Lysosomotropic active compounds—
hidden protection against COVID-19 / SARS-CoV-2 infection?

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Abstract

The COVID-19 pandemic is one of the largest challenges in medicine and health care worldwide in recent decades, and it is infecting and killing increasing numbers of people every day. In this paper, we discuss the possible relationships among lysosomotropism, increasing lysosomal pH, and the SARS-CoV-2 infection and disease process, and we deduce a possible approach for treatment and prophylaxis.

Lysosomotropism is a biological characteristic of small molecules, such as (hydroxyl)chloroquine, amitriptyline, NB 06, or sertraline, which is present in addition to intrinsic receptor-mediated or enzymatic pharmacological effects. Lysosomotropic compounds affect prominent inflammatory messengers, such as IL1B, CCL4, CCL20, and IL6, as well as cathepsin L dependent viral entry (fusion) into host cells. Therefore, this heterogeneous group of compounds is a promising candidate for the prevention and treatment of SARS-CoV-2 infections, as well as influenza A infections and cytokine release syndrome (CRS) triggered by bacterial or viral infections. Patients who have already taken medications with lysosomotropic compounds for other pre-existing conditions may benefit from this treatment in the COVID-19 pandemic.

Increased lysosomal pH levels play an important role in the disease process in common skin disorders, such as psoriasis and atopic dermatitis, thus suggesting that affected individuals might benefit from their particular conditions in the COVID-19 pandemic. We suggest data analysis of patients with these diseases, and who are treated with lysosomotropic compounds, and, if the results are promising, subsequent clinical testing of off-label therapy with clinically approved lysosomotropic compounds in the current COVID-19 pandemic and future influenza A pandemics.
Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified as the disease-causing pathogen of Coronavirus disease 2019 (COVID-19)\textsuperscript{1}. A total of 1 289 380 confirmed infections and 70 590 deaths worldwide were reported by the Johns Hopkins Center for Systems Science and Engineering, Baltimore, Maryland, USA, as of April 4, 2020.\textsuperscript{2} As of March 11, 2020, the status of the Covid-19 outbreak was reclassified from an epidemic to a pandemic, thus posing serious challenges to the health care systems in the EU, the US, and many Asian countries. Research to identify active compounds for the treatment of SARS-CoV-2 viral infection has focused to date on the virustatic agents ritonavir\textsuperscript{3} (off-label use) and remdesivir\textsuperscript{4,5} (GS-5734, compassionate use) or the antimalarial active compounds chloroquine\textsuperscript{5-7} and hydroxychloroquine\textsuperscript{6,8,9} (off-label use), both of which are well-known immune modulators. In 1984, the effects of the weak base and lysosomotropic compound chloroquine was investigated against Sindbis virus infection in BHK-21 cells. In established infections, chloroquine was found to inhibit the synthesis of viral RNA when added early in the process of pathogenesis\textsuperscript{10}. Lysosomotropism is an often neglected biological characteristic of small molecules, which is present in addition to their intrinsic receptor-mediated or enzymatic pharmacological effects and is sometimes responsible for severe adverse effects\textsuperscript{11-15}. Regardless of the medical indications for which they have been used, many (active) compounds possess lysosomotropic characteristics\textsuperscript{16-18} and therefore are potential active compounds for treatment of SARS-CoV-2 viral infection of airway epithelial cells (type II pneumocytes), such as chloroquine in Sindbis virus infection\textsuperscript{10}. Approved drugs comprising lysosomotropic compounds used for various medical indications offer a chance to treat the SARS-CoV-2 infection through more personalized approaches depending on drug profile. Given the wide application of drugs with lysosomotropic characteristics (Table 1), statistical testing of the protective effects of various drugs in the current pandemic appears straightforward. Here, we outline possible prevention and treatment options based on recent findings on the COVID-19 disease, which would be easy to apply, even for patients at risk.
SARS-CoV-2 uses Angiotensin-converting enzyme 2 (ACE2) as a cellular receptor

SARS-CoV-2 is an enveloped, non-segmented positive sense RNA human SARS-related coronavirus (SARS-CoV)\(^1\) with a single-stranded RNA composed of approximately 30 kb nucleotides encoding at least four major structural proteins: spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N)\(^2\). The S glycoprotein comprises two functional subunits, which are responsible for fusion of the viral and host cell membranes (S\(_2\) subunit), and binding to the host cell receptor (S\(_1\) subunit), thus mediating entry into host cells\(^1\). S forms homotrimers protruding from the surface of the SARS-CoV-2 virus\(^1,21\). The receptor binding motif (RBM; amino acids 437 to 508) is located in the receptor-binding domain in the S\(_1\) subunit and has high affinity toward human Angiotensin-converting enzyme 2 (ACE2; BRENDA:EC 3.4.17.23), a carboxypeptidase. The three-dimensional structure of the ACE2 receptor binding motif is similar in both SARS-CoV and SARS-CoV-2\(^20\). ACE2 functions as a cellular receptor for SARS-CoV-2, allowing the virus to gain entry into ACE2 expressing host cells. It is widely distributed in cells (such as those of the lung (airwave epithelial cells), heart, liver, testis, kidney, brain, intestine (pancreas and colon), and several other tissues), and it circulates in blood vessels (circulating plasma ACE2)\(^1,20,22–24\). Nevertheless, SARS-CoV mainly infects airway epithelial cells (pneumocytes) and macrophages; extrapulmonary spread of SARS-CoV in ACE2 expressing tissues has also been observed\(^25\).

**Mechanism of host cell entry**

Both types of SARS-CoV engage their receptor, ACE2, on the host cell surface for host cell entry\(^20\). In cells that do not express trypsin-like proteases (human airway trypsin-like protease (HAT))\(^26\) on their surfaces, SARS-CoV enters the cytoplasm through endosomes and travels along the endocytic pathway. In lysosomes, the last compartment of the endocytic pathway, active cathepsin L (BRENDA:EC3.4.22.15; optimum pH 5.0–5.5)\(^27\), induces the fusion of SARS particles bound to ACE2 with host cells\(^28\), and fusion is then triggered by cathepsin L cleavage at the S1/S2 cleavage site of the SARS S (fusion) protein\(^29\). The endosomal entry route of SARS particles bound to ACE2 and the subsequent maturation of early endosomes (EE) via late endosomes (LE) to endolysosomes (ELs), and finally lysosomes has been substantiated by evidence that the cysteine protease inhibitor E64d and bafilomycin A1 (inhibitor of vacuolar H\(^+\) ATPase (V-ATPase); BRENDA:EC 3.6.3.6) and vacuolar acidification block SARS-CoV S-protein
mediated endosomal entry\textsuperscript{28}. Bafilomycin A1, via preventing (endo)lysosomal acidification, and the cathepsin L inhibitor teicoplanin\textsuperscript{30}, a glycopeptide antibiotic, inhibit cathepsin L induced fusion of SARS-CoV particles bound to ACE2 with host cells. The required lysosomal pH for fusion-activating S protein cleavage by cathepsin L can therefore be provided only by intact lysosomes. EE, LE, less acidic EL, lysosomes containing lysosomotropic compounds and cells with depleted ATP levels are unable to provide active cathepsin L.

In experimental in vitro conditions, trypsin can override the need for cathepsin L mediated cleavage, thereby shifting the virus to an endosome independent route of entry, possibly through the plasma membrane\textsuperscript{31}. TMPRSS2 or TMPRSS11d, members of the transmembrane protease/serine subfamily (TMPRSS), have recently been reported to induce SARS-CoV fusion (S-protein priming) in cells\textsuperscript{25,29,31}.

**Current therapy guidelines**

The most recent guidelines (as of March 25, 2020) published by the National Health Commission of China (NHC) recommend treatment with IFN-\(\alpha\), lopinavir/ritonavir, ribavirin, chloroquine phosphate, and arbidol as anti-viral therapy\textsuperscript{32}, in off-label use (according to the legal approval status of the active compound). Chloroquine (phosphate) and hydroxychloroquine (sulfate) are disease-modifying antirheumatic drugs (DMARDs). In cell culture experiments, hydroxychloroquine has been demonstrated to prevent SARS-CoV-2 infection in vitro\textsuperscript{5,6,9}. Recent clinical trials have demonstrated that oral hydroxychloroquine (600 mg per day) is significantly associated with a reduction/disappearance of viral load in nasopharyngeal samples in patients with SARS-CoV-2\textsuperscript{8}. The Janus-associated kinase (JAK) inhibitor baricitinib, a new type of immunomodulatory active compound that is approved for treatment of rheumatoid arthritis, is expected to reduce the effects of elevated cytokine levels typically observed in patients with SARS-CoV-2 infection. It is, however, unlikely to reduce viral infectivity at tolerated doses\textsuperscript{33–35}.

There is a need for action in the field of therapy and prevention of SARS-CoV-2 infection, owing to the rapidly increasing number of cases. Therapies recommended to date represent a first step toward solving this immense challenge.
Research on the lysosomotropic compound chloroquine in SARS-CoV(-2) infection

Several in vitro studies have been conducted to elucidate the entry route and therapy options for treating SARS-CoV infection\textsuperscript{28,30,36,37}. An in vitro study conducted in 2005, focusing on the effects of chloroquine during pre- and post-infection periods, has provided profound insight into the progress of SARS-CoV infection. Chloroquine has been found to abolish SARS-CoV infection in Vero E6 cells starting from 0.1 μM chloroquine, in a dose dependent manner, whereas concentrations of 10 μM chloroquine completely inhibit SARS-CoV infection. As little as 0.1–1 μM chloroquine decreases the number of virus antigen-positive host cells by 50%. The IC\textsubscript{50} of SARS-CoV is 4.4 ± 1.0 μM\textsuperscript{36}/8.8 μM\textsuperscript{37}, and that of SARS-CoV-2 is 6.9 μM\textsuperscript{9}. The levels of host cell-surface ACE2 have been found to remain unchanged. Interestingly, chloroquine exerts antiviral effects during pre- and post-infection conditions\textsuperscript{36}. Furthermore, chloroquine impairs the terminal glycosylation of ACE2 (the cellular receptor of SARS-CoV) at anti-SARS-CoV concentrations and inhibits viral entry (fusion). This activity may affect endosome-mediated viral fusion with host cells and subsequent viral replication or assembly, thus releasing the virus and abrogating the infective process\textsuperscript{36}.

These findings suggest that both prophylactic and therapeutic effects of lysosomotropic compounds are likely. After the outbreak of COVID-19 in late 2019, successful in vitro experiments from 2005 that tested the effects of chloroquine on SARS-CoV infection of Vero E6 host cells\textsuperscript{36} were repeated, targeting SARS-CoV-2 instead of SARS-CoV. In addition to chloroquine, the less toxic compound hydroxychloroquine was tested in the same experimental setting\textsuperscript{6}. In SARS-CoV-2 infection, as in SARS-CoV infection, chloroquine displays similar inhibitory activity. Hydroxychloroquine shows a somewhat (approximately two times) lower inhibitory effect on viral infection of Vero E6 cells than chloroquine (determined as EC\textsubscript{50} per multiplicity of infection (MOI)) and thus may be a viable treatment option.

Therapeutic options to treat COVID-19 / SARS-CoV-2 infections

Lysosomotropic active compounds

Lysosomotropic compounds are small molecules selectively taken up by lysosomes, regardless of their chemical nature or mechanism of uptake\textsuperscript{38}. Typically, they are weak organic bases (pKa >6, lipophilic) that easily penetrate uncharged the lysosomal...
membrane and are protonated and consequently trapped in the lysosome lumen\textsuperscript{16,17}. Lysosomotropism is therefore a biological characteristic of active compounds that is independent of their pharmacological effects.

Because lysosomotropic compounds accumulate in lysosomes, they increase the lysosomal pH from 4.5–5 to 6–6.5\textsuperscript{39}. Lysosomotropic effects (IC\textsubscript{50}) can be determined, and compounds can be screened by quantifying the displacement of Red DND-99 (LysoTracker) from lysosomes\textsuperscript{40,41}. Effective compounds display a displacement IC\textsubscript{50} of approximately 10 \textmu M, which is in the range of the values for several receptor-mediated or enzyme inhibitory effects\textsuperscript{10,16,42}. Most lysosomal enzymes are inactivated through an increase in the lysosomal pH beyond their optimum pH range (pH 4.5–5.5).

In fact, various well-known clinically approved active compounds for various indications (psychotropic, antihypertensive, and antimycotic) share lysosomotropic characteristics. To date, amitriptyline, amlodipine, astemizole, benzatropine, bepridil, chlorpromazine, chlorprothixene clomiphene, desipramine, doxepine, fluoxetine, imipramine, maprotiline, norfluoxetine, nortriptyline, paroxetine, promazine, promethazine, sertraline, terfenadine, and triflupromazine have been classified as lysosomotropic compounds (Table 1, Fig. 1)\textsuperscript{17,18}.

Furthermore, other small molecules (NB 06 and NB 19) available for research applications can be used as tools for studying the biological effects of lysosomotropy and lysosome-dependent signaling pathways\textsuperscript{16}. Lysosomotropic characteristics may diminish target specificity if the target is located in lysosomes; e.g., a nitrogen-containing lipophilic selective cathepsin K inhibitor in cell culture experiments results in an apparent increase in inhibitor potency against antitarget enzymes (cathepsin B, L, and S) present in lysosomes\textsuperscript{43}.

**Current and achievable clinical impact of lysosomotropic compounds**

To date, lysosomotropism has been of scientific interest for its association with the occurrence of adverse effects during the application of particularly active compounds. Lysosomotropism in combination with dysfunction in elongation of very long-chain fatty acids is responsible for severe adverse effects when used orally or topically\textsuperscript{15}, in some cases such as hydroxychloroquine (rash or itching)\textsuperscript{14}, sertraline (exanthematous pustulosis)\textsuperscript{12}, and terbinafine (Lupus erythematoses or exanthematous pustulosis)\textsuperscript{11}.
Lysosomotropism appears at concentrations in the micromolar range; nevertheless, most drugs exhibit their desired primary pharmacological effects at low concentrations.

As described above, cathepsin L plays a crucial role in SARS-CoV-2 infection of host cells and subsequent dissemination. In general, cathepsin L can be inactivated through selective but not clinically approved inhibitors\textsuperscript{43,44}, or alternatively by clinically approved lysosomotropic compounds (see lysosomotropic active compounds) in off-label use. However, in the case of systemic administration of the active compound, the main indications must be considered, because they may represent undesired adverse effects in an anti-viral off-label use and therefore should not be ignored. According to current knowledge, inhibition of cathepsin L dependent viral entry (fusion) into host cells can be obtained only through off-label use of the active compounds listed in Table 1.

Teicoplanin and dalbavancin are solely intravenously applicable, clinically approved glycopeptide antibiotics. Both compounds display a pronounced inhibitory effect against SARS-CoV infection (fusion) in HEK293T cells (IC\textsubscript{50} teicoplanin: 3,76±1.1 µM; dalbavancin; 9,64±1.3 µM) but not against free cathepsin L enzyme (IC\textsubscript{50} > 200 µM)\textsuperscript{30}. This discrepancy indicates a lysosomotropic effect of teicoplanin and dalbavancin. Similarly to lysosomotropic aSMase inhibitors\textsuperscript{16}, lysosomal enzymes are generally inhibited by the tested compounds only in intact cells\textsuperscript{30}. In contrast, the targeted free enzyme displays no inhibitory effects. Similarly to other lysosomotropic compounds, both active compounds are accompanied by pruritus, urticaria, and rash as undesired adverse effects, thus indicating lysosomotropotropic characteristics. Furthermore, oritavancin (IC\textsubscript{50} 4,96±1.2 µM) and telavancin (IC\textsubscript{50} 3.45 ±1.2) are very promising clinically approved glycopeptide antibiotics with similar characteristics\textsuperscript{30}. A unique feature of glycopeptide antibiotics is that, if they are used off-label as lysosomotropic compounds, they retain their initial adverse effect profiles. In off-label use, the benefit-risk profile is indistinguishable from that in authorized applications.

**Clinical benefits of lysosomotropic compounds in COVID-19 (severe cases)**

SARS-Cov-2 infection is likely to cause both pulmonary and systemic inflammation, thus leading to multi-organ dysfunction (e.g., acute respiratory distress syndrome (ARDS), myocarditis, septic shock, sepsis, sepsis after bacterial superinfection, acute liver injury, and hepatitis) in high risk populations\textsuperscript{7,45,46}. One study has found that among deceased individuals, 46 (41%) went into septic shock. Notably, there was no shock diagnosis in the
group of recovered patients. Sepsis, a dysregulated host response (organ dysfunction) to an infection, was diagnostically confirmed in all deceased individuals and was considered the cause of mortality in all cases investigated (113; 100%). However, 66 (41%) of patients who recovered were diagnosed with sepsis. Concentrations of IL2R, IL8, TNFα, and IL6 are significantly higher in deceased patients (72.0 (35.6–146.8) pg/mL in deceased and 13.0 (4.0–26.2) pg/mL recovered patients), similarly to the occurrence of sepsis, in which chemokines (including IL6, IL8, and IFNγ), CCL2, CCL3, and CXCL10, are involved in early stages. Emerging evidence implicates IL6 as a central mediator of toxicity in cytokine release syndrome (CRS); therefore, these findings suggest that a rapid, severe, and serious deterioration during SARS-CoV-2 infection is associated with CRS/cytokine storm syndrome.

The hypothesis that chloroquine, owing to overactivation of the immune system triggered by SARS-CoV-2 infection, is able to suppress the CRS/cytokine storm syndrome and to attenuate the transition from mild to severe is supported by the results of gene expression experiments with the model compound NB 06, in a setting addressing the effects of lysosomotropic compounds in LPS-induced inflammation monocytic cells. NB 06, like chloroquine, modulates the gene expression of the prominent inflammatory messengers IL1B, IL23A, CCL4, CCL20, and IL6; likewise, it has beneficial effects in (systemic) infections involving bacterial endotoxins, such as LPS, by targeting the TLR4 receptor pathway in sepsis. High expression of IL1B, CCL4, and CXCL10 has been observed in murine lung lobes infected with influenza A (H1N1) virus. These findings are consistent with the reported inhibitory effects of hydroxychloroquine on antigen processing and MHC class II presentation, interference with Toll-like receptor (TLR) signaling (TLR9 and TLR7), and inhibition of TNFα, IFNα, IL6, and CCL4 production. The host inflammatory response to an infection, via TLR4, can induce a cytokine storm, thus resulting in acute pulmonary inflammation. Targeting the cellular TLR4 signaling pathway and inflammatory cytokine production has been demonstrated to be successful in vitro as well as in vivo in TLR4-null mice. TLR4-null mice are highly resistant to infection with the mouse-adapted influenza A virus. In contrast, resatorvid (TAK-242), a small molecule TLR4 antagonist, does not suppress cytokine levels in patients with sepsis and shock or respiratory failure.

NB 06 and the clinically approved lysosomotropic compounds listed in Table 1 might therefore serve as valuable active compounds to prevent or mitigate the cytokine storm in the lungs after viral (SARS-CoV-2) infection of airway epithelial cells in COVID-19 (Fig. 2).
Local application of lysosomotropic compounds (off-label)

The respiratory tract is the gateway for SARS-CoV-2 infection and the primary affected site. Clinically approved active compounds generally have market authorization for tablets, capsules, or liquids, and are usually orally administered. Oral (systemic) application of lysosomotropic compounds is sometimes accompanied by severe adverse effects and/or (in this case) undesirable pharmacological effects. Because the lung is an internal surface, local application is possible, preferably as inhalate (powder or spray) or via nebulizers (liquids) to avoid systemic effects. Preparations of solutions for use in nebulizers can be performed in any hospital pharmacy. Inhalative application of the active compounds listed in Table 1 is off-label; no guidelines or empirical data are currently available. Appropriate concentrations for inhalation therefore should be determined experimentally. If necessary, an application of mixtures of two lysosomotropic compounds for different indications may be used to avoid undesired pharmacological effects.

Prospects for the success of inhalative application

Inhalation therapy might be a simple method for managing COVID-19 and preventing SARS-CoV-2 infection in vulnerable individuals and those with undesirable systemic pharmacological effects. Individuals with COPD, asthma, or chronic bronchitis are usually familiar with the handling of inhalation devices. Numerous available active ingredients with lysosomotropic characteristics permit tailor-made therapy (dosage and composition). If required, a combination with clinically approved active compounds for inhalation could be used.

Systemic application (i.v.) of lysosomotropic compounds (off-label)

The use of glycopeptide antibiotics, such as teicoplanin, as lysosomotropic compounds is not covered by market authorization and would therefore be off-label. The adverse effect and benefit-risk profile, however, correspond to the specifications of the market authorization.
Systemic application (oral) of lysosomotropic compounds COVID-19 (off-label)

Conventional systemic therapy with lysosomotropic compounds (e.g., terbinafine) is likewise feasible. If systemic use is considered, a careful individual risk-benefit analysis must be performed.

Benefit-risk profile of (inhalative) treatment in COVID-19

Obtaining an efficacious blood level after inhalation is unlikely; if an efficacious blood level is obtained, the applied active compound triggers intrinsic pharmacological effects. However, in certain circumstances, intrinsic pharmacological effects are undesirable adverse effects that are expected after lysosomotropic administration.

Because the application is temporary, no permanent alterations resulting from the lysosomotropic effects are expected. Well-known adverse effects, such as drug-induced (sphingo-)-lipidoses or lupus erythematosides, are reversible upon termination of application\textsuperscript{11–13}.

Hypothesis regarding SARS-CoV-2 carriers, spreaders and non-infectable humans

The lysosomes, and particularly cathepsin L, appear to play a key role in COVID-19 and in SARS-CoV-2 infection of host cells. To provide maximum cathepsin L activity and thus maximal cleavage capacity of viral S protein, properly functioning (endo)lysosomes are essential. If the lysosomal pH increases, cathepsin L activity diminishes, thereby decreasing the rate of cleavage, fusion, and infection.

Two scenarios can trigger an increase in lysosomal pH: a lysosomal proton pump breakdown or the administration/presence of lysosomotropic compounds (Fig. 3). To date, a breakdown of the vacuolar H\textsuperscript{+} ATPase (V-ATPase) cannot be triggered by clinically approved active compounds. There is evidence, however, that in various skin disorders (e.g., psoriasis vulgaris, atopic dermatitis, exanthematous pustulosis, and pustular psoriasis), the function of the V-ATPase is more or less restricted by ATP deficiency caused by oxidative stress in cells (keratinocytes)\textsuperscript{15}. Therefore, depending on the severity of the skin disease, individuals might not be infected (high ATP depletion and V-ATPase breakdown), whereas with moderate ATP depletion, individuals may have a much more
moderate course of disease. Infection (fusion) of the airway epithelial cells is then reduced or impossible.

Individuals receiving lysosomotropic active compounds in high dosages (e.g., sertraline (up to 200 mg/d), terbinafine (250 mg/d), paroxetine (up to 60 mg/d), or amitriptyline 150 mg/d (given as maximum daily doses of each active compound)) exhibit a drug-induced increase in lysosomal pH. Cathepsin L is inactivated by lysosomotropic compounds, and a significant decrease in viral load or no SARS-CoV-2 infection (fusion) of airway epithelial cells is expected.

Depending on the increase in lysosomal pH, individuals with these characteristics may have a lower risk or no risk of acquiring COVID-19, as compared with the risk of untreated or healthy individuals with intact (endo)lysosomes. The severity of the infection (viral load) may also be reduced in these individuals.

Conclusions

The COVID-19 pandemic is one of the greatest challenges in medicine and health care in recent decades. Various studies have demonstrated similarities between SARS-CoV-2 and SARS-CoV: both viruses utilize ACE2 and share the same pathway of infection. Thus, findings on SARS-CoV are at least partially extrapolatable to SARS-CoV-2. Encouraging results of in vitro experiments with hydroxychloroquine have suggested that lysosomotropic compounds might be used as tools in fighting SARS-CoV-2 infections, by potentially triggering a variety of cellular modifications that impede alveolar cell infection and viral replication in this context. Modulating the effects of lysosomotropic compounds on cytokines and interleukins is likely to provide a means of preventing the development of CRS and concomitantly the rapid, severe, and serious deterioration in COVID-19 / SARS-Cov-2 infection. Clinically approved lysosomotropic compounds are used in various medical applications and might be selected according to individual characteristics. With glycopeptide antibiotics such as teicoplanin, available lysosomotropic compounds may prevent both viral and secondary bacterial infections. Therefore, for all active lysosomotropic compounds intended to be used off-label, attention must be paid to the pharmacological effects of their common indications and, if necessary, the administration route should be switched to a local one (e.g., inhalation). Timely application might aid in preventing viral infections with SARS-Cov-2 (and influenza A) as well as severe
progression. Patients already receiving lysosomotropic compounds e.g., sertralinine, terbinafine, or trimipramine for pre-existing conditions at appropriate dosages might be protected against viral infections.

Evidence indicates that in patients with skin disorders, such as psoriasis or atopic dermatitis, the lysosomal pH is increased. Therefore, these individuals are likely to be poor vectors, because conveying infection may be difficult.

Findings from various scientific fields jointly support a model from which a treatment strategy may be developed. Considerations described here await further support by statistical analysis of patient data. If further confirmed, the results may contribute another option for preventing and treating COVID-19 and SARS-Cov-2 infections.

**Competing interests:**

The authors declare no competing financial interest.

**Author contributions**

M.B. conceived the work; M.B., L.K., M.S., and H.P.D. wrote the manuscript.

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References


Figure 1. Chemical structures of some clinically approved lysosomotropic compounds\textsuperscript{6,10,17,18}. (A) Tricyclic antidepressants amitriptyline, imipramine, and doxepine. (B) Selective serotonin reuptake inhibitor (SSRI) antidepressants sertraline, fluoxetine, and paroxetine. (C) Selective estrogen receptor modulator (SERM)/trigger of ovulation clomiphene, phenothiazine antipsychotic promethazine, antifungal terbinafine, and the model compound NB 06\textsuperscript{16}. (D) Antiprotozoals and antirheumatics chloroquine and hydroxychloroquine.
<table>
<thead>
<tr>
<th>Drug class</th>
<th>Lysosomotropic active compound</th>
<th>Maximum daily dose</th>
<th>Lysosomotropic activity in vivo expected</th>
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<td>Amitriptyline, Nortriptyline, Imipramine, Desipramine, Trimipramine, Doxepine, Protriptyline, Maprotiline</td>
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<td><em>Calcium channel blockers</em></td>
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Table 1. Variety of clinically approved lysosomotropic compounds for various indications. Achievement of the desired lysosomotropic effect depends on the active compound, the dosage, and accumulation in lysosomes. Unless indicated, specified maximum daily doses are split into three doses of medication per day. Classification of lysosomotropic compounds: lysosomal effect within the therapeutic margin ++ occurs at maximum daily dosage and very likely in low or initial dosage, + very likely at maximum daily dosage and possible in low or initial dosage, o possible at maximum daily dosage and unlikely in low or initial dosage, and – unlikely even at maximum daily dosage; availability: * withdrawn from the market (in most countries), ** veterinary use only; dosage: # single dose per day.

<table>
<thead>
<tr>
<th>Category</th>
<th>Compound</th>
<th>Dosage</th>
<th>Classification</th>
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<td>Ovulation inducers</td>
<td>Clomiphene</td>
<td>50 – 100 mg</td>
<td>++</td>
</tr>
<tr>
<td>Vasodilator</td>
<td>Suloctidil *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1-antihistaminics</td>
<td>Astemizole *</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyproheptadine</td>
<td>≤ 12 mg</td>
<td>o</td>
</tr>
<tr>
<td></td>
<td>Pimethixene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticholinergics (H1-antihistaminics)</td>
<td>Benztropine</td>
<td>≤ 6 mg</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cloperastine</td>
<td>≤ 20 mg</td>
<td>o</td>
</tr>
<tr>
<td>Therapeutic target</td>
<td>Objectives Therapy and Prophylaxis</td>
<td>Effects</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td><strong>1</strong> (Unspecific) inhibition of <em>cathepsin L</em> and other lysosomal enzymes</td>
<td>Increase of (endo)lysosomal pH</td>
<td>Inhibition of viral fusion with host cells and replication terminal glycosylation of ACE2</td>
<td></td>
</tr>
<tr>
<td><strong>2</strong> Modulating gene expression inflammation-relevant genes</td>
<td>Gene expression of viral inflammation-relevant genes</td>
<td>Prevention of cytokine release syndrome (CRS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gene expression of bacterial inflammation-relevant genes</td>
<td>Prevention of TLR4 related cytokine outburst that results in an acute pulmonary inflammation</td>
<td></td>
</tr>
<tr>
<td><strong>3</strong> Inhibition of stress related $C_{16}$-ceramide synthesis</td>
<td>Inhibition of substrate supply of reverse activity of lysosomal aCERase ($C_{16}$-ceramide generation)</td>
<td>Blocking synthesis of proapoptotic $C_{16}$-ceramide in lysosomes</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** Therapeutic targets of lysosomotropic compounds in SARS-CoV-2 infection and prophylaxis$^{9,10,16,28,30,36}$. 
<table>
<thead>
<tr>
<th>Therapy / Disorder</th>
<th>Effect</th>
<th>Infectable? Carrier?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> Disorder treatment with lysosomotropic compounds</td>
<td>Increase of (endo)lysosomal pH and (Unspecific) inhibition of cathepsin L and other lysosomal enzymes</td>
<td><strong>No</strong> (dose dependent) Viral fusion with host cells, replication, and terminal glycosylation of ACE2 is dose dependent reduced or blocked (?)</td>
</tr>
<tr>
<td><strong>2</strong> Skin disorders moderate and severe forms of Atopic dermatitis Psoriasis vulgaris</td>
<td>Irreversible inactivation of lysosomal vacuolar H⁺-ATPase (V-ATPase) (irreversible formation of disulfide bond between Cys 254 + Cys 532), (NAD(P)H) and ATP depletion Increase of (endo)lysosomal pH and inhibition of cathepsin L</td>
<td><strong>No</strong> (dependend on severity) Viral fusion with host cells, replication, and terminal glycosylation of ACE2 is severity dependent reduced or blocked (?)</td>
</tr>
<tr>
<td><strong>3</strong> Skin disorders moderate and severe forms of dyshidrotic dermatitis (eczema)</td>
<td>Irreversible inactivation of lysosomal vacuolar H⁺-ATPase (V-ATPase) in affected areas</td>
<td><strong>Yes</strong> Only restricted areas of hands and feet affected No airway epithelial cells affected (?)</td>
</tr>
</tbody>
</table>

**Figure 3.** Disorder treatments and skin disorders, and their potential effects on SARS-CoV-2 infection and the risk/probability of acting as a carrier according to the described model of the path of SARS-CoV-2 infection.