti-SARS-CoV-2 action of auranoﬁn, it will constitute an additional factor in its favor. Furthermore, by inhibiting thioredoxin reductase, auranoﬁn can induce redox-sensitive apoptotic pathways and promote mitochondrial membrane permeability transition (27, 58). Studies will be needed to determine if auranoﬁn can target for apoptosis SARS-CoV-2-infected cells. Auranoﬁn also has an anti-fibrotic action in human hepatic stellate cells (47), but it is not yet elucidated if auranoﬁn can inhibit pulmonary ﬁbrosis in severe COVID-19 cases or as sequelae after COVID-19.

Auranoﬁn shows a peak plasma gold concentration 1-2 h following oral dosing (27, 59–61). Because of rapid metabolism, auranoﬁn is not detected intact in blood; pharmacokinetic data therefore rely on measurement of plasma gold concentration, where gold mostly binds to serum proteins (27, 59–61). Following 7 days of 6 mg/day auranoﬁn, plasma gold concentration ranged from 0.12 to 0.22 µg/ml (59). A clinical trial (Phase I and II Study of Auranoﬁn in Chronic Lymphocytic Leukemia (CLL); registration number NCT0149691) using 21 mg/day auranoﬁn to treat relapsed leukemia was approved by the FDA (59). At a dose of 21 mg/day auranoﬁn, plasma gold concentration increases proportionally and ranged from 0.42 to 0.77 µg/ml (59). Also, doubling the period of treatment from 7 to 14 days doubled the plasma gold concentration, which ranged from 0.22 to 0.42 µg/ml for 6 mg/day auranoﬁn and from 0.78 to 1.48 µg/ml for 21 mg/day auranoﬁn, respectively (59). Moreover, auranoﬁn has a long terminal half-life (59). Therefore, two weeks after the last treatment, plasma gold concentrations remained high ranging from 0.11 to 0.30 µg/ml for 6 mg/day auranoﬁn and from 0.39 to 1.05 µg/ml for 21 mg/day auranoﬁn, respectively (59). Because gold corresponds to 29% of the mass of auranoﬁn, the molarity of ‘auranoﬁn equivalent’ can be calculated from plasma gold molar concentration. But it is important to remember that auranoﬁn itself, as used for in vitro assays, is not present in blood and its metabolites might not have the same efﬁcacy in vivo, particularly because they are protein-bound (59–61). With this in mind, ‘auranoﬁn equivalent’ values can be extrapolated from plasma gold concentrations mentioned above (59). After 14 days of treatment with 21 mg/day auranoﬁn, plasma gold concentration reached 1.18 µM to 2.21 µM ‘auranoﬁn equivalent’ and remained between 0.58 µM to 1.5 µM ‘auranoﬁn equivalent’ two weeks after the last treatment (59).

The antimicrobial properties of auranoﬁn metabolites bound to serum proteins are unknown and should be further investigated. In other words, it may not be appropriate to assume that plasma gold concentration corresponding to a given ‘auranoﬁn equivalent’ molarity would be as eﬃcacious in vivo to inhibit infection as the same molar concentration of auranoﬁn in vitro; also, the stability of auranoﬁn in cell culture has not been reported. That being said, it is most likely that the concentration needed to inhibit SARS-CoV-2 papain-like protease (ICso 0.75 µM in vitro) and to reduce SARS-CoV-2 replication (ECso 1.4 µM in vitro) would be achievable by oral administration of auranoﬁn (33, 49). On the other hand, it is improbable that levels obtained after oral administration of auranoﬁn will reach the concentration needed to inhibit the spike-ACE2 interaction (ICso 22.2 µM in vitro) (49, 53, 54). Animal studies, particularly with humanized ACE2 transgenic mice, will better define the anti-SARS-CoV-2 effects of auranoﬁn in vivo. Notably, in the in vitro study reported by Rothan et al., the 50% cytotoxic concentration for human cells was approximately 5.7 µM, 4 times the ECso for SARS-CoV-2 (33). We recognize that this low margin of safety is a source of concern. Because treatment of COVID-19 would be short term, high doses of auranoﬁn may have an acceptable toxicity proﬁle; but clinical trials in COVID-19 patients are of course necessary to establish the appropriate dose.

Injectable gold compounds are also potential therapies for COVID-19 (49, 53, 54, 62). However, investigations remain to be done to determine if, like auranoﬁn (33), other gold metallodrugs inhibit NF-kB-induced IL-6 expression and SARS-CoV-2 replication. The main advantage of gold compounds administered intramuscularly is achieving high plasma gold levels, up to ten-fold higher than with oral administration of auranoﬁn (60, 61). Therefore, the high plasma concentrations needed to inhibit the spike-ACE2 interaction could potentially be achievable with injectable gold compounds (49); however, this hypothesis should be tested in animal models, particularly with humanized ACE2 transgenic mice. On the other hand, it might not be necessary to inhibit the spike-ACE2 interaction to have anti-SARS-CoV-2 effects and auranoﬁn could present other
advantages over injectable gold metallodrugs. Effectively, if further in vitro investigations followed by pre-clinical studies in animal models of COVID-19 prove that aurano can inhibit NF-kB-induced IL-6 expression as well as SARS-CoV-2 replication and SARS-CoV-2 papain-like protease, aurano could become a valuable addition to available therapies for COVID-19. Promising data obtained from pre-clinical studies would support evaluation of aurano in clinical trials with COVID-19 patients. Another point in the favor of aurano is that it is conveniently administered orally and for this reason, can be prescribed to patients recovering at home. If clinical trials prove that aurano can be safely administered to COVID-19 patients, then aurano could be prescribed outside of hospital settings. Finally, aurano is mostly excreted by the enteric route (60, 61). Because only 15% to 30% of an oral dose of aurano is absorbed, even the anti-rheumatic dose of 6 mg/day for 7 days leads to high gold concentration in feces following oral dosing (59–61). High ACE2 expression in enterocytes of the small intestine was proposed to explain enteric COVID-19 symptoms and sites of tissue damage (35). Therefore, we hypothesize that aurano can be particularly efficacious to treat SARS-CoV-2 infection in the intestines, but this hypothesis would need further investigation in animal models and, potenti ally, in clinical trials.

CONCLUDING REMARKS

While information on the action of aurano during SARS-CoV-2 infection is limited, knowledge of the mechanism of action of aurano in the treatment of autoimmune and infectious diseases allow to predict that this compound could be a promising candidate in the treatment of COVID-19. Due to the current pandemic, there is a necessity to perform further in vitro investigations and conduct pre-clinical studies in SARS-CoV-2-infected animals to evaluate the potential anti-inflammatory and antiviral effects of aurano during SARS-CoV-2 infection. If pre-clinical studies prove the anti-COVID-19 properties of aurano, these data will pave the way for clinical trials with SARS-CoV-2-infected patients to repurpose aurano for the treatment of COVID-19. Further studies could also determine if the inhibitory effect of aurano on thioredoxin reductase can induce redox-sensitive apoptosis of SARS-CoV-2-infected cells and if its anti-fibrotic action can prevent COVID-19-associated pulmonary fibrosis. Ultimately, this oral therapy could be added to supportive therapies administered to patients under monitoring in hospital settings and could benefit COVID-19 patients with mild to moderate symptoms recovering at home. We therefore join our voices with other researchers (33, 49, 53, 54, 62) to encourage the evaluation in animal models and clinical trials of chrysotherapy with aurano, or possibly other gold metallodrugs, alone or in combination with other immunomodulatory molecules for the treatment of COVID-19.

AUTHOR CONTRIBUTIONS

KS-D wrote the original draft of the manuscript. KS-D and MN reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. World Health Organization. Coronavirus Disease (COVID-19) Weekly Epidemiological Update (2021). Available at: https://www.who.int/publications/m/item/weekly-epidemiological-update—10-march-2021.
2. Callaway E. Could New COVID Variants Undermine Vaccines? Labs Scramble to Find Out. Nature (2021) 589(7841):177–8. doi: 10.1038/d41586-021-00031-0
3. Mishra SK, Tripathi T. One Year Update on the COVID-19 Pandemic: Where are We Now? Acta Trop (2021) 214:105778. doi: 10.1016/j.actatropica.2021.105778
4. Ali MJ, Hanif M, Haider MA, Ahmed MU, Sundas F, Hirani A, et al. Treatment Options for COVID-19: A Review. Front Med (Lausanne) (2020) 7:480. doi: 10.3389/fmed.2020.00480
5. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the Treatment of COVID-19 - Final Report. N Engl J Med (2020) 383(19):1813–26. doi: 10.1056/NEJMoa2007764
6. Young B, Tan TT, Leo YS. The Place for Remdesivir in COVID-19 Treatment. Lancet Infect Dis (2021) 21(1):20–1. doi: 10.1016/S1473-3099(20)30911-7
7. Bartoli A, Gabrielli F, Alicandro T, Nascimbeni F, Andreone P. COVID-19 Treatment Options: A Difficult Journey Between Failed Attempts and Experimental Drugs. Intern Emerg Med (2021) 16(2):281–308. doi: 10.1007/s11739-020-02569-9
8. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-Approved Drug Ivermectin Inhibits the Replication of SARS-CoV-2 In Vitro. Antiviral Res (2020) 178:104787. doi: 10.1016/j.antiviral.2020.104787
9. Sharun K, Dhama K, Patel SK, Pathak M, Tiwari R, Singh BR, et al. Ivermectin, a New Candidate Therapeutic Against SARS-CoV-2/COVID-19. Ann Clin Microbiol Antimicrob (2020) 19(1):23. doi: 10.1186/s12941-020-00368-w
10. Heidary F, Gharebaghi R. Ivermectin: A Systematic Review From Antiviral Effects to COVID-19 Complementary Regimen. J Antimicrob Chemother (2020) 75(9):593–602. doi: 10.1093/jac/dkaa296
11. Schmith VD, Zhou JJ, Lohmer LRL. The Approved Dose of Ivermectin Alone is Not the Ideal Dose for the Treatment of COVID-19. Clin Pharmacol Ther (2020) 108(4):762–5. doi: 10.1002/cpt.1889
12. Chaccour C, Hammann F, Ramon-Garcia S, Rabinovich NR. Ivermectin and COVID-19: Keeping Rigor in Times of Urgency. Am J Trop Med Hyg (2020) 102(6):1156–7. doi: 10.4269/ajtmh.20-0271
51. Huang Z, Chai J. Door to the Cell for COVID-19 Opened, Leading Way to Therapies. *Signal Transduct Target Ther* (2020) 5(1):104. doi: 10.1038/s41392-020-00215-6

52. Singh J, Dhindsa RS, Misra V, Singh B. SARS-CoV2 Infectivity is Potentially Modulated by Host Redox Status. *Comput Struct Biotechnol J* (2020) 18:3705–11. doi: 10.1016/j.csbj.2020.11.016

53. de Paiva REF, Marcal Neto A, Santos IA, Jardim ACG, Corbi PP, Bergamini FRG. What is Holding Back the Development of Antiviral Metallodrugs? A Literature Overview and Implications for SARS-CoV-2 Therapeutics and Future Viral Outbreaks. *Dalton Trans* (2020) 49(45):16004–33. doi: 10.1039/D0DT02478C

54. Cirri D, Pratesi A, Marzo T, Messori L. Metallo Therapeutics for COVID-19. Exploiting Metal-Based Compounds for the Discovery of New Antiviral Drugs. *Expert Opin Drug Discov* (2021) 16(1):39–46. doi: 10.1080/17460441.2020.1819236

55. Langsjoen RM, Auguste AJ, Rossi SL, Roundy HN, Kastis M, et al. Host Oxidative Folding Pathways Offer Novel Anti-Chikungunya Virus Drug Targets With Broad Spectrum Potential. *Antiviral Res* (2017) 143:246–51. doi: 10.1016/j.antiviral.2017.04.014

56. Lewis MG, DaFonseca S, Chomont N, Palamara AT, Tardugno M, Mai A, et al. Gold Drug Auranozin Restricts the Viral Reservoir in the Monkey AIDS Model and Induces Containment of Viral Load Following ART Suspension. *AIDS* (2011) 25(11):1347–56. doi: 10.1097/QAD.0b013e328347bd77

57. Chirullo B, Sgarbanti R, Limongi D, Shyta IL, Alvarez D, Das B, et al. A Candidate Anti-HIV Reservoir Compound, Auranozin, Exerts a Selective ‘Anti-Memory’ Effect by Exploiting the Baseline Oxidative Status of Lymphocytes. *Cell Death Dis* (2013) 4:e944. doi: 10.1038/cddis.2013.473

58. Rigobello MP, Scutari G, Boscolo R, Bindoli A. Induction of Mitochondrial Permeability Transition by Auranozin, a Gold(I)-Phosphine Derivative. *Br J Pharmacol* (2002) 136(8):1162–8. doi: 10.1038/sj.bjp.0704823

59. Capparelli EV, Bricker-Ford R, Rogers MJ, McKerrow JH, Reed SL. Phase I Clinical Trial Results of Auranozin, a Novel Antiparasitic Agent. *Antimicrob Agents Chemother* (2017) 61(1):e01947–16. doi: 10.1128/AAC.01947-16

60. Blocka K. Auranozin Versus Injectable Gold. Comparison of Pharmacokinetic Properties. *Am J Med* (1983) 75(6A):114–22. doi: 10.1016/0002-9343(83)90483-7

61. Blocka K, Paulus HE, Furst DE. Clinical Pharmacokinetics of Oral and Injectable Gold Compounds. *Clin Pharmacokinet* (1986) 11(2):133–43. doi: 10.2165/00003088-198611020-00003

62. Marzo T, Messori L. A Role for Metal-Based Drugs in Fighting COVID-19 Infection? *Case auranozin ACS Med Chem Lett* (2020) 11(6):1067–8. doi: 10.1021/acsmedchemlett.0c00190

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