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European Journal of Pharmacology 891 (2021) 173694

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

COVID-19 and antimalarials. Have we been doing it wrong all along?

Cosmin Andrei Cismaru [a,b,*], Gabriel Laurentiu Cismaru [c], Fazel Seyed Nabavi [d], Mohammad Seyed Nabavi [d,**], Ioana Berindan-Neagoe [a,e]

[a] Research Center for Functional Genomics, Biomedicine and Translational Medicine, The "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[b] Department of Functional Sciences, Immunology and Allergology, The "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[c] Department of Internal Medicine, Cardiology-Rehabilitation, The "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[d] Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[e] The Functional Genomics Department – The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania

ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Antimalarials
Macrolide antibiotics
Sequential
Coronavirus

ABSTRACT

In the context of the current SARS-CoV-2 pandemic, associations of drugs which interfere with specific steps of the viral infectious cycle are currently being exploited as therapeutic strategies since a specific treatment by vaccination is still unavailable. A widespread association of repurposed agents is the combination of the antimalarial drug Hydroxychloroquine and the macrolide antibiotic Azithromycin in the setting of clinical trials. But a closer analysis of their mechanism of action suggests that their concomitant administration may be impractical, and this is supported by experimental data with other agents of the same classes. However a sequential administration of the lysosomotropic antimalarial with the addition of the macrolide proton pump inhibitor after the first has reached a certain threshold could better exploit their antiviral potential.

In the context of the current SARS-CoV-2 pandemic, associations of drugs which interfere with specific steps of the viral infectious cycle are currently being exploited as therapeutic strategies since a specific treatment by vaccination is still unavailable. A widespread association of repurposed agents is the combination of theantimalarial drug Hydroxychloroquine and the macrolide antibiotic Azithromycin in the setting of clinical trials. However, assessing the effects of administering the two drugs together or in a sequential manner has not yet been addressed. This could dramatically change the course of the treatment as the mechanisms of action of the two classes of drugs suggests that their sequential administration could exploit the entire antiviral potential of such association.

The antimalarial activity of Chloroquine has long been documented and it is based on its ability of preferentially accumulating in lysosomes increasing their pH following protonation.(Homewood et al., 1972), (Kaufmann and Krise, 2007) while decreasing autophagosome-lysosome fusion (Mauthe et al., 2018).

Streptomyces derived macrolides such as Azythromycin have multiple biological effects ranging from direct inhibition of bacteria and fungi, to inhibition of the inflamasome and the autophagy system which is exploited by multiple pathogens including encapsulated viruses (López-Boado and Rubin, 2008). Much of their effects on autophagy are done through the ability of inhibiting the vacuolar proton pump (v-ATPase) which is responsible for maintaining an acidic pH in lysosomes (Huss and Wieczorek, 2009). This is why Azythromycin, an antibiotic with antimalarial properties is also the most potent macrolide antimalarial (Dahl and Rosenthal, 2007). Since coronaviruses rely on the formation of autophagosomes (double membrane vesicles) necessary for viral replication shielded from host immune responses (Knoops et al., 2008), both of these categories of antimalarials can interfere with viral replication, most of their antiviral effect being attributed to inhibiting autophagy. In COVID-19, it is likely that the sequence of drug administration could considerably increase the therapeutic index of this combination. Such sequence-dependent outcomes could be emphasized by the property of both of these cationic drugs to accumulate in acidic lysosomes increasing their pH, an ability known as lysosomotropism (Nujić et al., 2012). Lysosomotropic drugs accumulate in endosomes and lysosomes becoming trapped inside the organelle following protonation

* Corresponding author. Research Center for Functional Genomics, Biomedicine and Translational Medicine, The "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.
** Corresponding author.
E-mail address: cosmin.cismaru@umfcluj.ro (C.A. Cismaru).

https://doi.org/10.1016/j.ejphar.2020.173694
Received 5 August 2020; Received in revised form 21 September 2020; Accepted 26 October 2020
Available online 31 October 2020

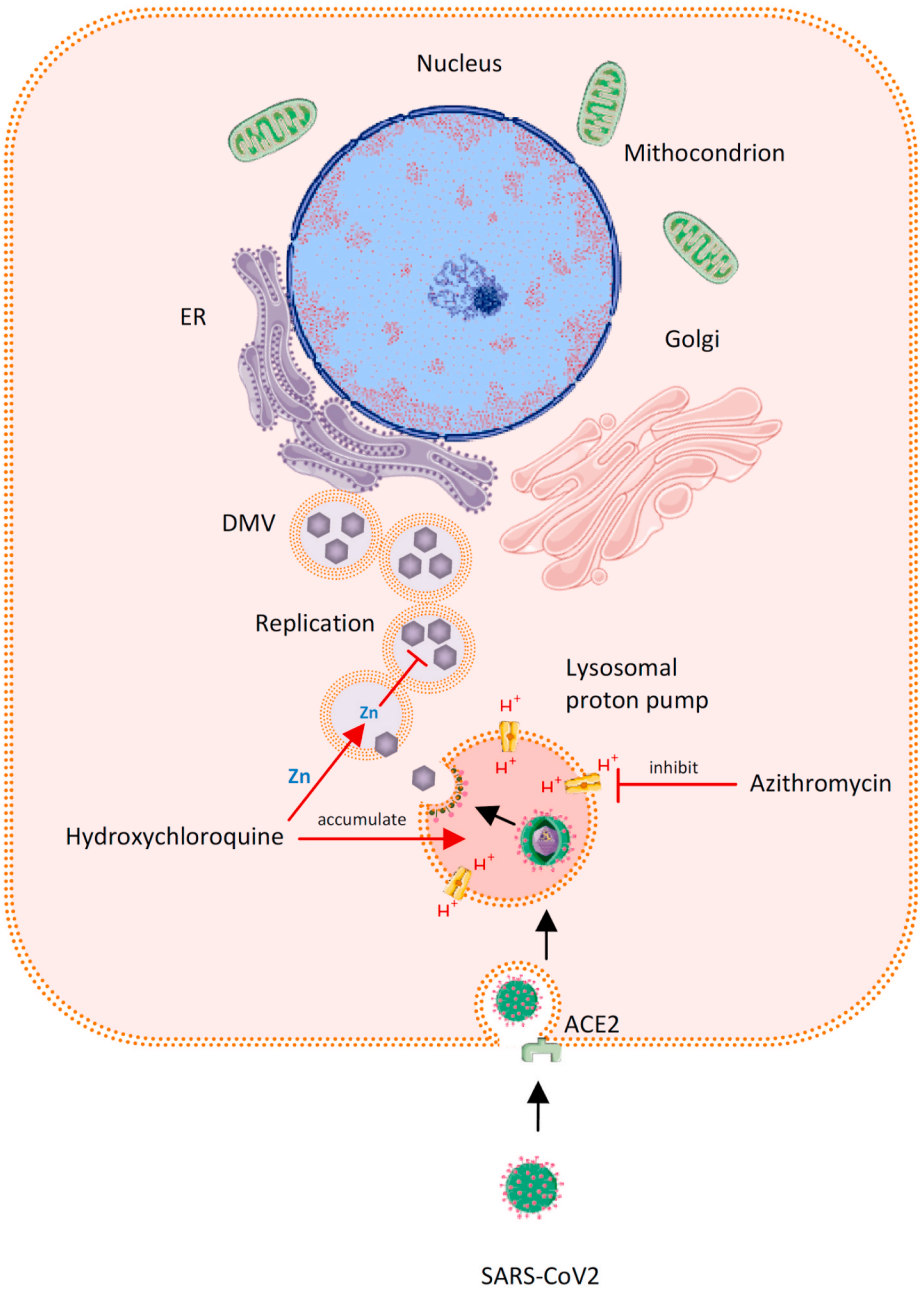

Fig. 1. Lysosomotropic drug Hydroxychloroquine accumulates into the lysosome increasing it's pH and inhibiting low pH dependent hydrolases necessary for the uncoating of the virus and the fusion of the envelope with the membrane. Macrolides such as Azithromycin are potent inhibitors of the lysosomal proton pump (v-ATPase) inhibiting the acidification of the organelle and the ion trapping oh hydroxychloroquine by protonation. Viral replication takes place in endosomal reticulum (ER) derived double membrane vesicles (DMVs), shielded from the host immune responses. By the property of being a metal ionophore, hydroxychloroquine gets Zinc (Zn) across the membranes of DMVs inhibiting viral replicases.

in a process called ion trapping (Kaufmann and Krise, 2007; Kuzu et al., 2017). This increases the endosomal pH to values where low pH dependent hydrolases no longer function properly leading to the inhibition of viral fusion with the organelles' membrane and egressing into the cytoplasm. As a consequence, a prolonged exposure of the virus to degradative lysosomal enzymes occurs in a higher lysosomal pH, with deletary effects on the virus (Simons et al., 1982). But the lysosomal proton pumps can restore the acidity of the lysosome by protonation (addition of hydrogen atoms). However, macrolide antibiotics are also potent inhibitors of the v-ATPases. Their addition leads to the inhibition of both protonation and restoration of the acidic pH in lysosomes. As protonation is required for the trapping of cationic drugs inside lysosomes, the concomitant administration of an inhibitor of the lysosomal v-ATPase becomes impractical as inhibiting protonation limits the sequestration of the cationic drug inside lysosomes. This was emphasized in experimental data showing that the administration of an inhibitor of the lysosomal protoin pump such as the macrolide antibiotic Concanamycin A almost abolishes the accumulation of the cationic LysoTracker, a lysosomotropic agent used for acidic cellular organelle staining (Nujić et al., 2012). This supports the hypothesis that a sequential administration of the two agents could better trap the cationic drug in the lysosomes after a certain threshold is obtained. Maintaining the increased pH in the lysosomes with the addition of the v-ATPase inhibitor could lead to hampering viral fusion with the organelles membrane and egress into the cytoplasm (Fig. 1).

Such mechanisms could explain the discrepant preliminary results from trials reporting no benefit from using the combination (Molina et al., 2020) while others are reporting benefits from adding Azithromycin to Hydroxychloroquine. In the EudraCT 2020-000890-25 trial on COVID-19, Azithromycin which was later added to the scheme, seemed to potentate the effect of hydroxychloroquine to a 100% cure rate in patients in the combination treatment arm (Gautret et al., 2020). This may be due to both the property of lysosomotropism and v-ATPase inhibitor of Azithromycin.(Arabi et al., 2019; Qiao et al.,

2018).

There are several ongoing clinical trials evaluating the effects of Hydroxychloroquine and Azithromycin either alone or in combination, (Search of: chloroquine, Azithromycin | COVID - List Results, n.d), (Ferner and Aronson, 2020) but neither of them are testing the sequential administration of these two drugs. Besides, the Hydroxychloroquine arm of the WHO Solidarity trial has been discontinued for failing to show benefits for patients in the current administration scheme ("Solidarity clinical trial for COVID-19 treatments," n.d.). However, the studies which have anticipated a crossover to the drug combination arm will be able to observe the aforementioned effect dynamically, while new trials may establish this as an endpoint from the beginning.

Although there are currently no results on their usage alone in COVID-19, macrolides were not associated with reduced mortality and viral clearance in a retrospective study on Middle East Respiratory Syndrome coronavirus (MERS-CoV) (Arabi et al., 2019). This implies that better results could be obtained by the association and the administration of the two classes of drugs in a rather sequential manner, such as starting with the lysosomotropic drug followed by adding the v-ATPase inhibitor later in the therapeutic scheme.

The endo-lysosomal alkalization obtained from inhibiting the v-ATPase alone, may even be more efficient than that provided by the lysosomotropic antimalarials and many old and new macrolide antibiotics are potent v-ATPase inhibitors (Huss and Wieczorek, 2009). This is supported by experimental data which shows that Azythromicin is a better lysosomal acidity inhibitor that Chloroquine while Concamycin A showed the most pronounced effects (Nujić et al., 2012). However, lysosomotropic antimalarials such as hydroxychloroquine or even methylthioninium chloride (methylene blue) have other effects besides lysosomotropism which stress the importance of their usage in combination with a v-ATPase inhibitor, and these include the ability of a metal ionophore - being able to transport metals such as Zinc across lipid bilayer membranes (Xue et al., 2014; Jonnalagadda and Gollapalli, 2008) as well as to inhibit the viral replicases in double membrane vesicles (te Velthuis et al., 2010) and binding heme and preventing the SARS-CoV-2 usage of porphyrin as recently described in a possibly new mechanism of viral pathogenesis (Wenzhong and Hualan, 2020). By releasing the breaks on this potent combination from administering the lysosomotropic drugs and v-ATPase inhibitors in a sequential manner, better results could be obtained in COVID-19 trials, based on the aforementioned mechanisms. The usage of safer antimalarials such as methylene blue could address some of the safety concerns of the drug combination that led to the termination of some trials, while still addressing multiple aspects of the viral infectious cycle. Since a validation of this effect should be emphasized by randomized, controlled clinical trials, this stepwise approach is worth pursuing since this drug combination might have been wrongly used all along from the beginning of the SARS-CoV-2 pandemic while no specific or effective treatment against COVID-19 has been released since its outbreak 10 months ago.

Author agreement

All authors have read and approved this version of the manuscript.

Funding

No applicable funding from public or private resources was assigned for the writing of this manuscript.

Credit author contribution statement

Cosmin Andrei Cismaru: Conceptualization, Writing - original draft, Writing - review & editing, Equal contribution. **Gabriel Laurentiu Cismaru:** Conceptualization, Writing - original draft, Writing - review & editing, Equal contribution. **Fazel Seyed Nabavi:** Conceptualization, Writing - review & editing, Supervision, Equal contribution. **Mohammad Seyed Nabavi:** Conceptualization, Writing - review & editing, Supervision, Equal contribution. **Ioana Berindan-Neagoe:** Conceptualization, Writing - review & editing, Supervision, Equal contribution.

Declaration of competing interest

Authors declare no conflict of interest.

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