The eye as the discrete but defensible portal of coronavirus infection

Minas Theodore Coroneo [a,b,*]

[a] *Department of Ophthalmology, Prince of Wales Hospital/University of New South Wales, Sydney, Australia*
[b] *Ophthalmic Surgeons, 2 St Pauls St, Randwick, NSW, 2031, Australia*

ABSTRACT

Oculo-centric factors may provide a key to understanding invasion success by SARS-CoV-2, a highly contagious, potentially lethal, virus with ocular tropism. Respiratory infection transmission via the eye and lacrimal-nasal pathway elucidated during the 1918 influenza pandemic, remains to be explored in this crisis. The eye and its adnexae represent a large surface area directly exposed to airborne viral particles and hand contact. The virus may bind to corneal and conjunctival angiotensin converting enzyme 2 (ACE2) receptors and potentially to the lipophilic periocular skin and superficial tear film with downstream carriage into the nasopharynx and subsequent access to the lungs and gut. Adenoviruses and influenza viruses share this ocular tropism and despite differing ocular and systemic manifestations and disease patterns, common lessons, particularly in management, emerge. Slit lamp usage places ophthalmologists at particular risk of exposure to high viral loads (and poor prognosis) and as for adenoviral epidemics, this may be a setting for disease transmission. Local, rather than systemic treatments blocking virus binding in this pathway (advocated for adenovirus) are worth considering. This pathway is accessible with eye drops or aerosols containing drugs which appear efficacious via systemic administration. A combination such as hydroxychloroquine, azithromycin and zinc, all of which have previously been used topically in the eye and which work at least in part by blocking ACE2 receptors, may offer a safe, cost-effective and resource-sparing intervention.

Background

Unexpectedly, ophthalmology may be playing a central role in the current Coronavirus Disease 2019 (COVID-19) epidemic. The harbinger of the third zoonotic coronavirus epidemic in as many decades [1], was Dr Li Wenliang, an ophthalmologist, who died following infection with COVID-19 [2,3]. It was thought he was infected during examination of a patient with angle closure glaucoma in the second week of January 2020. He suspected an outbreak after seeing patients with SARS-like symptoms and the system failure to heed his warning may well have changed the course of world history. The second instructive case is that of the respiratory physician Guangfa Wang [4]. Days before pneumonia onset, his earliest symptoms related to left conjunctivitis, then catarrhal symptoms and fever which developed after 2–3 h, slower than might be expected from older studies tracing the passage of bacteria through the lacrimal drainage system [5]. While an N95 respirator was worn, offering protection from infection via oro-nasal pathways, eye protection was not.

Evidence that ocular and periocular tissue may be uniquely placed as an entry point for viral invasion will be reviewed. At the height of the 1918 world influenza epidemic, a landmark paper appeared, proposing transmission of acute respiratory infections via the eye and lacrimal-nasal pathway [5] (Fig. 1). It was noted that this pathway had been "disregarded in planning measures for the prevention of the spread of contagious diseases" and it would appear that little has changed.

A third factor in this perfect storm is the well-established but not well recognized coronavirus ocular tropism [6]. The study of oculotropic influenza and adenoviruses [7–9] sets precedents for disease patterns, bought into focus by coronaviruses. Whereas influenza viruses generally represent a respiratory pathogen and only occasionally cause ocular complications, adenoviruses mirror image this disease pattern, causing severe ocular surface disease, and are often highly contagious (known as "eye hospital eye [10]") with fewer, seldom lethal, systemic manifestations. Thus, all three virus types share a common ocular entrée but vary in their degree of contagion, ocular versus respiratory/system impact and lethality. Taken as a whole, these disease patterns have implications for more effective management strategies.

Why Are Ophthalmologists/Eye Care Providers At Occupational Risk of COVID-19?

Dr Li Wenliang's death and the apparent high infection rate in ophthalmologists (and ENT surgeons) [11], attributed to the high viral shedding from the nasal cavity, should not be unexpected. A high initial viral load is associated with poor prognosis [12] and ophthalmologists are particularly at risk, since ophthalmic practice involves very close ophthalmologist-patient physical proximity. This is necessitated by optical imperatives to optimise image quality by close alignment of imaging devices to the eye. Slit lamp biomicroscopy is the cornerstone of ophthalmic practice and the slit lamp is also used to carry out surgical

---

procedures. The back focal distance (the distance of the subject from the front lens surface of the microscope), is set to give the surgeon sufficient space for manipulation ~ 11 cm (Fig. 2A). With the instrument body length added, the distance from patient to the surgeon's eye is at a convenient working distance of ~28–30 cm. This proximity and the possibility of cross infection between examiner and examinee has long been recognized and a protective, transparent, typically Perspex® "breath shield" was added to slit lamps. It is evident in slit lamps from as early as 1919 [13] and may well have gained popularity during the era of the 1918 influenza pandemic. The necessity for such shields is readily apparent from studies in which exposure between two face-to-face breathing manikins is measured [14] – Fig. 2B and C. Tracer gas experiments to investigate the range of exhalation, either by mouth (Fig. 2B) or nose (Fig. 2C) show that at ~30 cm, one would be within the "line of fire". This study concluded that air exhaled from human respiration contains contaminants and is able to penetrate the breathing zone of other nearby persons. Of interest is that exhalation flow may stratify in a horizontal layer at breathing zone height under certain conditions. Thus, with slit lamp examination, the layer of air between patient and ophthalmologist may remain stable, exposing both individuals for the entire time of the examination. Breath shields are often missing from slit lamps either because they are not fitted or because, as they get in the way, are removed.

That slit lamps and their accessory lenses are a source of infection transmission has long been known [15]. In epidemic adenoviral keratoconjunctivitis ((AKC), shipyard/eye hospital eye) [10,16], ophthalmologists are not infrequently infected although this is not well documented [17,18]. While AKC can cause considerable incapacity and has resulted in some risk-minimization measures, unlike coronavirus it does not usually result in death. The earlier severe acute respiratory syndrome (SARS) epidemic resulted in recommendations for how to manage eye facilities and include advice in relation to slit lamp cleaning
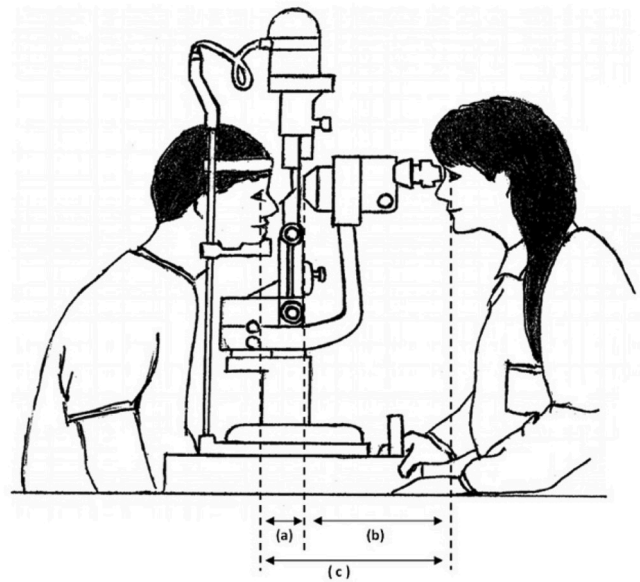

Fig. 2a. Typical configuration during slit lamp examination indicating distances between patient and physician: (a) Back focal distance - 11 cm, (b) Distance between front lens surface and ophthalmologist's eyes - 17–19 cm, (c), Distance between ophthalmologist and patient eyes - 28–30 cm.

and eye protection for staff [19,20]. Slit lamp biomicroscopy is an almost optimal method of transferring material in breaths between two individuals, short of actual facial contact. A very recent study provides evidence for human-to-human transmission [21]. Pathways include direct transmission, such as cough, sneeze, droplet inhalation
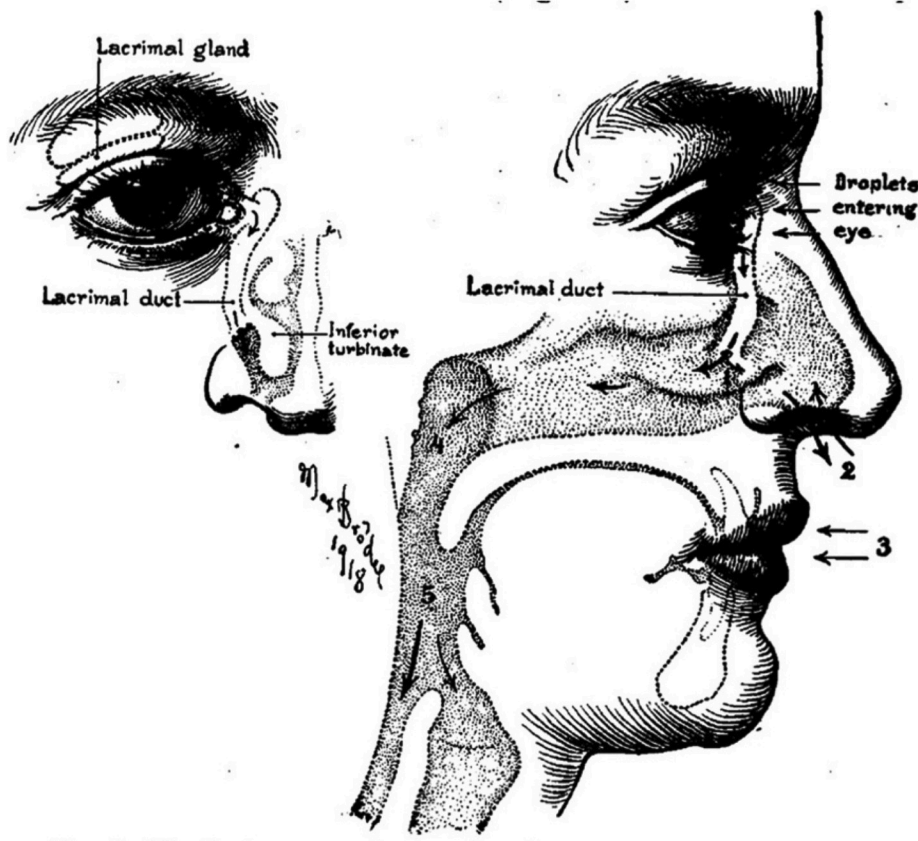

Fig. 1. The lacrimatory-nasal mechanism for the mechanical disposition of organisms entering the upper respiratory tract [5].

Fig. 2b. Smoke visualisation of exhalation flow from mouth of mannequin on the right. The breathing zone of mannequin the left mannequin is obviously penetrated despite a distance of 1.2 M [14].

Fig. 2c. Smoke visualisation of exhalation flow from nose of the mannequin on the right – separation of mannequins was 0.4 M. The breathing zone of the mannequin on the left is again penetrated but its closer proximity to the exhaled smoke would suggest a greater degree of breathing zone penetration [14].

transmission as well as contact transmission including contact with oral, nasal, and eye mucous membranes [22]. SARS-CoV-2 aerosol and fomite transmission is highly likely, since the virus can remain viable and infectious in aerosols for hours and on different surfaces up to days [23]. While transmission of coronaviruses occurs via an airborne route [24], because infected, symptomatic patients tend to develop severe lower, as opposed to upper respiratory tract infections, it has been supposed that airborne agent virus has to be small enough to penetrate directly into the lower respiratory tract to preferentially replicate there before causing disease. That transmission could occur via the ocular-nasolacrimal pathway was not considered.

Is the ocular surface an entry point for SAR-CoV2?

Human eyes are located at a coign of vantage in the body, simultaneously providing information from our highest bandwidth sense but also being exposed to risk of exposure including to airborne virus. The surface area of the eye(s) is large compared to that of the mouth and nares – (Fig. 2D) and was recognized early as a target for "promiscuous spraying" whereby coughing could project material at least 10 feet away [5]. Since this 1919 study [5], a number of other studies have investigated ocular surface area and reported as a total (for two eyes) as 226–426 [25] and 300–640 mm^2 [26], indicating that the figure from Maxcy's early study [5] was a good estimate (Table 1) of the "visible" ocular surface. The later study [26] also explains variability in measurements, since palpebral aperture is dependent on eye gaze direction. However, the total ocular surface area has been estimated at
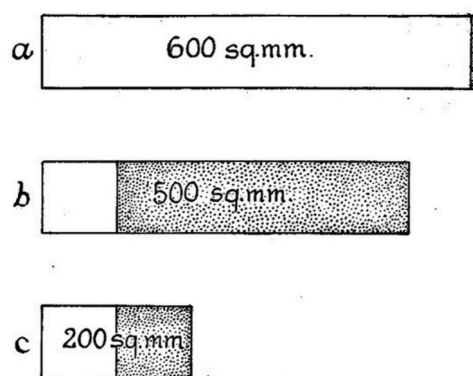

Fig. 2d. Comparison of eye exposure to direct droplet spray to that of the mouth and nares: (a), average total eye surface exposed; shaded area represents proportion of time not exposed, due to winking; (b), average total mouth area exposed in talking; shaded area represents proportion of time not exposed, due to closure; (c), average total area of cross-section of nares exposed; shaded area represents proportion of time not exposed, owing to protected position and expiration [5].

Table 1
External ocular surface areas.

Palpebral Aperture mm2	Methodology	Reference
600	Unreported	[5]
226–426	Digitized video images	[25]
300–640	Digitized video images	[26]
Total Ocular Surface Area		
3200	Designed instrument	[27]
3738	Molds of cadaver eyes	[38]
Total Orbital Aperture Area		
2509 (estim)	CT Scan	[32]
1793–1987	Radiographs	[33]

~1600–1869 sq. mm/eye [27,28], including the cornea, this representing a maximal absorptive area of ~3738 sq.mm, accounting for the tarsal conjunctiva and including the palpebral fornices. Even this consideration underestimates the potential ocular/periocular landing zone for a viral particle. It is not an uncommon observation that makeup, applied around the eye, can "migrate" onto the ocular surface [29], similar to the phenomenon by which noxious agents in minute quantities are easily transferred from fingertip to periocular skin and then the eye. This may be due to subtle actions of Riolan's muscle [30]. In fact, this mechanism of transport of agents from the periocular eye lid skin to the eye (obviating the need for eyedrops) has been termed supracutaneous and has been developed as an efficient delivery system for management of dry eye syndrome [31]. This supracutaneous mechanism, might provide a substantial periocular area in which viral particles could land and be "funnelled" onto the ocular surface and beyond. Table 1 provides available information on the size of the orbital opening [32] and reviewed in [33], which likely underestimates the area of eyelid skin. We have estimated eyelid skin to be ~4000 mm^2 and that of the brow to be ~3000 mm^2, so that in total with the ocular surface area, a landing site of ~10,000 mm^2 would be available, – 2 orders of magnitude greater than for the nares and mouth. This does not take into account the surface area that could be attributed to the hair of the eyebrows or the eyelashes, for which estimates have not been made. Eyelash aerodynamics may also play a role [34]. Eyelashes have been shown to divert airflows, acting as a passive ocular dust controlling system. They reduce evaporation and particle deposition up to 50%. In a comparative study [35], Asian eyelashes had lower lift-up and curl-up angles, fewer numbers and a thicker transverse diameter as compared to Caucasian eyelashes. Whether these differences play any role in influencing the rate at which particles land on the ocular surface is unknown. However, it is possible

that a deficiency in this mechanism could increase the risk of infection.

In the 1919 study [5], Bacillus prodigiosus (Serratia marcescens) was instilled into the lacrimal sac of 5 volunteers and subsequently was recoverable from the nose, throat and stool after 5 min, 15 min and 24 h respectively. It was concluded that via this ocular lacrimatory-nasal mechanism (Figs. 1 and 2), the upper respiratory tract of a person wearing a properly constructed mask may be infected by exposing the eye briefly to direct droplet spray.

Role of Ocular Surface Cellular Receptors

Identification of ocular surface cellular receptors utilized by respiratory viruses has provided information as to the permissiveness of ocular tissue to infection with these agents [6,36]. Central to COVID-19 pathogenicity is that cellular entry is via the cell surface angiotensin converting enzyme 2 (ACE2) receptor [37,38]. It is the only mammalian group I coronavirus known to use ACE2 as its receptor [39]. ACE2 was previously identified as the receptor for SARS-Cov and NL63 [40]. Virus infectivity studies have shown that ACE2 is essential for SARS-CoV-2 to enter HeLa cells [41].

While ACE2 mRNA is known to be present in virtually all organs, surface expression of ACE2 protein was described in lung alveolar epithelial cells and small intestinal enterocytes [42] and it was postulated that ACE2 might provide the coronavirus entry route. The eye and its adnexae were not investigated in this study. It was subsequently shown that ACE2 protein is more abundantly expressed on the apical than the basolateral surface of polarized airway epithelia [43] and thus accessible by topical agents. An immunohistochemical study revealed both extra- and intraocular localisation of ACE in human eyes [44]. Of particular interest was the localisation of ACE to the epithelial cells of both the cornea and conjunctiva. Apical epithelia location and whether receptors are ACE2 remains to be confirmed. Recently, more widespread distribution of SARS-CoV-2 entry factors, ACE2 as well as TMPRSS2 protease and cathepsin B/L activity (for post-binding spike protein priming) have been documented using single-cell RNA-sequencing data [45]. While it was recognized that binding affinity of the spike protein and ACE2 is the major determinant of SARS-CoV replication rate and disease severity and that viral entry also depends on protease activity, ACE2, rather than protease activity, may be a limiting factor for initial viral entry. This study confirmed evidence of ACE2 in the limbus, corneal epithelium (basal/suprabasal, superficial and wing cells) and conjunctiva (basal and superficial cells) and co-location with TMPRSS2 in superficial conjunctival cells. Of interest is that this study demonstrated evidence of ACE2 in nasal goblet cells which also express genes associated with immune functions including innate and antiviral immune functions. There should be some priority for re-evaluation of these elements in the ocular surface [46].

The density of SARS-CoV-2 entry factors and their presence or absence in the lacrimal drainage system would be of interest, since viral particles that land in the tear film need not necessarily bind to an epithelial cell immediately but could be presented with a second opportunity (a "wash through" effect) in the lacrimal drainage system. Tear film resilience may well protect the underlying corneal and conjunctival epithelium so that viral adherence to the tear film could prevent access to the apical epithelial surface, which may account for the relatively low incidence of keratitis/conjunctivitis reported to date [47,48].

The tear film lipid layer plays an important role in retarding tear film evaporation and tear spillage [49]. However, this lipophilicity and that of the periocular skin [50] may potentially play a role in how coronaviruses and perhaps other enveloped viruses access the ocular surface. In the skin, extracellular surface lipids provide a barrier function [51] and while a similar function might keep viruses from accessing ocular surface receptors, tear and supracutaneous flow could result in a "second chance" for the virus to bind to receptors downstream in the lacrimal drainage pathway and beyond. The tear film, comprised of nonpolar lipids, is generally hydrophobic [52]. The coronaviruses with a crown of spike proteins around a lipid bilayer envelope [53] may be well suited to adhering to the ocular surface. Coronavirus cell entry and adhesion require lipid rafts and the presence of cell membrane cholesterol [54,55] and in a sense, the cholesterol containing tear lipid layer [56] might act as "lipid raft", facilitating initial viral adhesion. The precise nature of potential viral adhesion to the tear film requires elucidation since this represents another potential means of intervention via local rather than systemic means.

The possibility of ocular surface viral replication and transmission should be further explored given the finding of likely sustained SARS-CoV-2 replication in three patients with systemic infection and conjunctivitis [57–59]. While it was found that no viral RNA was detected in the tear fluid and conjunctival secretions of infected patients without conjunctivitis in one study, it was felt that this did not eliminate the risk of transmission via this pathway [57]. The finding that human conjunctival explant cultures were more extensively infected by SARS-CoV-2 than by SARS-CoV is also significant [46]. Taken together, these findings are also consistent with the concept that the tear film may protect the ocular surface from epithelial infection and convey virus downstream.

The nasolacrimal system, via the nasopharynx (considered crucial for viral replication [46]) thus provides a bridge between ocular and respiratory tissues, serving as a conduit for virus-containing fluid exchange between these sites [60]. Furthermore, beyond the anatomical linkage, it was recognized that the structure and distribution of cellular receptors in these systems is likely contributing to the tissue tropism of respiratory viruses [60]. Furthermore, there is respiratory-ocular mucosal immune interdependence with linkage via the nasolacrimal lymphoid tissue [6,61].

Despite early suggestions of ocular involvement [62] in this process, it appears that this was not taken into consideration in framing early guidelines for protection against infection. More recently, American Academy of Ophthalmology recommendations include protection for the mouth, nose and eyes when caring for patients potentially infected with this virus [63].

Thus, the pathophysiology of ocular surface-SARS-CoV-2 interactions requires re-evaluation, not only to determine the dynamics of ocular surface viral exposure and binding but also transmission via the lacrimal drainage system. Less pathogenic coronaviruses as well as other respiratory viruses could be used in animal models to better elucidate this relatively unexplored pathway.

Potential for intervention

Thus, the ocular surface, representing a large surface area, exposed to and likely receptive to coronavirus, bearing the appropriate SARS-CoV-2 entry factors, may be an ideal point of intervention. Efforts in directly targeting the ACE2 receptor in this way are limited [64]. Potentially, blocking the ACE2 receptor would deprive coronaviruses from their main point of tissue binding. P4 and P5 peptides and NAAE, a small molecule targeting ACE2 have been developed but there have been concerns about a narrow spectrum of activity and effects on blood pressure regulation [65]. This strategy has previously been employed, using antibodies [66] - anti-ACE2 but not anti-ACE1 antibody blocked viral replication in a model system. Blocking of TMPRSS2 protease and cathepsin B/L activity could also be considered, however it appears that ACE2, rather than protease activity, may be the viral entry rate limiting factor [45].

Possible drug interventions targeting the ACE2 receptor include ACE inhibitors and chloroquine. ACE inhibitors are widely used in the treatment of systemic diseases including hypertension and are generally well tolerated [67]. This class of drugs has not previously been considered as having an anti-viral role. Crystallography studies demonstrate that while the ACE inhibitor lisinopril binds in a region near the centre of the receptor [68,69], the virus binding sites are on the outer surface of

the receptor near the N terminal [39], so direct blocking by lisinopril of the virus attachment site seems unlikely. However, the potent ACE2 inhibitor MLN-4760 induces a large receptor conformational change, a hinge bending motion, important for both inhibitor binding and catalysis and could prove to be unfavourable for viral binding to the receptor and/or syncytial formation [69]. It was thought that metallopeptidase inhibitors such as MLN-4760 may prove useful for prevention of viral binding to ACE2 [70].

A search of FDA-approved drug libraries identified a number of drugs including chloroquine as potentially having *anti*-coronavirus actions [71] and therefore potentially able to be repurposed. Table 2 summarises the drugs that have been identified as potential treatments for coronavirus infection, their efficacy (in vitro and in vivo) and for which there is data (for that agent or a related compound) for previous topical ocular surface usage. It is apparent that while for each of these agents, there is evidence of in vitro *anti*-coronavirus activity, there is a paucity of in vivo studies. This has been raised as a matter for concern in that there are precedents for paradoxical untoward drug systemic effects that result in increased disease severity when in vitro testing alone might suggest efficacy [85].

Chloroquine has long been used in the treatment of malaria and subsequently, autoimmune disorders (such as rheumatoid arthritis) as well as in oncology and for pediatric inflammatory disease. However, chloroquine has direct antiviral effects, inhibiting pH-dependent steps of the replication of flaviviruses, retroviruses, and coronaviruses [86], exhibiting strong antiviral effects on coronavirus infection of primate cells [72,87]. The drug can be effective either before or after exposure to the virus, so could act both therapeutically and prophylactically. Several mechanisms have been proposed but of particular interest is that the drug appears to interfere with terminal glycosylation of ACE2 [87–89]. Chloroquine also has immuno-modulatory effects [86,90], suppressing the production/release of tumour necrosis factor and interleukin 6, which may mitigate the severe inflammatory cascade associated with severe COVID-19 disease [86]. Recently, hydroxychloroquine was found to be more potent than chloroquine at inhibiting SARS-CoV-2 in vitro [91], fortuitous, since it appears that lower toxicity has been attributed to this derivative [92].

A recent systematic review of clinical trials utilising chloroquine or hydroxychloroquine [74] concluded that 5/7 trials had shown favorable outcomes for patients using these drugs and 2/7 demonstrated no significant change compared to control. It was noted that all 7 trials carried varying degrees of poor study design and bias. One such study, a pilot observational study [93] showed that use of hydroxychloroquine and azithromycin demonstrated apparent improved clinical outcomes in 65/80 patients, expanding earlier work suggesting that combined therapy resulted in rapid reduction in viral load. Clearly, more robust clinical studies are needed and have been called for [74].

While systemic chloroquine and its derivatives appear to be associated with relatively minor side effects in the shorter term, electrocardiogram QT interval prolongation has been reported [94] and this may be exacerbated by combination treatment with azithromycin [74]. The well-known association with irreversible visual loss due to retinal toxicity is considered a late manifestation [95], however early-onset (~2 months) has been reported in a case where the ideal dosage was exceeded for one month [96]. A number of predisposing factors to retinal toxicity include genetic [97] - polymorphisms in the cytochrome P450 gene, drug interactions [98] and racial factors [99]), may play a role. Chloroquine's anticancer activity in concert with zinc have been explained by the fact that chloroquine acts as a zinc ionophore [100], significantly, inhibiting cellular autophagy and enhancing apoptotic cell death via inhibition of lysosome function. It transpires that zinc inhibits coronavirus polymerase activity [101], blocking viral replication. For this reason, combination treatment for Covid-19 with chloroquine and zinc has been suggested [102], although this intervention has yet to be formally reported.

Possibility of topical/local treatment for SARS-CoV-2

It is perhaps unsurprising, given the passage of time, that an understanding of viral oculotropism from the 1918 influenza epidemic has faded. Yet if SARS-CoV-2 oculotropism is confirmed, this invasion entry point also represents an opportunity for intervention. Given the potential toxicity of apparently efficacious drugs and issues relating to drug bioavailability, initially in the upper airways and respiratory system, local, topical therapy offers potential significant advantages.

Many of the proposed systemic therapies such as chloroquine, zinc and ACE inhibitors have all been used topically in the eye (Table 2). Chloroquine as 0.03% chloroquine phosphate eye drops have apparently been efficacious in the management of dry eye syndrome in humans [75, 76]. Significant side effects were not reported, however there is a report of chloroquine keratopathy in workers chronically exposed to chloroquine dust [103]. Chronic usage is unlikely in the setting here proposed.

ACE inhibitors have been used topically in animal models of glaucoma [104]. In this class of drugs, agents such as telmisartan are long acting, with a mean half-life of 24 h [105]. Zinc has traditionally been used in astringent eye drops or as an excipient, zinc sulphate (0.25%) [83]. Since chloroquine and ACE2 inhibitors seem to act at different parts of the receptor, in combination, synergy is possible or at the very least an additive effect, permitting a reduction in dosage and risk of potential side effects. However, another issue is that ACE2 inhibitors may upregulate this receptor [106], perhaps increasing the risk of coronavirus infection. Early reports have suggested that hypertension may be a risk factor for infection but these are unconfirmed [107]. Azithromycin has also previously been safely used topically in the human eye as a 1–1.5% solution to treat ocular infections [78,79]. In the case of hydroxychloroquine, preliminary calculations suggest that with topical application, a dosage one to two orders of magnitude higher than plasma levels (reached with systemic treatment [108]) could be achieved.

Conclusion

At this point, clinical studies are needed expeditiously to investigate the safety and efficacy of local prophylactic regimens following exposure to SARS-CoV-2. For instance, investigations can include initial local delivery of a combination of chloroquine, zinc and azithromycin to the eyelids and ocular surface with eye drops or a spray/aerosol. This would potentially inhibit binding of SARS-CoV-2 to the likely major entry point into the human body, would reduce the risk of systemic side effects and conserve drugs that may become scarce, since the dosage required would be much less than for systemic treatment. A spray or aerosol preparation would also allow treatment of the nasal passages [45] and mouth [109], given high ACE2 receptor populations in these locations. However, if the virus is truly oculotropic, this may be superfluous. If a

Table 2
Proposed Agents For Initial Ocular Surface *anti*-SARS-CoV-2 Intervention.

Proposed Agents For Initial Ocular Surface *anti*-SARS-CoV-2 Intervention				
Agent	Anti- SARS-CoV-2 Effect		Ocular Surface Application (for SARS-CoV-2-unrelated disease)	
	In Vivo	In Vitro	Clinical	Human	Animal
Chloroquine Phosphate	+[72]	-[a73]	±[74]	+[75,76] (0.03%)	0
OHChloroquine	+[72]	0	±[74]	0	0
Azithromycin	+[77]	0	±[77]	+[78,79] (1–1.5%	+[80,81] (0.2–1.5%)
Zinc	+aa[82]s	0	0	+[83] (0.25% as SO_4)	+[84] (0.5%)

+ denotes efficacy, - denotes inefficacy, ± denotes equivocal results, 0 denotes lack of studies.

[a] For SARS-CoV.

patient has active infection of the respiratory tree, then this combined medication could also be given in an inhaled form. The advantage of this ACE2 targeting approach is that ACE2 receptors from the outer cornea, through the lacrimal drainage system to the respiratory and gastrointestinal systems as well as both nasal and oral cavities, are directly accessible to a high topical drug dose. Thus, the portals that make us susceptible to coronavirus attack and invasion can be used to provide protection that is likely to be safe, extensive, convenient and at low cost. The ocular surface, offering all of the benefits of local treatment familiar to ophthalmologists, may thus provide a convenient testing ground for rapid, safe and likely cost-effective trials of newer drugs that may be developed to combat this modern plague.

Declaration of competing interest

The author has filed patent applications on the treatment methods and concerning slit lamp design, described in this paper.

Acknowledgments

References

[1] Perlman S. Another decade, another coronavirus. N Engl J Med 2020;382:760–2.

[2] Green A. Li Wenliang. Obituary. Lancet 2020;395:682.

[3] accessed, https://www.msn.com/en-us/news/world/chinese-doctor-who-issued-early-warning-on-virus-dies/ar-BBZJrw6?ocid=spartandhp. [Accessed 8 February 2020].

[4] Lu Cheng-wei, Xiu-fen Liu, Jia Zhi-fang. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet 2020;395(10224):e39.

[5] Maxcy KF. The transmission of infection through the eye. J Am Med Assoc 1919;72:636–9.

[6] Belser JA, Rota PA, Tumpey TM. Ocular tropism of respiratory viruses. Microbiol Mol Biol Rev 2013;77:144–56.

[7] Belser JA, Lash RR, Garg S, Tumpey TM, Maines TR. The eyes have it: influenza virus infection beyond the respiratory tract. Lancet Infect Dis 2018;18:e220–7.

[8] Belser JA, Davis CT, Balish A, Edwards LE, Zeng H, Maines TR, Gustin KM, Martínez IL, Fasce R, Cox NJ, Katz JM, Tumpey TM. Pathogenesis, transmissibility, and ocular tropism of a highly pathogenic avian influenza A (H7N3) virus associated with human conjunctivitis. J Virol 2013;87:5746–54.

[9] Chandra N, Frängsmyr L, Imhof S, Caraballo R, Elofsson M, Arnberg N. Sialic acid-containing glycans as cellular receptors for ocular human adenoviruses: implications for tropism and treatment. Viruses 2019;11(5):395.

[10] Eye hospital eye. Lancet. 1983;2:1065–6.

[11] Accessed, https://www.enttoday.org/article/otolaryngologists-may-contract-covid-19-during-surgery/. [Accessed 6 April 2020].

[12] Chu CM, Poon LL, Cheng VC, et al. Initial viral load and the outcomes of SARS. CMAJ (Can Med Assoc J) 2004;171:1349–52.

[13] Von Haugwitz T. The history of optical instruments for the examination of the eye. Hirshberg's history of ophthalmology, eleven. Part 2. Bonn : Wayenborgh: Translated Frederick C. Blodi; 1986.

[14] Bjørn E, Nielsen PV. Dispersal of exhaled air and personal exposure in displacement ventilated rooms. Indoor Air 2002;12:147–64.

[15] Sobolewska B, Buhl M, Liese J, Ziemssen F. Slit lamps and lenses: a potential source of nosocomial infections? Eye 2018;32:1021–7.

[16] OYong K, Killerby M, Pan CY, et al. Outbreak of epidemic keratoconjunctivitis caused by human adenovirus type D53 in an eye Care clinic - los angeles county, 2017. MMWR Morb Mortal Wkly Rep 2018;67:1347–9.

[17] Chams H, Mohammadi SF, Moayyeri A. Frequency and assortment of self-report occupational complaints among Iranian ophthalmologists: a preliminary survey. MedGenMed 2004;6(4):1.

[18] Qader UJA. The prevalence of adenoviral conjunctivitis in Tikrit district and the role of traditional medicine "OM AL KAASH" in transmission of the disease and in the severity of complications. Tikrit Medical Journal 2011;17:30–40.

[19] Chan WM, Liu DT, Chan PK, et al. Precautions in ophthalmic practice in a hospital with a major acute SARS outbreak: an experience from Hong Kong. Eye 2006;20:283–9.

[20] Li JO, Lam DSC, Chen Y, Ting DSW. Novel Coronavirus disease 2019 (COVID-19): the importance of recognising possible early ocular manifestation and using protective eyewear. Br J Ophthalmol 2020;104:297–8.

[21] Li Q, Guan X, Wu P, et al. Early transmission dynamics in wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020;382:1199–207.

[22] Peng X, Xu X, Li Y, Cheng L, Zhou X, Ren B. Transmission routes of 2019-nCoV and controls in dental practice. Int J Oral Sci 2020;12:9.

[23] WHO | Update 27 - one month into the global SARS outbreak: status of the outbreak and lessons for the immediate future Accessed 6 April 2020.

[24] Tellier R, Li Y, Cowling BJ, Tang JW. Recognition of aerosol transmission of infectious agents: a commentary. BMC Infect Dis 2019;19:101.

[25] Zaman ML, Doughty MJ, Button NF. The exposed ocular surface and its relationship to spontaneous eyeblink rate in elderly caucasians. Exp Eye Res 1998;67:681–6.

[26] Sotoyama M, Villanueva MBG, Jonai H, Saito S. Ocular surface area as an informative index of visual ergonomics. Ind Health 1995;3:43–56.

[27] Ehlers N. On the size of the conjunctival sac. Acta Ophthalmol 1965;43:205–10.

[28] Watsky MA, Jablonski MM, Edelhauser HF. Comparison of conjunctival and corneal surface areas in rabbit and human. Curr Eye Res 1988;7:483–6.

[29] Ng A, Evans K, North RV, Purslow C. Migration of cosmetic products into the tear film. Eye Contact Lens 2015;41:304–9.

[30] MacKeen DL, Roth HW, Doane MG, MacKeen PD. Supracutaneous treatment of dry eye patients with calcium carbonate. Adv Exp Med Biol 1998;438:985–90.

[31] Tsubota K, Monden Y, Yagi Y, Goto E, Shimmura S. New treatment of dry eye: the effect of calcium ointment through eyelid skin delivery. Br J Ophthalmol 1999;83:767–70.

[32] Özer CM, Öz II, Şerifoğlu I, Büyükuysal MÇ, Ç Barut. Evaluation of eyeball and orbit in relation to gender and age. J Craniofac Surg 2016;27:e793–800.

[33] Kanjani V, Rani A, Kanjani D. Morphometric analysis of the orbital aperture in north Indian population: a retrospective digital forensic study. Int J Appl Basic Med Res 2019;9(2):85–8.

[34] Amador GJ, Mao W, DeMercurio P, et al. Eyelashes divert airflow to protect the eye. J R Soc Interface 2015;12:20141294.

[35] Na JI, Kwon OS, Kim BJ, Park WS, Oh JK, Kim KH, Cho KH, Eun HC. Ethnic characteristics of eyelashes: a comparative analysis in Asian and Caucasian females. Br J Dermatol 2006;155:1170–6.

[36] Tayyari F, Marchant D, Moraes TJ, Duan W, Mastrangelo P, Hegele RG. Identification of nucleolin as a cellular receptor for human respiratory syncytial virus. Nat Med 2011;17:1132–5.

[37] Xu X, Chen P, Wang J, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 2020;63:457–60.

[38] Zhao, Y., Zhao, Z., Wang, Y., Zhou, Y., Ma, Y., Zuo, W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. bioRxiv 2020.01. vol. 26.919985; doi: https://doi.org/10.1101/2020.01.26.919985.

[39] Wu K, Li W, Peng G, Li F. Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. Proc Natl Acad Sci U S A 2009;106:19970–4.

[40] Li W, Sui J, Huang IC, Kuhn JH, et al. The S proteins of human coronavirus NL63 and severe acute respiratory syndrome coronavirus bind overlapping regions of ACE2. Virology 2007;367:367–74.

[41] Zhou P, Yang X, Wang X, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3.

[42] Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004;203:631–7.

[43] Jia HP, Look DC, Shi L, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. J Virol 2005;79:14614–21.

[44] Savaskan E, Löffler KU, Meier F, Müller-Spahn F, Flammer J, Meyer P. Immunohistochemical localization of angiotensin-converting enzyme, angiotensin II and AT1 receptor in human ocular tissues. Ophthalmic Res 2004;36:312–20.

[45] Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med 2020 Apr 23. https://doi.org/10.1038/s41591-020-0868-6. Epub ahead of print.

[46] Hui KPY, Cheung MC, Perera RAPM, et al. Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. Lancet Respir Med 2020 May 7:30193–4. S2213-2600(20.

[47] van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, Wertheim-van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B. Identification of a new human coronavirus. Nat Med 2004;10:368–73.

[48] Vabret A, Mourez T, Dina J, et al. Human coronavirus NL63, France. Emerg Infect Dis 2005;11:1225–9.

[49] Clayton JA. Dry Eye. N Engl J Med. 2018;378:2212–23.

[50] Pappas A. Epidermal surface lipids. Dermatoendocrinol 2009;1:72–6.

[51] Chapman SJ, Walsh A, Jackson SM, Friedmann PS. Lipids, proteins and corneocyte adhesion. Arch Dermatol Res 1991;283:167–73.

[52] Butovich IA. Tear film lipids. Exp Eye Res 2013;117:4–27.

[53] Rey FA, Lok SM. Common features of enveloped viruses and implications for immunogen design for next-generation vaccines. Cell 2018;172:1319–34.

[54] Choi KS, Aizaki H, Lai MM. Murine coronavirus requires lipid rafts for virus entry and cell-cell fusion but not for virus release. J Virol 2005;79:9862–71.

[55] Glende J, Schwegmann-Wessels C, Al-Falah M, Pfefferle S, Qu X, Deng H, Drosten C, Naim HY, Herrler G. Importance of cholesterol-rich membrane microdomains in the interaction of the S protein of SARS-coronavirus with the cellular receptor angiotensin-converting enzyme 2. Virology 2008;381:215–21.

[56] Dartt DA, Willcox MD. Complexity of the tear film: importance in homeostasis and dysfunction during disease. Exp Eye Res 2013;117:1–3.

M.T. Coroneo

[57] Xia J, Tong J, Liu M, et al. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J Med Virol 2020 Feb 26. https://doi.org/10.1002/jmv.25725 [Epub ahead of print] [PMID: 32100876] doi:.

[58] Colavita F, Lapa D, Carletti F, et al. SARS-CoV-2 isolation from ocular secretions of a patient with COVID-19 in Italy with prolonged viral RNA detection. Ann Intern Med 2020 Apr 17:M20–1176.

[59] Chen L, Liu M, Zhang Z, et al. Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. Br J Ophthalmol 2020 Apr 7. https://doi.org/10.1136/bjophthalmol-2020-316304. bjophthalmol-2020-316304.

[60] Paulsen F. Functional anatomy and immunological interactions of ocular surface and adnexa. Dev Ophthalmol 2008;41:21–35.

[61] Chentoufi AA, Dasgupta G, Nesburn AB, et al. Nasolacrimal duct closure modulates ocular mucosal and systemic CD4(+) T-cell responses induced following topical ocular or intranasal immunization. Clin Vaccine Immunol 2010; 17:342–53.

[62] Chan WM, Yuen KS, Fan DS, Lam DS, Chan PK, Sung JJ. Tears and conjunctival scrapings for coronavirus in patients with SARS. Br J Ophthalmol 2004;88:968–9.

[63] accessed, https://www.aao.org/headline/alert-important-coronavirus-context. [Accessed 31 March 2020].

[64] Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. Drug Dev Res 2020 Mar 4. https://doi.org/10.1002/ddr.21656. Epub ahead of print. PMID: 32129518.

[65] Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses - drug discovery and therapeutic options. Nat Rev Drug Discov 2016;15:327–47.

[66] Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003;426:450–4.

[67] Abraham HM, White CM, White WB. The comparative efficacy and safety of the angiotensin receptor blockers in the management of hypertension and other cardiovascular diseases. Drug Saf 2015;38:33–54.

[68] Natesh R, Schwager SL, Sturrock ED, Acharya KR. Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature 2003;421:551–4.

[69] Wu K, Chen L, Peng G, Zhou W, Pennell CA, Mansky LM, Geraghty RJ, Li F. A virus-binding hot spot on human angiotensin-converting enzyme 2 is critical for binding of two different coronaviruses. J Virol 2011;85:5331–7.

[70] Towler P, Staker B, Prasad SG, et al. ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. J Biol Chem 2004; 279:17996–8007.

[71] de Wilde AH, Jochmans D, Posthuma CC, et al. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. Antimicrob Agents Chemother 2014;58:4875–84.

[72] Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30: 269–71.

[73] Barnard DL, Day CW, Bailey K, et al. Evaluation of immunomodulators, interferons and known in vitro SARS-coV inhibitors for inhibition of SARS-coV replication in BALB/c mice. Antivir Chem Chemother 2006;17:275–84.

[74] Chowdhury MS, Rathod J, Gernsheimer J. A rapid systematic review of clinical trials utilizing chloroquine and hydroxychloroquine as a treatment for COVID-19. Acad Emerg Med 2020 May 2. https://doi.org/10.1111/acem.14005.Mar9: ciaa237.

[75] Bhavsar Ankita Samir, Bhavsar Samir G, Jain Sunita M. Evaluation of the effects of chloroquine phosphate eye drops in patients with dry eye syndrome. Int J Biomed Adv Res 2011;2:198–214.

[76] Titiyal JS, Kaur M, Falera R, Bharghava A, Sah R, Sen S. Efficacy and safety of topical chloroquine in mild to moderate dry eye disease. Curr Eye Res 2019;44: 1306–12.

[77] Damle B, Vourvahis M, Wang E, et al. Clinical pharmacology perspectives on the antiviral activity of azithromycin and use in COVID-19. Clin Pharmacol Ther 2020 Apr 17. https://doi.org/10.1002/cpt.1857.

[78] Opitz DL, Harthan JS. Review of azithromycin ophthalmic 1% solution (AzaSite (®)) for the treatment of ocular infections. Ophthalmol Eye Dis 2012;4:1–14.

[79] Amza A, Goldschmidt P, Einterz E, et al. Elimination of active trachoma after two topical mass treatments with azithromycin 1.5% eye drops. PLoS Neglected Trop Dis 2010;4(11):e895.

[80] Kuehne JJ, Yu AL, Holland GN, et al. Corneal pharmacokinetics of topically applied azithromycin and clarithromycin. Am J Ophthalmol 2004;138:547–53.

[81] Tabbara KF, Kotb AA, Hammouda EF, et al. Effects of dehydration on corneal tissue absorption of topical azithromycin in rabbits. Curr Eye Res 2005;30:915–8.

[82] te Velthuis AJ, van den Worm SH, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS Pathog 2010;6(11):e1001176.

[83] Hubbard GB, Herron BE, Andrews JS, Elliott JH. Influence of topical and oral zinc upon corneal wound healing. Br J Ophthalmol 1969;53:407–11.

[84] Bellows JG. Influence of local antiseptics on regeneration of corneal epithelium of rabbits. Arch Ophthalmol 1946;36:70–81.

[85] Sharma A. Chloroquine paradox may cause more damage than help fight COVID-19. Microb Infect 2020;(20):S1286–4579. 30071-X.

[86] Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect Dis 2003;3: 722–7.

[87] Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2005;2:69.

[88] Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun 2004;323:264–8.

[89] Savarino A, Di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. Lancet Infect Dis 2006;6:67–9.

[90] Perricone C, Triggianese P, Bartoloni E, et al. The anti-viral facet of anti-rheumatic drugs: lessons from COVID-19. J Autoimmun 2020 Apr 17:102468.

[91] Yao X, Ye F, Zhang M, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2020:ciaa237. https://doi.org/10.1093/cid/ciaa237.

[92] Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discovery 2020;6:16–9.

[93] Gautret P, Lagier JC, Parola P, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: a pilot observational study. Trav Med Infect Dis 2020 Apr 11:101663.

[94] Saleh M, Gabriels J, Chang D, et al. The effect of chloroquine, hydroxychloroquine and azithromycin on the corrected QT interval in patients with SARS-CoV-2 infection. Circ Arrhythm Electrophysiol; 2020 Apr 29.

[95] Yusuf IH, Sharma S, Luqmani R, Downes SM. Hydroxychloroquine retinopathy. Eye 2017;31:828–45.

[96] Pasaoglu I, Onmez FE. Macular toxicity after short-term hydroxychloroquine therapy. Indian J Ophthalmol 2019;67:289–92.

[97] Shroyer NF, Lewis RA, Lupski JR. Analysis of the ABCR (ABCA4) gene in 4-ami-noquinoline retinopathy: is retinal toxicity by chloroquine and hydroxychloroquine related to Stargardt disease? Am J Ophthalmol 2001;131: 761–6.

[98] Hedner T, Samuelsson O, Währborg P, Wadenvik H, Ung KA, Ekbom A. Nabumetone: therapeutic use and safety profile in the management of osteoarthritis and rheumatoid arthritis. Drugs 2004;64:2315–43. ; discussion 2344-5.

[99] Giocanti-Aurégan A, Couturier A, Girmens JF, Le Mer Y, Massamba N, Barreau E, Audo I, DHU Vision Handicaps task force rétine. Différences ethniques parmi les patients souffrant de toxicité maculaire aux antipaludéens de synthèse [Variability of chloroquine and hydroxychloroquine retinopathy among various ethnicities]. J Fr Ophtalmol 2018;41:363–7.

[100] Xue J, Moyer A, Peng B, Wu J, Hannafon BN, Ding WQ. Chloroquine is a zinc ionophore. PloS One 2014;9:e109180.

[101] Kaushik N, Subramani C, Anang S, et al. Zinc salts block hepatitis E virus replication by inhibiting the activity of viral RNA-dependent RNA polymerase. J Virol 2017;91. e00754-17.

[102] Shittu MO, Afolami OI. Improving the efficacy of Chloroquine and Hydroxychloroquine against SARS-CoV-2 may require Zinc additives - a better synergy for future COVID-19 clinical trials. Inf Med 2020;28:192–7. Ahead of print.

[103] Eriksen LS. Chloroquine keratopathy in chloroquine workers after topical dust exponation. A case report. Acta Ophthalmol 1979;57:823–5.

[104] Watkins RW, Baum T, Cedeno K, et al. Topical ocular hypotensive effects of the novel angiotensin converting enzyme inhibitor SCH 33861 in conscious rabbits. J Ocul Pharmacol 1987;3:295–307.

[105] Burnier M, Maillard M. The comparative pharmacology of angiotensin II receptor antagonists. Blood Pres Suppl 2001;1:6–11.

[106] Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension 2004;43:970–6.

[107] Leung C. Clinical features of deaths in the novel coronavirus epidemic in China. Rev Med Virol 2020 Mar 16:e2103. https://doi.org/10.1002/rmv.2103. Epub ahead of print. PMID: 32175637.

[108] Tett SE, Cutler DJ, Day RO, Brown KF. Bioavailability of hydroxychloroquine tablets in healthy volunteers. Br J Clin Pharmacol 1989;27:771–9.

[109] Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, Li T, Chen Q. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020;12(1):8.