The lysosome: A potential therapeutic juncture between the COVID-19 Pandemic and Niemann-Pick type C disease

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List of Abbreviations

NPC: Niemann-Pick type C disease

ACE2: Angiotensin-converting enzyme 2

TMPRSS2: Transmembrane serine protease 2

ADAM17: Disintegrin and metallopeptidase domain-containing protein 17, also known as TACE for Tumor necrosis factor (TNF)-alpha converting enzyme

SARS-CoV: Severe acute respiratory syndrome-related coronavirus

MERS-CoV: Middle east respiratory syndrome-related coronavirus

FCoV-I: Feline infectious peritonitis-related coronavirus 1

COVID-19: Coronavirus disease; previously known as nCoV19 (“2019 novel coronavirus”)

SARS-CoV-2: Severe acute respiratory syndrome-related coronavirus 2

HCoV-229E: Human coronavirus-229E

LSD: Lysosomal storage disease

ROS: Reactive oxygen species

7-KC: 7-ketocholesterol

25-HC: 25-hydroxycholesterol

SP-A: Surfactant protein-A

Abstract

In the face of the newly emergent COVID-19 pandemic, researchers around the world are racing to identify efficacious drugs capable of preventing or treating its infection. They are doing that by testing already available and approved antimicrobials for their rapid repurposing against COVID-19. Using the data emerging on the comparable efficacy of various compounds having different mechanisms of action and indications, I suggest in this report, their potential mechanistic convergence. Specifically, I highlight the lysosome as a key possible therapeutic target for COVID-19, proposing one of the lysosomal storage disorders, Niemann-Pick type C disease (NPC), as a prototypical condition with inherent resistance or an "unfavorable" host cell environment for viral propagation. The included reasoning evolves from previously generated data in NPC, along with the emerging data on COVID-19. The aim of this report is to suggest that pharmacological induction of a "transient" NPC-like lysosomal dysfunction, could hold answers for targeting the ongoing COVID-19 pandemic.

Keywords: *Coronavirus, SARS-CoV-2, lysosomal storage diseases, lipid rafts, cholesterol, angiotensin-converting enzyme-2 (ACE2), cathepsins.*

What is known about SARS-CoV-2

SARS-CoV-2, the newly emergent coronavirus implicated in the current COVID-19 pandemic, was first discovered in Wuhan city in China, in late November 2019 [1]. Following its identification, SARS-CoV-2 was found to have around 80% sequence homology with 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 been treated as being "similar" to SARS-CoV at the molecular level, and underlying infectivity process. Specifically, SARS-CoV-2 carries the same spike (S) protein in its envelope, as SARS-CoV virus, which mediates viral binding and subsequent entry into host cells [1, 3]. The S protein mediates viral binding to the angiotensin-converting enzyme 2 (ACE2) receptor [4], a transmembrane protein that is particularly abundant in the plasma membrane of type II pneumocytes [5, 6]. Upon binding ACE2, SARS-CoV-2 undergoes internalization and trafficking into the endosomes and eventually, the lysosomes, which constitute key intracellular players in viral uncoating and fusion [7-10].

However, after binding to ACE2 and prior to internalization, the S protein is subject to enzymatic modification by the transmembrane serine protease 2 (TMPRSS2), a membrane protein residing in the vicinity of ACE2 [11, 12]. Specifically, TMPRSS2 induces a cleavage-mediated conformational changes in the S protein, as well as in ACE2, which allows the host cell membrane to invaginate, which is a crucial step in initiating viral endocytosis [11].

The S protein is then subject to a series of enzymatic cleavages and modifications by cathepsin L, and to a lesser degree by cathepsin B, lysosomal cysteine proteases encountering the virus in the endo-lysosomes. This steps is key for viral membrane fusion and subsequent release of its RNA genome into host cytoplasm [10, 13, 14]. In fact, the latter step has been shown to be highly pH-dependent, with fusion only occurring after reaching highly acidic compartments (i.e., the lysosomes), which possess the highest cathepsin L activity [8]. This has been further

supported by the observation that the use of various lysomotropic agents, inhibit successful infection by interfering with lysosomal processing of viral membrane proteins necessary for fusion [9].

Thus, it is reasonable at this point to speculate that, disrupting the binding of the S protein to ACE2, or preventing its subsequent cleavage by cathepsin L intracellularly [15], potentially constitute two key targets for therapeutic intervention against COVID-19. However, what if there actually is a "real" physiological model that combines both cellular characteristics, as well as possesses many other "qualities" that grant its bearer "intrinsic resistance", or at least "reduced susceptibility", to COVID-19? This will be discussed in this report, in which I postulate that patients with lysosomal storage disorders, specifically those with Niemann-Pick type C disease (NPC), likely constitute "unfavorable hosts" for SARS-CoV-2, owing to their unique deleterious intracellular biochemical characteristics, which paradoxically grants them reduced susceptibility to the virus.

Perturbations in ACE2 and ADAM17 in NPC

The ACE2 protein has been previously shown to reside mainly within the cholesterol-enriched microdomains of the plasma membrane, known as lipid rafts [16]. These domains are easily perturbed by processes that affect the synthesis and/or trafficking of their predominant lipid constituents, namely cholesterol and sphingomyelin [16]. Interestingly, the intracellular trafficking of both, cholesterol and sphingomyelin, is disrupted in NPC [17, 18]. NPC is an autosomal recessive lysosome storage disorder caused by a defect in the NPC1 or NPC2 proteins (90% and 4%, respectively), leading to the entrapment and accumulation of cholesterol and

sphingolipids within the lysosomes [19]. Consequently, the partitioning of certain proteins to lipid rafts, and their retention of function, have been shown to be affected in NPC, as a result of the disease-related abnormal enrichment of cholesterol within these rafts [20]. In fact, *Takeuchi et al.* have successfully demonstrated cholesterol-enrichment of the plasma membrane in NPC cells, which has been further implicated in disrupting the internalization and trafficking of proteins residing within these domains [20]. As such, it could be argued that since ACE2 is a raft-resident membrane protein, its internalization, which is necessary for SARS-CoV-2 entry into the endo-lysosomal system, would be impaired in NPC. Indeed, this has been previously suggested, albeit indirectly, by *Glende et al.*, who showed that cyclodextrin-induced disruptions of lipid rafts, abolish the binding of SARS-CoV to ACE2, reducing infectivity by around 50% [16]. Thus, since SARS-CoV-2 employs a similar infection mechanism as SARS-CoV, it is also likely that alterations in the composition of lipid rafts, specifically those that render them less "fluid", as occurs in NPC, impair or markedly reduce infectivity. In fact, it is worth mentioning here that, while SARS-CoV does not require a functional NPC1 protein for establishing infectivity, in the same way that a functional NPC1 protein is essential for successful Ebola viral infection [21], trafficking of SARS-CoV to the NPC1-positive sub-compartments of the endo-lysosomal system has been shown to be crucial for successful viral infection [8]. Similarly, SARS-CoV-2 would also be expected to require entry into NPC1-positive compartments, prior to establishing infection [8].

Intriguingly, the same cells that highly express ACE2, and are targeted by SARS-CoV-2 (i.e., type II alveolar cells) [4, 6], rely heavily on the functionality of the NPC1 and NPC2 proteins, which play a key role in modulating the lipid composition of the primary extracellular product of these cells i.e., pulmonary surfactant [22]. NPC1-deficient type II alveolar cells have

been shown to contain enlarged lipid-rich lamellar bodies within their cytoplasm, with their secreted surfactant being particularly abundant in cholesterol and phospholipids, and markedly enriched in surfactant protein A (SP-A), a key endogenous antimicrobial peptide [23, 24]. Thus, the elevated SP-A content of pulmonary surfactant in NPC constitutes yet another barrier against the attachment of SARS-CoV-2 to the type II pneumocytes.

Furthermore, the previously mentioned TMPRSS2 is also predominantly a raft-resident protein, with some reports highlighting it as playing a potentially more important role in viral entry into host cell, compared with cathepsins [25]. Interestingly however, ADAM metallopeptidase domain 17 (ADAM17), also known as TACE (tumor necrosis factor-α-converting enzyme), is another plasma membrane protein that has been found to compete with TMPRSS2 on inducing shedding of the ACE2 protein, with only the TMPRSS2-mediated modification of ACE2 facilitating viral entry, while ADAM17-mediated shedding appeared to preclude entry of viral particles [26]. Both membrane proteases, TMPRSS2 and ADAM17, partition mainly into the detergent-resistant membrane domains i.e., lipid rafts [27, 28]. While no formal evaluation of TMPRSS2 expression or activity levels has been made in NPC, plasma membrane levels of ADAM17 have been suggested to be increased in NPC cells [29-31]. As a result, it may be argued that the increased levels of ADAM17 in NPC cells grants them further "protection" against coronavirus infection, by increasing ACE2 shedding, and opposing the TMPRSS2-mediated enzymatic processing necessary for viral entry.

Abnormal intracellular localization and disrupted activity of Cathepsins in NPC

Besides their previously described deficiency in cholesterol egress from the lysosomes to the plasma membrane, NPC cells have been shown to have an abnormal distribution, and consequently, reduced activities, of several lysosomal enzymes, including cathepsins L and B [32-35]. Specifically, the NPC-related accumulation of various substrates within the lysosomes has been shown to disrupt lysosomal membrane integrity, resulting in the leakage of many lysosomal enzymes, including cathepsins L and B into the cytosol [32, 33]. Moreover, the substrates accumulating within the lysosome of NPC cells have also been reported to alter the intra-lysosomal pH, which inadvertently affects the activity of the remaining enzymes, while simultaneously hindering the entry of newly synthesized lysosomal enzymes, such as cathepsins, into the lysosome [32-35]. Thus, here too, it can be argued that the NPC-induced reduction in activity of cathepsins B and L, and their abnormal extra-lysosomal displacement, pose "barriers" against proper processing of the S protein, which is required for successful fusion.

The oxysterols that accumulate in NPC possess potent antiviral activities

Among the various oxysterols that accumulate in NPC, two are worth highlighing in the context of COVID-19. These include 7-ketocholesterol (7KC) and 25-hydroxycholesterol (25-HC) [36], both of which have been shown to possess potent antiviral activities [37-40]. Increased intracellular 25-HC levels have been shown to reduce infectivity by several members of the coronaviridae, filoviridae (e.g. Ebola virus), and flaviviridae families, including the Zika and hepatitis C viruses [37-40]. In contrast, elevated intracellular 7-KC levels interfere with viral maturation and subsequent budding and release from host cells [41]. Nonetheless, the precise mechanisms underlying the antiviral activity of these oxysterols remain unknown. However, they appear to be partly mediated by their induction of increased reactive oxygen species (ROS)

production in neutrophils, which is relevant to bacterial killing [42], as well as inducing perturbations in intracellular cholesterol trafficking, and preventing viral entry, intracellular transport, and subsequent fusion [39, 43].

Making the link between COVID-19 and the lysosomes based on the demonstrated preliminary efficacy of tested drugs

Several approved drugs are currently undergoing repurposed testing for efficacy against COVID-19, with the intention of rapidly bringing "efficacious" ones to treat affected patients (https://www.nature.com/articles/d41587-020-00003-1).

Among the various drugs undergoing testing, the Chinese government appears to endorse Choloroquine, and its derivatives, the most, based on preliminary clinical trial data [44, 45]. In fact, chloroquine and its derivatives, are undergoing testing in over 10 different clinical trials to evaluate their efficacy in treating active COVID-19 infection (ChiCTR2000029935, ChiCTR2000029898 etc.) [46]. Additionally, a phase III trial is currently investigating the use of hdroxychloroquine for prophylaxis against COVID-19 infection, in at-risk healthcare workers (NCT04303507). However, just as anyone else involved in lysosomal storage diseases, I had to pause and ask myself how, an anti-malarial agent such as chloroquine, could demonstrate efficacy against a viral pathogen like SARS-CoV-2. Nonetheless, anyone in the lysosomal storage disease community has directly worked with, or at least heard of the use of, chloroquine in *in vitro* studies of LSDs, given its ability to inhibit lysosomal fusion with endosomes, as well as inhibiting the activity of its enzymes [47-49]. In fact, this is the very mechanism of action that is believed to underlie chloroquine's anti-malarial activity; it is suggested that after its entry into

cells, chloroquine becomes protonated and entrapped within the lysosomes, with its accumulation preventing lysosomal fusion with the "food vacuoles" (i.e., phagosomes) of *Plasmodium* trophoizoites, which impairs the activity of the resident heme polymerase [50, 51]. However, because of its propensity to concentrate within intracellular acidic organelles, chloroquine also enters into the host cell lysosomes, where it can induce similar aberrations in lysosomal function, primarily by elevating lysosomal pH and inducing partial lysosomal membrane permeabilization [51, 52]. These conditions are all "intrinsic" to NPC, as previously discussed, which suggests that chloroquine's demonstrated efficacy against SARS-CoV-2 may indeed be due to chloroquine's ability to induce NPC-like lysosomal abnormalities that interfere with intracellular viral transport and fusion. In agreement with this hypothesis, chloroquine has been previously shown to exert antiviral activity against several caliciviridae through exerting similar inhibitory effect on cathepsin L activity, compared with cathepsin L-specific inhibitors [53], as well as impairing the trimming of the N-glycosylated chain of ACE2, the receptor involved in SARS-CoV and SARS-CoV-2 docking on the host cell membrane [54]. Interestingly, N-glycosylation has been shown to be altered in NPC [55], which further suggests a chloroquine-like "protective" effect of the altered modification of N-glycosylation of ACE2 observed in NPC, against SARS-CoV-2 infection. In fact, the results of an open-label trial conducted in France (EU CTR 2020-000890-25) and posted on March 20th, 2020 on medrxiv [56], have gained remarkable attention shortly afterwards, not only from the media, but also from the office of the president of the United States. The study reported a statistically significant difference in the rates of clearance of the SARS-CoV-2 virus from nasal swabs of patients with COVID-19 receiving a combination of hydroxychloroquine, a chloroquine-derivative, and azithromycin, a macrolide antibiotic, compared with those receiving neither [56]. Thus, it is imperative at this point to discuss the

lysomotropic activity of azithromycin, the *add-on* therapy combined with hydroxychloroquine in that trial. Interestingly, just like chloroquine, azithromycin has also been shown to accumulate intracellularly within the lysosomes, increasing their luminal pH, and thereby negatively impacting the activity of its resident enzymes [57]. In fact, a combination of both drugs has previously been shown to demonstrate synergy with regards to increasing intra-lysosomal pH [57]. Moreover, chronic azithromycin treatment in patients with cystic fibrosis has been shown to increase susceptibility to mycobacterial infection, which usually relies heavily on adequate phagocytosis and containment of the bacteria in phagosomes [58]. Specifically, azithromycin has been shown to block the acidification of the phagosomes containing mycobacteria, allowing the latter to escape the phagosome and multiply uncontrollably [58]. However, unlike mycobacteria, which require intact lysosomal function for containment of the infection [59, 60], SARS-CoV-2, as discussed earlier, relies on an intact lysosomal function for complete entry and fusion. Thus, it is reasonable to expect that azithromycin would show efficacy against COVID-19, through a similar mechanism to chloroquine – that is, disrupting the function of lysosomal proteases. Furthermore, azithromycin's tropism towards lysosomes has been shown to induce an accumulation of neutral lipids, namely free cholesterol and various phospholipids, predominantly within the lysosomes [61, 62]. Thus, it is clear from these findings that azithromycin induces an intracellular status that phenocopies NPC, which most likely accounts for its demonstrated potent antiviral activity, despite being an antibiotic with the primary function of inhibiting prokaryotic ribosomes [63].

Another promising drug demonstrating preliminary efficacy against COVID-19 is remdesivir (NCT04292899), an adenosine analogue originally developed for Ebola [44, 64]. However, following its testing against Ebola viral infections, remdesivir was also found to

possess marked efficacy against a member of the coronaviridae family, a feline coronavirus in particular i.e., feline infectious peritonitis type I (FCoV-I) [65]. In addition, remdesivir has also been found to have moderate efficacy against COVID-19-related coronaviridae, the middle east respiratory coronavirus (MERS-CoV) and SARS-CoV [66, 67], including bat coronaviruses [67], as well as other human RNA viruses [68]. However, infection by FCov-I, the coronavirus species that appeared to respond the most to remdesivir, was shown to be abolished by pre-treating host cells with U18666A, a pharmacological agent that induces a cellular NPC phenotype with lysosomal accumulation of cholesterol and impaired trafficking [69, 70], suggesting a possible mechanistic convergence or synergy between NPC1 inhibition and remdesivir, besides the latter's primary mechanism of inhibiting viral RNA-dependent RNA polymerase [71]. Moreover, various adenosine analogs, including those used as antivirals or antineoplastic agents, have been shown to possess adenosine receptor-binding activities, particularly the ribose-modified types [72], which demonstrate differential affinities to the A1 and A2a adenosine receptors [73, 74]. The reason for highlighting this is that, A2a receptor stimulation has been shown to rescue the cholesterol entrapment seen in NPC, with that effect being abolished by A2a antagonism [75]. Thus, although no data exists to date on whether remdesivir can bind any of the adenosine receptors, it is worth entertaining the possibility that due to its structural similarity to adenosine, remdesivir could also act as an A2a receptor antagonist, thereby inducing a transient NPC-like cellular state. In fact, several adenosine analogs have been shown to inhibit lysosomal activity in neutrophils [76], further supporting the need to test a possible lysosomal-inhibitory effects of remdesivir, which could partly account for its demonstrated antiviral efficacy against COVID-19.

A yet another class of drugs expected to show potent antiviral efficacy against COVID-19 (supplementary file of https://www.nature.com/articles/d41573-020-00016-0), are the triazole antifungals, namely posaconazole and itraconazole, and their derivatives, based on their previously demonstrated efficacy against several coronaviraidae, including FCoV-I, human coronavirus-229E (HCoV-229E), MERS-CoV, and SARS-CoV [77-81]. While triazoles are known to interfere with ergosterol synthesis, an essential component of fungal cell membranes, their antiviral activities are mediated by their differential inhibition of viral helicases, which interferes with viral replication [77, 80]. However, triazole antifungals are also renowned for their interference with mammalian cell cholesterol trafficking, owing to the latter's molecular similarity to ergosterol, as well as inhibition of lysosomal cholesterol efflux, specifically by interfering with activity of the NPC1 transporter [82-84]. Thus, besides their possible role in interfering with viral replication via inhibiting viral helicase, the antiviral activity of triazoles could also be partly mediated by their interference with lysosomal cholesterol egress and NPC1-interaction, further supporting the idea of targeting the lysosomes as therapeutic interventions against the growing COVID-19 pandemic.

Furthermore, another class of drugs that was initially suggested [85], and subsequently confirmed to be efficacious against SARS-CoV-2 (https://www.biorxiv.org/content/10.1101/2020.02.05.935387v1), is the glycopeptide antibiotics, such as vancomycin, teicoplanin, or their modified derivatives. These agents have previously demonstrated efficacy against several members of the coronaviridae family, including FCoV-I, SARS-CoV and MERS-CoV [86, 87], as well as the Ebola virus [88]. However, since viruses, unlike bacteria, lack a cell wall that these compounds are known to act on, their antiviral activity has been shown to result from their intracellular effects on host cells, including direct cathepsin

L inhibition [87], as well as their concentration in lysosomes and subsequent "overloading" of these organelles [89, 90]. Additionally, glycopeptide antibiotics have also been shown to induce increased ROS production within eukaryotic cells, which in the case of viruses however, may not be relevant [91]. Nonetheless, such findings are fascinating because, not only is there reduced activity and abnormal localization of cathepsin L in NPC, as previously discussed, but NPC cells also have increased baseline ROS levels [92]. These combined "natural characteristics" of NPC cells therefore, grant them an intrinsic glyopeptide-like effect that is likely to hamper viral infectivity. As a side note, it is worth mentioning that within our NPC community, several patients receiving glycopeptide antibiotics for hospital-acquired pneumonia primarily, were found to develop focal pyogenic skin abscesses, whose underlying mechanisms remain to be investigated (unpublished data). However, since glycopeptide antibiotics are known to accumulate within, and disrupt the function of lysosomes [89, 90], it is likely that these compounds further burden the already overloaded lysosomes of NPC-affected phagocytes, hampering their ability to destroy engulfed debris, resulting in abscess formation [59, 93].

Finally, the finding that lysosomal proteases are key mediators of coronavirus tropism and infection in bats, the natural reservoirs of the virus [94], is another reason highlighting the importance of considering the lysosome as a potential target of therapeutic intervention against COVID-19.

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Figure 1. An illustration of SARS-CoV-2 entry into healthy (i.e., wildtype) versus NPC1-deficient cell. In healthy cells, SARS-CoV-2 binds via its Spike (S) protein to host cell ACE2. The latter complex is further modified by TMPRSS2, followed by endocytosis of the ACE2-bound viral particle. Within the host cytoplasm, the endosome carrying the virus fuses with a mature cathepsin L-containing lysosome, which activates viral membrane proteins, allowing for viral fusion *(left image)*. In contrast, in NPC1-deficient cells, following its S protein-mediated binding to ACE2, SARS-CoV-2 entry into the cell is jeopardized at several points: 1- ACE2 is "tethered" within the cholesterol-enriched plasma membrane of NPC cells, which hinders membrane invagination; 2- Increased levels of ADAM17 within the plasma membrane of NPC cells competes with TMPRSS2 on differently cleaving the S protein-ACE2 complex; 3- If successfully endocytosed, the viral particles within the endosome fail to undergo necessary enzymatic cleavages by cathepsin L, due to the reduced activity, and cytosolic leakage of the latter enzyme from the NPC1-defective, cholesterol-laden lysosomes of NPC cells. These factors combined, reduce the likelihood of successful viral entry, transport and subsequent fusion, thereby reducing the likelihood of a successful infection *(right image)*.