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The lysosome: A potential juncture between SARS-CoV-2 infectivity and Niemann-Pick disease type C, with therapeutic implications

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Abstract

Drug repurposing is potentially the fastest available option in the race to identify safe and efficacious drugs that can be used to prevent and/or treat COVID-19. By describing the life cycle of the newly emergent coronavirus, SARS-CoV-2, in light of emerging data on the therapeutic efficacy of various repurposed antimicrobials undergoing testing against the virus, we highlight in this review a possible mechanistic convergence between some of these tested compounds. Specifically, we propose that the lysosomotropic effects of hydroxychloroquine and several other drugs undergoing testing may be responsible for their demonstrated in vitro antiviral activities against COVID-19. Moreover, we propose that Niemann-Pick disease type C (NPC), a lysosomal storage disorder, may provide new insights into potential future therapeutic targets for SARS-CoV-2, by highlighting key established features of the disorder that together result in an "unfavorable" host cellular environment that may interfere with viral propagation. Our reasoning evolves from previous biochemical and cell biology findings related to NPC, coupled with the rapidly evolving data on COVID-19. Our overall aim is to suggest that pharmacological interventions targeting lysosomal function in general, and those particularly capable of reversibly inducing transient NPC-like cellular and biochemical phenotypes, constitute plausible mechanisms that could be used to therapeutically target COVID-19.

KEYWORDS

angiotensin-converting enzyme-2 (ACE2), cathepsins, cholesterol, COVID-19, lipid rafts, lysosomal storage diseases, pandemic

Abbreviations: 25-HC, 25-hydroxycholesterol; 7-KC, 7-ketocholesterol; ACE2, angiotensin-converting enzyme 2; ADAM17, disintegrin and metallopeptidase domain-containing protein 17, also known as TACE for Tumor necrosis factor (TNF)-alpha-converting enzyme; COVID-19, coronavirus disease, previously known as nCoV19 ("2019 novel coronavirus"); FCoV-I, feline infectious peritonitis-related coronavirus 1; HCoV-229E, human coronavirus-229E; LSD, lysosomal storage disease; MERS-CoV, middle east respiratory syndrome-related coronavirus; NPC, Niemann-Pick disease type C; ROS, reactive oxygen species; SARS-CoV, severe acute respiratory syndrome-related coronavirus; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2; SP-A, surfactant protein-A; TMPRSS2, transmembrane serine protease 2.

1 | THE SARS-COV-2 INFECTION CYCLE REQUIRES INTACT LYSOSOMAL FUNCTION

SARS-CoV-2 is the newly emergent coronavirus implicated in the current COVID-19 pandemic that appeared first in Wuhan, Hubei province, China, in late December 2019.[1] Shortly after its discovery, SARS-CoV-2 was shown to carry nearly 80% sequence homology to SARS-CoV, another member of the coronaviridae family that was responsible for the SARS epidemic in 2002-2003.[2,3] Since then, SARS-CoV-2 has increasingly been viewed as molecularly "similar" to SARS-CoV, especially in regards to carrying a similar spike (S) protein in its envelope, which mediates viral binding to host cells.[1,3] Specifically, the S protein docks the viral particle onto angiotensin-converting enzyme 2 (ACE2),[4] a membrane protein particularly abundant in the plasma membrane of type II pneumocytes.[5,6] Upon binding ACE2 via its S protein, SARS-CoV-2 gets internalized and undergoes intracellular trafficking within endosomes, which eventually fuse with mature lysosomes, a requirement for viral uncoating and fusion (Figure 1A).[7-9]

However, after binding ACE2, but prior to undergoing internalization, the S protein in the viral envelope must undergo enzymatic activation, formally referred to as "priming", by the transmembrane serine protease 2, TMPRSS2, a membrane-bound enzyme that resides within the vicinity of ACE2 in lipid rafts.[10-13] This TMPRSS2-mediated cleavage of the S protein has been shown to constitute an important prerequisite for the endocytic entry of SARS-CoV-2 into the host cell, a process that is abrogated when cells are treated with selective inhibitors of TMPRSS2.[10,12,14,15]

Within the lysosome, the S protein undergoes another series of enzymatic cleavages and modifications, mediated primarily by cathepsin L, and to a lesser degree, by cathepsin B, which are crucial for viral membrane fusion and subsequent release of the viral RNA genome into the host cytoplasm.[9,16,17] It is important to note that the aforementioned modifications are highly pH-dependent, such that the fusion of the viral envelope can only occur after the endocytosed viral particle has reached a highly acidic intracellular compartment, namely the lysosome, which possesses the highest intracellular concentration and activity of cathepsin L.[7] In support of this notion, several lysosomotropic agents, that is, compounds that interfere with normal lysosomal fusion or function, have been shown to interfere with the intracellular processing of key proteins within the viral envelope that mediate fusion, thereby halting viral infectivity.[8] In fact, SARS-CoV entry into host cells has been shown to be prevented by pre-treating target cells with lysosomotropic agents.[15]

Thus, it is reasonable to propose that, shedding ACE2 so as to reduce its presentation at the host cell membrane, disrupting its internalization, and/or interfering with its trafficking upon binding SARS-CoV-2 and subsequent cleavage within the lysosomes,[18] constitute three possible points of therapeutic intervention against COVID-19. However, testing either of these therapeutic strategies may be time-consuming and complicated, especially in the absence of an "appropriate" or representative physiological model. But, what if, there is already an existing cellular model that possesses these characteristics, that is, reduced ACE2 presentation and internalization, and impaired trafficking and fusion of ACE2-bound SARS-CoV-2 particles in the lysosomes, added to several other intracellular biochemical characteristics that create an "unfavorable" host cell environment to SARS-CoV-2?

Here, we hypothesize that the inherent cellular and biochemical abnormalities of lysosomal storage diseases (LSDs) in general, and Niemann-Pick disease type C (NPC) in particular, create possibly "unfavorable" environments for SARS-CoV-2 infectivity in the corresponding host cells (Figure 1B). Moreover, the fact that lysosomal proteases are the key mediators of coronavirus tropism and infection rates in bats, the natural reservoir of these viruses,[19] supports the importance of investigating the lysosome as a potential therapeutic target of intervention against COVID-19.

2 | NPC-RELATED PERTURBATIONS IN ACE2 AND ADAM17 INTERFERE WITH VIRAL INTERNALIZATION

The ACE2 protein has been previously shown to reside mainly within the lipid raft domains of the plasma membrane, which are tightly packed and cholesterol-enriched microdomains within the lipid bilayer.[20] Because they mainly consist of cholesterol and sphingomyelin, along with several other sphingolipid species, these plasma membrane microdomains (ie, lipid rafts) are easily perturbed by intrinsic or extrinsic processes that affect the synthesis and/or intracellular trafficking of either lipid species.[21] As a result, lipid rafts are disrupted in NPC, a condition notable for having impaired intracellular trafficking of both, cholesterol and sphingomyelin,[22] due to autosomal recessive mutations in *NPC1* or *NPC2* (90% and 4%, respectively) that result in the entrapment and accumulation of cholesterol and various sphingolipids within late endosomes and lysosomes.[23] Consequently, the partitioning of various proteins to lipid rafts, which is often a requirement for their normal functionality, is disrupted in NPC.[24] Specifically, Lusa et al, followed by Garver et al, have each independently shown that NPC cells have reduced numbers of lipid rafts in their plasma membrane, with the available ones being relatively cholesterol-depleted, compared with those of wild-type (ie, healthy) cells.[25,26] Moreover, membrane proteins that normally reside within these domains

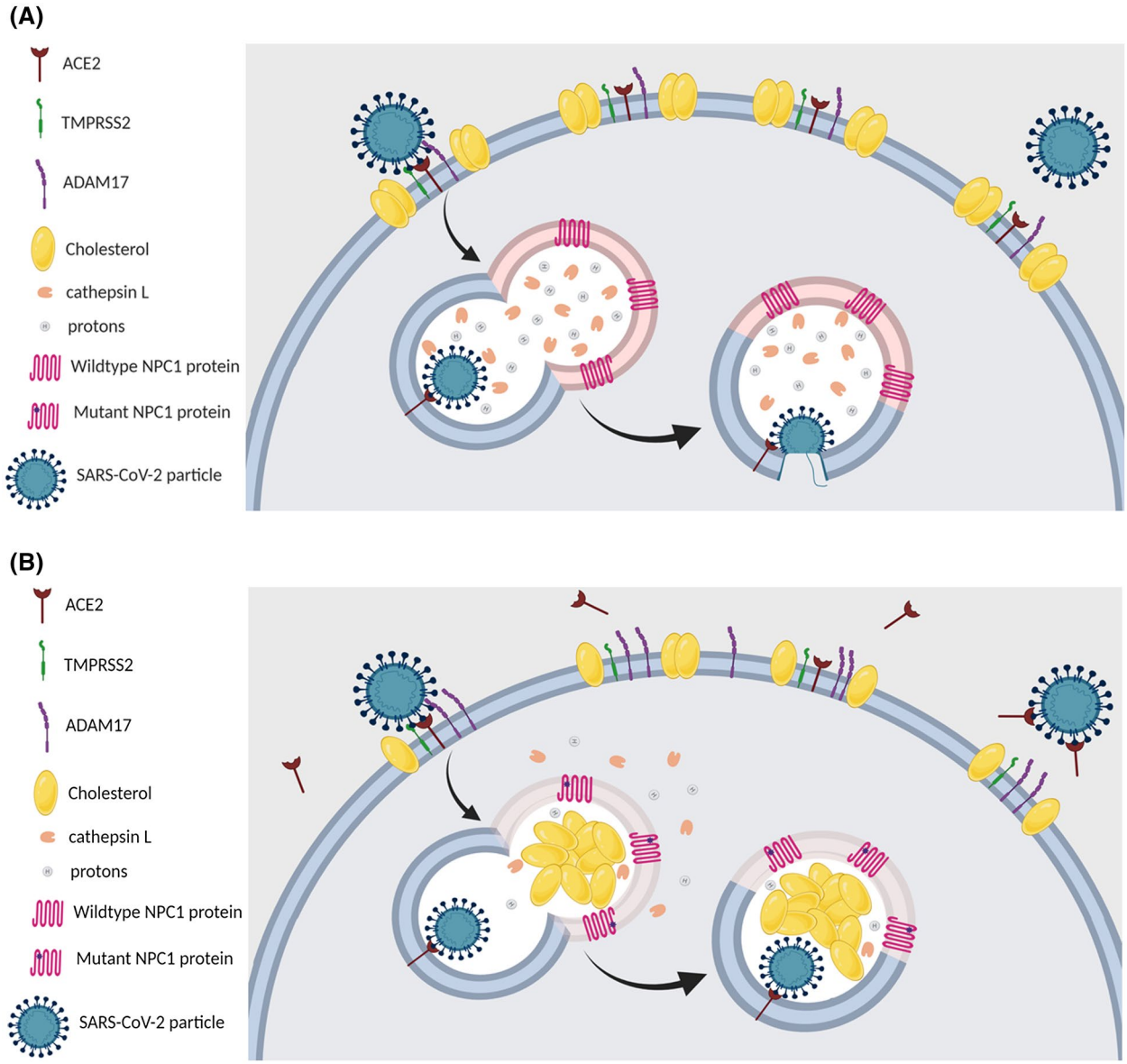

FIGURE 1 An illustrative diagram showing SARS-CoV-2 entry into wild-type vs NPC1-deficient cells. In wild-type human cells, SARS-CoV-2 binds via its spike (S) protein to ACE2 on the host cell plasma membrane. This complex subsequently undergoes proteolytic modification by TMPRSS2, which facilitates its endocytosis. The plasma membrane containing the ACE2-bound viral particle invaginates intracellularly, releasing an endosome containing the viral particle that is then transported to fuse with a lysosome. Upon fusing, the lysosome releases cathepsin L into the endosome containing the viral particle, which proteolytically activates certain proteins within the viral envelope, allowing viral fusion and release of the viral RNA genome into the host cytoplasm to occur (Figure 1A). In contrast, SARS-CoV-2 entry and infection of NPC1-deficient cells is negatively affected at several stages:

1. The reduced number and cholesterol-depleted nature of lipid rafts in the plasma membrane of NPC cells influence the stability of ACE2 and TMPRSS2 which reside within these membrane domains.
2. The NPC-related increase in plasma membrane levels of ADAM17 induces increased the shedding of ACE2, which hinders viral attachment/docking to host cells.
3. The NPC-related abnormalities in the localization and activities of cathepsin L would blunt the chances of a successful viral fusion, after the endosome carrying the viral particle fuses with the NPC1-deficient lysosome.
4. The elevated levels of the antiviral oxysterols 25-HC and 7-KC in NPC cells, also impede viral fusion and subsequent replication.

Altogether, these NPC-related intracellular aberrations may reduce the likelihood of successful entry, trafficking, and fusion of SARS-CoV-2 in NPC cells (Figure 1B)

have also been shown to exhibit higher affinity for cholesterol within the membranes of NPC cells, which is believed to at least partially account for their altered functions/activities in NPC.[24] These NPC-related alterations in lipid raft numbers and reduced cholesterol composition would, therefore, be expected to adversely impact both, the presentation and subsequent internalization and trafficking of the raft-resident protein ACE2, which in turn, would impede SARS-CoV-2 entry and subsequent infection of host cells. Moreover, it has also been shown that membrane cholesterol levels play an important role in the S protein-mediated binding of SARS-CoV to ACE2 in the membrane of host cells, a model that finds its support in the previous work of Glende et al, who showed that cyclodextrin-induced cholesterol-depletion of lipid rafts in the plasma membrane abolishes the S protein-mediated binding of SARS-CoV particles to ACE2, reducing viral infectivity in a dose-dependent manner.[27] Moreover, while it is not known whether SARS-CoV requires a functional NPC1 protein to proceed through its infectious cycle as the Ebola virus does for instance,[28] it has been shown that the intracellular trafficking of the SARS-CoV particle to NPC1-positive compartments of the endo-lysosomal system is important for establishing successful infection.[7] Therefore, SARS-CoV-2, which has a similar infectious life cycle as SARS-CoV would also be expected to require entry into NPC1-positive subcellular compartments, that is, late endosomes and lysosomes, in order for it to successfully establish infection.[7]

Importantly, the same cells notable for their high expression of ACE2 and therefore highly susceptible to infection by SARS-CoV-2, that is, the type II pneumocytes,[4,6] also rely heavily on the functionality of the NPC1 and NPC2 proteins. The latter play key roles in modulating the lipid composition of the predominant secretory product of these cells, that is, pulmonary surfactant.[29,30] In fact, NPC1-deficient type II alveolar cells contain enlarged lipid-rich lamellar bodies within their cytoplasm, such that the surfactant these cells secrete is particularly abundant in cholesterol and surfactant protein A (SP-A), a notable endogenous antimicrobial peptide with potent antiviral activities.[30-34] Thus, type II pneumocytes deficient for the NPC1 protein would be expected to not only be "altered" and possibly less recognizable to SARS-CoV-2 particles, but the SP-A-enriched pulmonary surfactant that they secrete may pose another barrier that precludes SARS-CoV-2 attachment/binding to these pneumocytes.

In contrast to TMPRSS2, which plays an important role in inducing key conformational changes in the S protein that promote the endocytic entry of the viral particle,[35] ADAM metallopeptidase domain 17 (ADAM17), also known as TACE (tumor necrosis factor-α-converting enzyme), competes with TMPRSS2 to counteract its viral entry-promoting role. Specifically, by inducing the shedding of ACE2, ADAM17 prevents TMPRSS2 from modifying ACE2 in the manner required for viral entry. In addition, the shed ACE2 avidly binds to the S protein in the envelope of SARS-CoV-2 particles, thereby preventing their attachment to host cells. ADAM17 activity may, therefore, reduce the likelihood for entry of SARS-CoV-2 particles into host cells.[36] Intriguingly, both membrane proteases, TMPRSS2 and ADAM17, partition primarily to the detergent-resistant membrane domains, that is, lipid rafts of the plasma membrane.[13,37] While no formal evaluation for the expression and/or activity level of TMPRSS2 has been reported in NPC, it is suggested that the plasma membrane levels of ADAM17 are elevated in NPC,[38-40] presumably due to the previously mentioned NPC-related alterations in lipid rafts. As a result, it is possible to speculate that this elevated level of ADAM17 in the plasma membrane of NPC cells increases ACE2 shedding and counteracts TMPRSS2-mediated entry-favoring modifications, which grants these cells an increased "protection" against the viral binding.

3 | NPC-INDUCED ABERRATIONS IN CATHEPSIN LOCALIZATION AND ACTIVITY INTERFERE WITH VIRAL FUSION

In addition to their defective egress of intra-lysosomal cholesterol, NPC cells are also known to have impaired localization and activities of various lysosomal enzymes, including cathepsins L and B, a finding also reported in other LSDs.[41,42] Specifically, the chronic accumulation of various substances within the lysosomes of NPC cells, with sphingomyelin and sphingosine being notable examples of these substances, has been found to disrupt the integrity of the lysosomal membrane, leading to the leakage of several lysosomal enzymes, such as cathepsins, into the cytosol.[41,43-45] Other studies have also shown direct inhibitory effects of the lipids accumulating in NPC toward lysosomal cathepsins.[46] Moreover, the same lipid substrates that accumulate within the lysosomes of LSD cells, have also been shown to disrupt normal acidification of the lysosomes, thereby increasing the intra-lysosomal pH and adversely affecting the activities of enzymes within those lysosomes.[47-50] In fact, these substrates have also been found to simultaneously impede normal fusion of vesicles transporting lysosomal cargo, including enzymes, such as cathepsins, into the lysosome. This further depletes the intra-lysosomal stores of key hydrolytic enzymes.[41,47,51] Thus, it is also reasonable to speculate that these NPC-related aberrations in lysosomal enzyme localization, transport into the lysosomes, and intracellular activity levels, especially those of cathepsins B and L, pose additional "barriers" in NPC that could prevent the trafficking and intracellular processing of viral membrane proteins, a step that is required for successful viral fusion. Therefore, it may also be worth testing various inhibitors of cathepsins B and L, such as recombinant cystatins or stefins,

for their therapeutic potential against SARS-CoV-2 infection, given their ability to induce an NPC-resembling lysosomal dysfunctional state.[17,18,52]

4 | THE OXYSTEROLS THAT ACCUMULATE IN NPC POSSESS POTENT ANTIVIRAL ACTIVITIES

Among the several oxysterols that accumulate in NPC, two are particularly worth highlighting, given their potential relevance to COVID-19, namely 7-ketocholesterol (7-KC) and 25-hydroxycholesterol (25-HC).[53] Both 7-KC and 25-HC have been previously reported to possess potent antiviral activities, against various viral families.[54-57] Specifically, elevated intracellular levels of 25-HC, have been previously shown to reduce infectivity by several members of the coronaviridae, filoviridae (eg, Ebola virus), and flaviviridae (eg, Zika and hepatitis C viruses).[54-57] Similarly, increased intracellular levels of 7-KC have been shown to interfere with viral maturation, and subsequent budding and release from host cells.[58] While the precise mechanism(s) underlying their antiviral activities remain a subject of ongoing debate, these oxysterols have been shown to perturb the normal trafficking of cholesterol, such that they replace the latter within the lipid rafts, altering their properties, which impairs subsequent viral entry, endocytic transport of viral particles, and their eventual fusion intracellularly.[56,59]

5 | THE LYSOSOMOTROPIC ACTIVITIES OF DIFFERENT DRUGS UNDERGOING TESTING AGAINST COVID-19

Multiple available drugs are undergoing repurposed testing to evaluate their safety and efficacy against COVID-19, in an attempt to expedite drug discovery and approval for use in treating patients with COVID-19.[60] In what follows, we discuss a subset of these drugs currently being tested for COVID-19, highlighting some of their lysosomotropic effects.

5.1 | Chloroquine ± Azithromycin

Among the various repurposed drugs currently under investigation for the treatment of COVID-19, chloroquine and its derivatives, such as hydroxychloroquine, emerged as the first potentially efficacious existing drug for COVID-19, based on documented pre-clinical efficacy and expert consensus by several Chinese scientific authorities.[61-63] As a result, there has been cumulative interest in testing the efficacy of chloroquine and its derivatives, in the treatment of COVID-19, such that there are now, over 20 different related clinical trials in trial registries.[64] Additionally, two different phase III clinical trials are currently investigating the use of hydroxychloroquine for pre- and post-exposure prophylaxis against COVID-19 in healthcare workers (NCT04303507 and NCT04328285). However, it remains unclear how an antimalarial drug-like chloroquine could also exert antimicrobial activity against a viral pathogen-like SARS-CoV-2, raising the question of whether the same mechanism underlying chloroquine's antimalarial activity may also be responsible for its antiviral effects.

Importantly, chloroquine has been used for many years in lysosomal storage disease (LSD) research, given its ability to inhibit lysosomal fusion with endosomes, as well as inhibit the activity of various lysosomal enzymes. These properties of chloroquine allowed it to be used in vitro to pharmacologically induce transient LSD-like cellular pathology.[65-67] In fact, this lysosomotropic activity of chloroquine is what is thought to be responsible for its anti-malarial mode of action. Specifically, chloroquine is believed to undergo trafficking into the lysosomes of *Plasmodium* trophozoites, where it gets protonated and entrapped, thereby disrupting the fusion of these lysosomes with the "food vacuoles" (ie, phagosomes) of the trophozoites, hampering the latter's ability to feed on engulfed red blood cells.[68,69] However, this same propensity of chloroquine to traffic into, and concentrate within intracellular acidic organelles, also cross-reacts with mammalian cells, inducing similar disruptions in the functions of their lysosomes as the ones it induces for protozoal food vacuoles, that is, interfering with endo-lysosomal fusion, elevating intra-lysosomal pH, and inducing partial permeabilization of lysosomal membranes, which altogether mirror the lysosomal pathology intrinsic to several LSDs.[65,69,70]

Such lysosomal "disruptions" are actually intrinsic to NPC in particular, as previously discussed, which further supports the possibility of a lysosome-mediated antiviral activity for chloroquine against SARS-CoV-2, since chloroquine is capable of inducing transient NPC-like lysosomal abnormalities that may interfere with intracellular viral trafficking and fusion.

In support of this hypothesis, previous studies have successfully shown that the antiviral activity of chloroquine against several caliciviridae, another family of RNA viruses, occurs through chloroquine's ability to inhibit cathepsin L.[71] Furthermore, chloroquine also inhibits the transport of cholesterol out of the lysosomes, including to the plasma membrane, which would be expected to reduce the abundance, and alter the composition of membrane rafts,[72] thereby mimicking the raft alterations seen in NPC. Additionally, chloroquine has also been shown to interfere with the trimming of the N-glycosylated side chain of ACE2, which may affect the internalization of ACE2, and subsequently, viral entry.[73]

Interestingly, N-glycosylation has actually been shown to be altered in NPC,[74] further suggesting that NPC cells likely possess inherent chloroquine-like effects of altered N-glycosylation modification of ACE2, which potentially further offers these cells with "protection" against SARS-CoV-2 infection. In that regard, a small open-label non-randomized clinical trial conducted in France (EU CTR 2020-000890-25) has recently gained considerable interest, after it showed a statistically significant difference in the rates of SARS-CoV-2 viral clearance from the nasal swabs of COVID-19-positive patients receiving a combination of hydroxychloroquine and azithromycin, the latter being a macrolide antibiotic, compared with those receiving hydroxychloroquine only ($P = .002$ at Day 3 post-inclusion), or no antimicrobial therapy whatsoever ($P = .005$ at Day 3 post-inclusion).[75] In this context, it is also important to highlight the lysosomotropic activity of azithromycin itself, the drug combined with hydroxychloroquine in that trial, as an *add-on* therapy.[75] Similar to chloroquine or its derivatives, azithromycin also undergoes trafficking to, and accumulation within the lysosomes, where it alkalinizes the luminal pH of these organelles, thereby inhibiting the activity of resident enzymes.[76] In fact, the combination of both drugs, chloroquine and azithromycin, has been previously shown to exhibit synergistic lysosomotropic effects, especially with regards to increasing lysosomal pH.[76] Moreover, chronic azithromycin treatment in patients with cystic fibrosis has been shown to increase susceptibility to mycobacterial infections, which usually rely heavily on adequate phagocytosis and bacterial containment within phagosomes.[77] Azithromycin has been particularly shown to block the lysosome-mediated acidification of phagosomes containing the mycobacteria, allowing the latter to escape the phagosomes and multiply uncontrollably.[77] However, in contrast to mycobacteria where the intact lysosomal function is required to contain/control the infection,[78,79] in SARS-CoV-2 infections, the intact lysosomal function is actually needed for successful viral fusion and establishment of infection, as discussed earlier. Thus, it is possible that the observed synergistic efficacy of azithromycin combination with hydroxychloroquine, in the treatment of SARS-CoV-2, is the result of their similar lysosome-mediated antiviral activities, that is, their independent inhibition of endosomal-lysosomal fusion and lysosomal proteases, which are key for successful viral fusion. In addition, azithromycin's tropism toward the lysosomes has also been shown to induce an accumulation of neutral lipids, namely free cholesterol and phospholipids, within these organelles,[80,81] which phenocopies the "natural" cellular phenotype of NPC cells.[23]

5.2 | Remdesivir

Another promising drug with documented pre-clinical efficacy against COVID-19, and undergoing testing in multiple different phase III clinical trials, is remdesivir (NCT04292899, NCT04292730, etc), an adenosine analog originally developed to treat Ebola.[61,82] However, besides its efficacy against the Ebola virus, remdesivir has been shown to possess remarkable in vivo antiviral activity against several members of the coronaviridae family, including the feline coronavirus that is implicated in feline infectious peritonitis type I (FCoV-I),[83] as well as the middle east respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV,[84,85] two human coronaviruses that belong to the same genus as SARS-CoV-2, that is, the beta coronaviruses.[86]

Interestingly, infection by FCoV-I, the coronavirus species showing the greatest response to remdesivir treatment, was inhibited in host cells pre-treated with U18666A, a well-known direct pharmacological inhibitor of the NPC1 protein that is used to induce NPC-like lysosomal dysfunction, with a resultant lysosomal accumulation of cholesterol and sphingolipids.[87,88] This raises the possibility of a mechanistic convergence or synergy, albeit being a partial one, between the U18666A-mediated direct inhibition of NPC1, and remdesivir's demonstrated activity against SARS-CoV-2, besides its primary mechanism of action being the inhibition of the viral RNA-dependent RNA polymerase.[89] We, therefore, propose evaluating the antiviral potential of U18666A against SARS-CoV-2, given its capacity to induce an NPC cellular phenocopy, as an easy starting point to support our hypothesis. Although the safety of U18666A has not been established yet in humans, it has been shown to be without major toxicity in at least two animal models, that is, rats and cats.[88,90]

In addition, various adenosine analogs, especially the ribose-modified subtypes such as those used as antivirals or antineoplastic agents, have been shown to possess cross-binding and activation or inhibition capacities for adenosine receptors,[91] with different affinities toward the A1 and A2a adenosine receptor types.[92,93] The reason for mentioning this is to highlight that stimulating A2a receptors has been previously shown to rescue the cholesterol entrapment seen in NPC, with that effect being abolished by A2a antagonism.[94] Thus, despite a current lack of data on remdesivir's ability to bind and subsequently, activate or inhibit any of the adenosine receptors, it is worth entertaining the possibility that, given its structural similarity to adenosine, remdesivir may potentially act as an A2a receptor antagonist, causing it to induce a transient NPC-like cellular environment as part of its antiviral activity against SARS-CoV-2. In fact, several adenosine analogs have been previously shown to directly inhibit lysosomal activity in neutrophils,[95] as well as compete with intracellular adenosine on binding adenosine deaminase, an intra-lysosomal adenosine-metabolizing enzyme,[96] leading to the accumulation of adenosine within the lysosomal lumen[97] and inducing an LSD-like cellular pathology with impaired regulation of lysosomal calcium stores.[98] Such features have all been previously shown to be part of the cytopathological findings in NPC.[99]

5.3 | Triazoles

Another class of drugs that is being considered for testing against COVID-19, is the triazole antifungals,[100] namely posaconazole, itraconazole, and their modified derivatives. This is based on previous studies that have demonstrated potent antiviral activities for these compounds, against a wide array of coronaviridae members, including FCoV-I, human coronavirus-229E (HCoV-229E), MERS-CoV, and SARS-CoV.[101-105] While triazoles are widely used as antifungal agents owing to their inhibition of ergosterol synthesis, which is an essential component of fungal cell membranes, they have also been shown to possess antiviral activities that were attributed to their differential inhibitory actions on viral helicases, including coronavirus helicase, thereby interfering with viral replication.[101,104] Interestingly, however, triazole antifungals are also notable for their cross-interference with mammalian cholesterol homeostasis, including both cholesterol synthesis and trafficking, due the molecular resemblance between cholesterol and ergosterol.[106] In fact, triazoles have been actually shown to inhibit lysosomal cholesterol efflux, through directly binding to, and inhibiting the activity of NPC1 within the lysosomal membrane.[107,108] Thus, besides their inhibition of viral helicase, which would interfere with SARS-CoV-2 replication, the predicted antiviral activities of triazole antifungals against SARS-CoV-2 could also be mediated by their direct interference with lysosomal cholesterol egress and its eventual trafficking to the plasma membrane and membrane rafts, via NPC1-targeted inhibition.

5.4 | Glycopeptides

An additional class of drugs undergoing testing against COVID-19 is the glycopeptide antibiotics, primarily vancomycin, teicoplanin, and their modified derivatives.[100,109] In vitro studies evaluating the antiviral potentials of these compounds against SARS-CoV-2 have successfully demonstrated their efficacy in blocking viral entry into host cells.[110] Speculations of a possible antiviral efficacy exhibited by these compounds against SARS-CoV-2 were based on their previously demonstrated activities against several members of the coronaviridae family, including FCoV-I, SARS-CoV, and MERS-CoV.[111,112] Unlike their established anti-bacterial activity that is mediated by their inhibition of cell wall synthesis, the antiviral activity of glycopeptide antibiotics is attributed to their various intracellular effects on host cells; these mainly include their direct inhibition of cathepsin L [110,112] and their trafficking to, and accumulation within the lysosomes, such that they "overload" the latter organelles.[113,114] Additionally, glycopeptide antibiotics have also been shown to induce increased ROS production within eukaryotic cells, which arguably may not be necessarily relevant for viral killing as they normally are for bacterial killing.[115] However, such findings further support our hypothesis, because NPC cells not only have an already reduced cathepsin L activity and overloaded lysosomes, but they also have elevated baseline levels of ROS intracellularly.[116] These combined intracellular effects of glycopeptides, therefore, create intracellular characteristics that mimic those intrinsic to NPC, which possibly accounts for their demonstrated antiviral activity against SARS-CoV-2. It is, however, worth mentioning that within the NPC patient community, several patients receiving glycopeptide antibiotics to treat hospital-acquired pneumonia were subsequently found to develop focal pyogenic skin abscesses, whose underlying mechanism remains unclear (unpublished data). This anecdotal yet consistent finding could be due to the lysosomal accumulation of glycopeptide antibiotics, and their disruption of lysosomal function,[113,114] which causes these drugs to further burden the already overloaded lysosomes in NPC cells, including phagocytes, thereby hindering the latter's ability to destroy engulfed debris, resulting in abscess formation.[78,117]

5.5 | Cepharanthine

Finally, another repurposed drug being tested against COVID-19 is cepharanthine, a plant-derived alkaloid with prominent anti-inflammatory effects.[118] In fact, cepharanthine demonstrated the highest potency among 2406 different clinically approved drugs that were screened against COVID-19, with preclinical data suggesting it targets the entry of SARS-CoV-2.[118] Interestingly, cepharanthine has also been shown to undergo intracellular trafficking to the lysosomes, where it physically interacts with and inhibits the NPC1 protein, resulting in lysosomal cholesterol accumulation and elevated intra-lysosomal pH.[119] It is, therefore, possible that cepharanthine's exhibited activity against SARS-CoV-2 is mediated, at least partially, by its lysosomotropic effects of directly inhibiting the NPC1 protein and inducing a cellular phenocopy of NPC.

6 | CONCLUSION

To summarize, this report raises the hypothesis that the intracellular biochemical abnormalities inherent to LSDs in general and NPC in particular, may pose an "unfavorable" host cell environment for the entry, trafficking, and fusion of SARS-CoV-2. Specifically, we postulate that the altered composition of the plasma membrane and lipid rafts in NPC may affect the trafficking of ACE2, the primary host cell membrane receptor responsible for viral docking, thereby interfering with viral infection. Moreover, the increased levels of ADAM17 in the plasma membrane of NPC cells

promote ACE2 shedding, thereby inhibiting viral docking at the plasma membrane of host cells. Additionally, the NPC-related lysosomal membrane permeabilization, which leads to cathepsin L leakage, and the increased intra-lysosomal pH seen in NPC, impair the activity of cathepsin L, a key protease required for the successful fusion of SARS-CoV-2. Furthermore, we highlight how two key oxysterols whose levels are notably elevated in NPC, 25-HC, and 7-KC, possess potent antiviral activities, which further grants NPC cells the characteristic of being an unfavorable host cell environment for successful SARS-CoV-2 infectivity. We also discuss how the different repurposed drugs demonstrating preliminary efficacy in the treatment of COVID-19 (chloroquine, azithromycin, remdesivir, triazoles, glycopeptide antibiotics, and cepharanthine) possess lysosomotropic activities, which we propose as being the unifying mechanism underlying their demonstrated and shared antiviral activity against SARS-CoV-2. Overall, we propose that pharmacologically targeting one or more of the metabolic facets that comprise the NPC cellular phenotype, may prove beneficial in identifying and rapidly developing treatments for COVID-19 (Figure 1).

Finally, it is important to note that most of the evidence we present here in support of a role of the lysosomes in SARS-CoV-2 infectivity is mainly circumstantial and inferred from studies originally designed to test other hypotheses. Thus, going forward, it will be important to test some of our proposed connections between LSD-related lysosomal dysfunction, especially those pertaining to NPC, and reduced SARS-CoV-2 infectivity, for example, by comparing the rates of successful infection of human NPC vs wild-type cells, following incubation with labeled SARS-CoV-2 pseudovirions. Alternatively, it would also be interesting to compare infection rates in wild-type pneumocytes before and after the transient induction of an NPC cellular phenotype, for example, via U18666A treatment or treatment with other lysosomotropic agents. It would also be important to establish the safe yet efficacious dosage ranges for the different lysosomotropic compounds discussed here, that would be capable of sufficiently preventing SARS-CoV-2 infection. Such information will be key for determining the precise mechanisms of action underlying the demonstrated antiviral activities of the different agents undergoing testing against COVID-19, which may expedite drug design and development for proper therapeutic targeting of this pandemic. Ultimately, however, large-sized, randomized, and double-blinded controlled clinical trials are needed to determine the safety and efficacy of any drug that may be used in the future for COVID-19, including for the various agents/compounds we described here.

CONFLICT OF INTEREST

All authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

AUTHOR CONTRIBUTIONS

R.A. Ballout conceived and researched the hypothesis proposed in this manuscript and drafted the entire preliminary version of it. A.T. Remaley revised and edited the first draft, and with R.A. Ballout, added a conclusion section. A.T. Remaley then consulted a virologist (M. Bukrinsky), and an expert in intracellular lipid metabolism and lipid rafts (D. Sviridov), for them to revise, update and edit the sections of the draft falling within their areas of expertise. R.A. Ballout consulted with two outside NPC experts, who revised and edited the draft, shared some valuable reference with R.A. Ballout, and helped in updating the figure. However, they opted to remain unknown at their own discretion, after we offered them co-authorship status, or at least, to acknowledge them for all their efforts.

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