Received: 30 July 2021 | Revised: 9 September 2021 | Accepted: 12 October 2021

DOI: 10.1002/jcb.30166

VIEWPOINT

Journal of Cellular Biochemistry WILEY

Harnessing autophagy to fight SARS-CoV-2: An update in view of recent drug development efforts

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Abstract

Drug repurposing is an attractive option for identifying new treatment strategies, in particular in extraordinary situations of urgent need such as the current coronavirus disease 2019 (Covid-19) pandemic. Recently, the World Health Organization announced testing of three drugs as potential Covid-19 therapeutics that are known for their dampening effect on the immune system. Thus, the underlying concept of selecting these drugs is to temper the potentially life-threatening overshooting of the immune system reacting to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. This viewpoint discusses the possibility that the impact of these and other drugs on autophagy contributes to their therapeutic effect by hampering the SARS-CoV-2 life cycle.

KEYWORDS

autophagy, Covid-19, drug repurposing, pharmacology, SARS-CoV2, virophagy

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has gained notoriety for causing the currently raging coronavirus disease 2019 (Covid-19) pandemic.[1] Humongous efforts are ongoing worldwide to cope with the impact on health and society. Although vaccines could be developed and marketed with unprecedented swiftness, drug development will take significantly longer. In light of the obvious exigency, drug repurposing is a promising strategy that is being followed by many scientists in preclinical and clinical research.[2] For example, the World Health Organization (WHO) launched the research program "Solidarity" in 2020 to test four compounds as options for antiviral treatment, namely remdesivir (originally developed as an inhibitor of viral RNA polymerase to treat hepatitis C, Ebola, or Marburg virus infection), interferon β1a (boosting the host response to viral infection), hydroxychloroquine (a malaria drug), and a combination of lopinavir and ritonavir (both HIV drugs). Unfortunately, an interim report of the study, including 11 330 in-patients with Covid-19 at 405 hospitals in 30 countries, revealed little or no effect.[3]

A more recent initiative in the Solidarity program evaluates three established immune-modulatory drugs for Covid-19 treatment.[4] The selection of these drugs was based on a different rationale, that is, instead of trying to fight the virus directly, the aim is to confine the damage of an exaggerated immune response to the own body. A previous study showed that limiting the host defense can have beneficial effects in critically ill patients with Covid-19.[5] The selected drugs are

Viewpoint for the Journal of Cellular Biochemistry

infliximab, imatinib, and artesunate. The aim of this short review is to point to a potential involvement of autophagy in the action of these drugs, which may play a more prominent role than generally acknowledged. The review additionally includes the drug ivermectin, which received media attention as it promising results were reported in clinical trials,[6] and also covers antidepressants.

2 | AUTOPHAGY

In general, autophagy is an evolutionary conserved intracellular degradation process pivotal for cellular protein, energy, and organelle homeostasis.[7] It is active under the basic condition at a low level ensuring continuous turnover and can be activated under certain stress conditions such as proteotoxicity or starvation.[8] Material destined for degradation or recycling is engulfed by or transported into a double-membrane structure called "autophagosome." Through additional membrane remodeling processes, these autophagosomes fuse with lysosomes producing autolysosomes, with prior fusion with late endosomes as a potential intermediate step.[9] As detailed in excellent reviews, this process is tightly controlled and executed by a vast array of proteins, ATG proteins in particular, but also EPG proteins required for the more complex autophagy in multicellular organisms.[7–11] Autophagic flux refers to the activity through all consecutive steps of autophagy and typically is defined as a measure of autophagic degradation activity.[12] Analytical tools assessing autophagic flux need to be chosen with great care to avoid erroneous conclusions.[13] Several compounds currently are being developed, targeting different proteins in the autophagic cascade, given its involvement in various physiological and pathophysiological conditions, including viral infection.[14,15]

3 | AUTOPHAGY AND CORONAVIRUSES

The link between autophagy and invading pathogens is anything but new and both pro- and antiviral roles of autophagy were identified.[16] For example, evidence suggests that double-membrane structures derived from the endoplasmic reticulum both are required for the initial steps of autophagy and serve as replication sites for coronaviruses[17–22] (see also Figure 1). Later on, several coronavirus proteins were shown to induce the formation of double-membrane structures, such as the nonstructural proteins 2, 3, 4, and 6.[23] The broad activity against coronavirus replication of compounds that interfere with the generation of these structures further corroborates their importance.

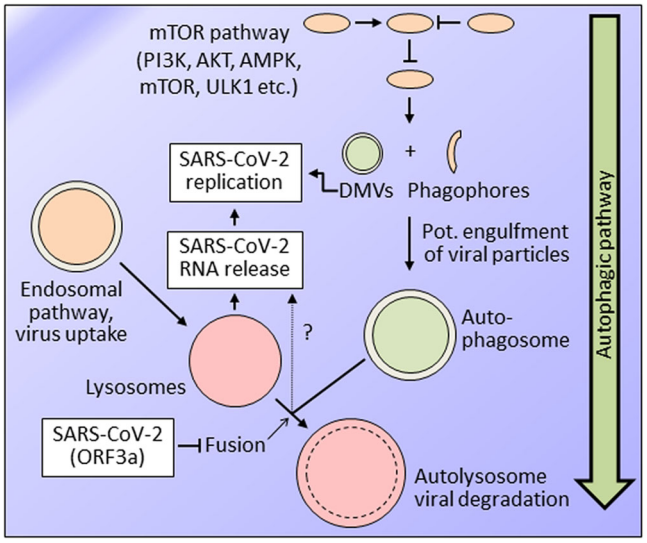

FIGURE 1 Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) replication and endosomal/autophagic pathways, simplified scheme. Current knowledge supports both beneficial and detrimental effects of the autophagic pathway for SARS-CoV-2 replication. A major entry route for the virus is endocytic uptake, which requires lysosomal acidification for viral RNA release. The autophagic pathway is a multifactorial and multistep pathway with a vast range of possibilities for pharmacological targeting. In the more initial phases, phagophores, and double-membrane vesicles (DMVs) are formed, most likely from the endoplasmic reticulum, possibly also promoted by some coronavirus proteins. SARS-CoV-2 replication takes place at the endoplasmic reticulum as well, at very similar, if not identical, membrane structures. SARS-CoV-2 inhibits the last step of autophagy leading to viral degradation, that is, the fusion of autophagosomes with lysosomes to form autolysosomes, thus inhibiting autophagic flux. Accordingly, compounds impacting autophagy are expected to be efficient in fighting SARS-CoV-2 only if they enhance autophagic flux

In contrast to this appearing congruence of viral mechanisms and early steps of autophagy, there is also firm evidence that coronaviruses interfere with late steps of autophagy to evade degradation. Very recently, for example, ORF3a (the protein derived from open reading frame 3a) of SARS-CoV-2 has been demonstrated to inhibit the fusion of autophagosomes with lysosomes, thereby increasing the number of autophagosomes but decreasing autophagic flux,[24,25] in line with the effect of coronavirus infection.[26,27] However, the exact details of how coronaviruses in general, and SARS-CoV-2 in particular, are intertwined with autophagy await further elucidation.[23,28,29] Nevertheless, it appears plausible that coronaviruses may benefit from earlier steps of the autophagic pathway, but are vulnerable to the increased autophagic flux that clears out viral particles. This is supported by reports showing that induction of autophagy has the potential to fight coronavirus infection.[26,27]

Chloroquine inhibits autophagy by interfering with autophagosome-lysosome fusion.[30] However, chloroquine and hydroxychloroquine exert additional effects like disorganizing the endo–lysosomal system that might have been the basis for the initial hope put on this drug for Covid-19 treatment.[31,32] However, with more studies coming up, no overall beneficial effect of this drug on Covid-19 was apparent,[33–35] and the drug now is abandoned in the WHO Solidarity program. Therefore, it appears likely that autophagy-targeting drugs need to promote autophagy flux rather than other aspects of autophagy. Although COVID-19 primarily is a respiratory disease, multiple organs are affected, either through cytokines or directly upon invasion of SARS-CoV-2.[36–38] As autophagy is a conserved mechanism operative in most cells, pharmacological induction of autophagy has the potential to fight SARS-CoV-2 in all organs that are reached by the compound. However, the effect on overall health may depend on existing comorbidities, such as cancer, for example, where the effect of autophagy depends on the circumstances.[39,40]

4 | ARTESUNATE

Like the other two drugs infliximab and imatinib, artesunate was added to the WHO Solidarity program because of its effects on the immune system.[4] Artesunate is a derivate of artemisinin with established antimalaria features, but also potent anticancer effects. For considering its potential effects on autophagy, it is important to differentiate general effects on autophagy from effects on autophagy flux, given the complex interaction of SARS-CoV-2 with autophagy. The vast majority of publications assessing artesunate for its effects on autophagy report induction[41–44] not all publications, however, assess autophagic flux following the established guidelines.[13] Nevertheless, some flux assays have been performed such as the use of the late autophagy blockers chloroquine or bafilomycin A, where artesunate still enhances the autophagy marker LC3BII/I.[45–47] Although all these studies support autophagy promoting function of artesunate, an inhibitory effect of artesunate has been observed using the tandem fluorescence tagged LC3B stably transfected into HeLa cells,[48] which is recognized as a valid method to determine autophagic flux.[13] The reason for this seeming discrepancy is not known, which makes further studies mandatory.

5 | INFLIXIMAB

Infliximab is a chimeric antibody targeting TNF-α used in clinical practice to treat autoimmune diseases such as Crohn's disease. Recently, it has been put forward that a range of drugs, including infliximab, that are either approved or in a clinical trial with great promise to treat Crohn's disease induces autophagy as a relevant mechanism at least contributing to their effect.[49,50] At least for infliximab, however, there is a scarcity of studies investigating the effect on autophagy directly,[51,52] and no reports were found presenting autophagic flux assays for infliximab.

6 | IMATINIB

Imatinib is an ABL tyrosine kinase inhibitor used to treat chronic myeloid leukemia. Like chloroquine, it is a cationic amphiphilic drug and thus should have the potential to inhibit autophagy by accumulating in lysosomes and disturbing their function.[53] However, several studies report an autophagy-inducing effect of imatinib, including the assessment of autophagic flux using chloroquine as an inhibitor.[54–57] Nevertheless, more studies are needed applying a broader range of autophagic flux assessments to solidify the conclusion that imatinib induces autophagy. Furthermore, it has been argued that compounds prone to induce phospholipidosis such as cationic amphiphilic drugs should be excluded from drug repurposing for SARS-CoV-2 treatment.[58] Nevertheless, it should be noted, that several other cationic amphiphilic drugs such as some antidepressants also induce autophagic flux.[59] Thus, this compound class may as well elicit more specific effects.

7 | ANTIDEPRESSANTS

Evidence is accumulating that patients with Covid-19 benefit from antidepressant treatment: A multicentric observational retrospective study with 7230 adults hospitalized for Covid-19 reported that those receiving antidepressant treatment had a reduced risk of intubation or death.[60] Similarly, a study with 3238 Covid-19 patients revealed a beneficial effect of the antidepressant fluvoxamine, reducing the need for emergency room observation or hospitalization.[61] A small randomized clinical trial with 152 COVID-19 outpatients revealed a lower likelihood of clinical deterioration for patients receiving fluvoxamine.[62] Furthermore, a recent preclinical study found the antidepressant fluoxetine as an inhibitor of SARS-CoV-2 in human lung tissue.[63] The beneficial effects of antidepressants frequently are conceptualized as cytokine effects[64] thus reducing the risk of a fatal cytokine storm.[65] However, antidepressants are known to induce autophagy as well.[59,66] Thus, their effect on autophagy might not only be important for treating

depression but also to fight SARS-CoV-2. In fact, tricyclic antidepressants inhibit lysosomal acidic sphingomyelinase, thereby not only enhancing autophagy but also reducing SARS-CoV-2 entry into epithelial cells.[67,68]

8 | IVERMECTIN

Ivermectin is an antihelmintic macrolide of the avermectin group.[69] It is investigated as a potential anti-SARS-CoV-2 treatment with promising initial results, but also very recent dispute.[6,71] Several mechanisms are discussed for its apparent antiviral activity[70,71] and this viewpoint argues for adding autophagy to this panel. A number of publications report an autophagy-inducing effect of ivermectin,[72–74] including a study carefully determining autophagic flux.[75] Therefore, autophagy should be considered as a mediator of the manifold effects of ivermectin in general,[70] and of its antiviral activity in particular. Of note, another antihelmintic drug, niclosamide, not only is known for its autophagy-inducing action but also has been demonstrated to reduce replication of the Middle East Respiratory Syndrome Coronavirus[26] as well as of SARS-CoV-2.[27]

9 | CONCLUSION

The point of this article is to draw attention to autophagy as a potential contributing mechanism of selected drugs currently under investigation for repurposing to Covid-19 treatment. In other words, it is possible that the three drugs recently added to the WHO Solidarity program may not just prevent a life-threatening overreaction of the body during a SARS-CoV-2 infection, but also actually limit SARS-CoV-2 replication through activating autophagy. The interaction of SARS-CoV-2 with the autophagic pathway is complex (Figure 1), with evidence for both the virus taking advantage of the autophagic pathway and trying to tame the full activity of this pathway to prevent its degradation. It is obvious from this scenario that it will be essential to learn how exactly the SARS-CoV-2 life cycle is intertwined with the autophagic pathway. Future research should include all known forms of autophagy, such as macroautophagy, microautophagy, chaperone-mediated autophagy, secretory autophagy, and so forth. This also applies to better understanding the action of to be repositioned or new drugs at each level of the autophagic pathway.

ACKNOWLEDGMENTS

Open Access funding enabled and organized by Projekt DEAL.

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How to cite this article: Rein T. Harnessing autophagy to fight SARS-CoV-2: An update in view of recent drug development efforts. *J Cell Biochem*. 2022;123:155-160. doi:10.1002/jcb.30166