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 promising.

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 interventions, which work at least in part by blocking ACE2 receptors, may offer a safe, cost-effective and resource-sparing intervention.
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 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 lacrimary-nasal mechanism for the mechanical disposition of organisms entering the upper respiratory tract [5].

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.
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 mm² [25] and 300–640 mm² [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 ~1600–1869 sq. mm/eye [27,28], including the cornea, this representing a maximal absorptive area of ~3738 sq.mm, accounting for the pericoroidal conjunctiva and including the palpebral fornices. Even this consideration underestimates the potential ocular/periacular 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 periorcular eye lid skin to the eye (obviating the need for eyedrops) 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 mm² and that of the brow to be ~3000 mm², so that in total with the ocular surface area, a landing site of ~10,000 mm² 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

Table 1

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Palpebral Aperture mm²</th>
<th>Total Ocular Surface Area mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methodology Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>Unreported</td>
<td>3200</td>
</tr>
<tr>
<td>3200</td>
<td>Designed instrument</td>
<td></td>
</tr>
<tr>
<td>226–426</td>
<td>Digitized video images</td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>Mold of cadaver eyes</td>
<td></td>
</tr>
<tr>
<td>1793–1987</td>
<td>CT Scan</td>
<td></td>
</tr>
<tr>
<td>2509 (estim)</td>
<td>Radiographs</td>
<td></td>
</tr>
</tbody>
</table>

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. The breathing zone of mannequin the left is again penetrated but its closer proximity to the exhaled smoke would suggest a greater degree of breathing zone penetration [14].

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].
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 lacrimary-nasal mechanism (Figs. 1 and 2), the upper respiratory tract of a patient 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 pericorneal 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 catal-
ysis and could prove to be unfavourable for viral binding to the receptor
and/or syncytial formation [69]. It was thought that metalloproteinase
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 summa-
rises 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 sig-
nificant 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
chloroquine dust [103]. Chronic usage is unlikely in the setting here proposed.

ACE inhibitors have been used topically in animal models of glau-
coma [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 ACE 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

| Proposed Agents For Initial Ocular Surface Anti-SARS-CoV-2 Intervention. |

<table>
<thead>
<tr>
<th>Agent</th>
<th>Anti- SARS-CoV-2 Effect</th>
<th>Ocular Surface Application (for SARS-CoV-2-unrelated disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In Vivo</td>
<td>In Vitro</td>
</tr>
<tr>
<td>Chloroquine Phosphate</td>
<td>+/−2</td>
<td>+/−7</td>
</tr>
<tr>
<td>OHChloroquine</td>
<td>+/−2</td>
<td>0</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>+/−7</td>
<td>0</td>
</tr>
<tr>
<td>Zinc</td>
<td>+/as²</td>
<td>0</td>
</tr>
</tbody>
</table>

+ denotes efficacy, - denotes inefficacy, ± denotes equivocal results, 0 denotes lack of studies.
² For SARS-CoV.
patient has active infection of the respiratory tract, 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 protection that is likely to be safe, extensive, convenient and at low cost.

The ocular surface must not be ignored. Lancet 2020;395(10224):e39.

Declaration of competing interest

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

Acknowledgments

The very helpful suggestions and encouragement from Professor Ivan R Schwab MD, FACS and Dr Steven G Safran MD, are gratefully acknowledged.

The kera-net group provided a critical commentary of this evolving field during the writing of the manuscript.

References


