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Natural protection of ocular surface from viral infections – A hypothesis

Keith Zimmerman[a], Fiona Kearns[b], Radouil Tzekov[a,c,d,*]

[a] Department of Ophthalmology, University of South Florida, Tampa, FL, USA
[b] Department of Chemistry, University of South Florida, Tampa, FL, USA
[c] Department of Pharmaceutical Sciences, University of South Florida, Tampa, FL, USA
[d] Department of Medical Engineering, University of South Florida, Tampa, FL, USA

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ABSTRACT

A pandemic outbreak of a viral respiratory infection (COVID-19) caused by a coronavirus (SARS-CoV-2) prompted a multitude of research focused on various aspects of this disease. One of the interesting aspects of the clinical manifestation of the infection is an accompanying ocular surface viral infection, viral conjunctivitis. Although occasional reports of viral conjunctivitis caused by this and the related SARS-CoV virus (causing the SARS outbreak in the early 2000s) are available, the prevalence of this complication among infected people appears low (~1%). This is surprising, considering the recent discovery of the presence of viral receptors (ACE2 and TMPRSS2) in ocular surface tissue. The discrepancy between the theoretically expected high rate of concurrence of viral ocular surface inflammation and the observed relatively low occurrence can be explained by several factors. In this work, we discuss the significance of natural protective factors related to anatomical and physiological properties of the eyes and preventing the deposition of large number of virus-loaded particles on the ocular surface. Specifically, we advance the hypothesis that the standing potential of the eye plays an important role in repelling aerosol particles (microdroplets) from the surface of the eye and discuss factors associated with this hypothesis, possible ways to test it and its implications in terms of prevention of ocular infections.

Background

Ocular surface infections caused by viruses are a considerable public health problem worldwide. For example, an analysis of the 1985 National Ambulatory Medical Care Survey found that ~1% of all primary care office visits in the United States were related to conjunctivitis [1]. If one assumes that the proportion remained the same, that would translate to ~2.3 M visits in 2019. Similar proportion has been reported for UK [2]. Infectious conjunctivitis[1] is more prevalent than the other types (e.g. allergic, chemical, etc.) and viral conjunctivitis is estimated to be the most common cause of infectious conjunctivitis, at up to 80% of all cases [3].

Of all different types of viruses as candidates of causing infectious conjunctivitis, adenoviruses have been found to be the most common pathogen, in up to 90% of all cases [4]. However, it has to be kept in mind that exhaustive testing related to the causation of conjunctivitis is not always possible, which may introduce bias in reported results. Apart from adenoviruses, other types of viruses reported to be isolated from the conjunctiva and implicated in the development of conjunctivitis are herpes simplex virus, enterovirus 70 and coxsackie A24 variant virus [5].

Somewhat surprisingly, the majority of the viruses responsible for respiratory viral infections are not a major cause of infectious conjunctivitis [6]. Thus, even the adenovirus causing the majority of infectious conjunctivitis cases is subtype D, while strains that cause respiratory infections are subtypes B, C and E and they rarely cause conjunctivitis (Table 1 in [6]). Similarly, only one of the influenza viruses subtypes (H7, causing avian influenza), is a significant cause of conjunctivitis, with ~80% of the cases with this infection presenting with conjunctivitis [7], while the much more common subtypes causing human infection with influenza virus subtypes that are more commonly associated with respiratory illness are an infrequent cause of the disease [6].

Recently, the worldwide attention has been focused on a viral

* Corresponding author at: Department of Ophthalmology, University of South Florida, 12901 Bruce B. Downs Boulevard, MDC 021, Tampa, FL 33612, USA.
E-mail address: rtzekov@usf.edu (R. Tzekov).

[1] In this work, we will use "infectious conjunctivitis" as an umbrella term to denote ocular surface inflammation caused by a viral infection; however, in reality, the inflammation of the ocular surface often involves more than just the conjunctiva, and can spread over the cornea, a condition which is described as "kerato-conjunctivitis" and rarely can involve only the cornea, a condition described as "keratitis".

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infection caused by a coronavirus, resulting in a global pandemic. This infection is most often associated with respiratory symptoms and its major complication is overwhelmingly a disease of the lower respiratory tract, a bilateral viral pneumonia [8] and, therefore, can be classified as a respiratory viral infection. It is caused by a coronavirus (SARS-CoV-2) [9], while the disease has been named COVID-19 [10]. This virus belongs to the subfamily of coronaviruses, a term proposed in 1968 to describe a group of enveloped, positive-sense, single-stranded RNA viruses with similar form, including a characteristic appearance of the envelope glycoproteins in electron microscopy observation, recalling the solar corona [11]. This proposal was accepted and currently this type of viruses are classified as subfamily Orthocoronavirinae (of family Coronaviridae) and contains 4 major groups (genera): alpha-, beta-, gamma- and deltacoronaviruses, a total of 25 species, the majority of which are found in animals, but not humans [12]. The viruses found either exclusively or not in humans typically cause respiratory tract illnesses [13]. Seven types of coronaviruses are known to cause disease in humans. The two types that were discovered in the 1960s, HCoV-OC43 (a betacoronavirus) and HCoV229E (an alphacoronavirus), were both causing mostly mild upper respiratory tract illnesses in adults. They are still in circulation, together with three types identified in the 2000s: HCoV-NL63, HCoV-HKU1 (both alphacoronaviruses) and MERS-CoV (a betacoronavirus), the first two casing mild upper and lower respiratory tract infections in adults, while the third one causing more severe infections. Another virus, SARS-CoV (a betacoronavirus), identified also in the early 2000s, causing severe acute respiratory syndrome (SARS), is no longer circulating in humans after strict and coordinated public health measures [13]. The new virus, SARS-CoV-2, has closest similarity (40–90% identity) to the first SARS-CoV virus [14] and shows similar clinical manifestations, although with a lower mortality rate [15].

Generally, coronaviruses infecting humans have been rarely associated with ocular surface infections [6]. Only rare reports of conjunctivitis have been associated with HCoV-NL63 [16] and no reports have been presented for SARS-CoV [17]. Whether and to what extent these types of viruses can be spread through ocular surface exposure remains a subject of debate and uncertainty. For example, the SARS-CoV virus was detected in the conjunctiva from 3 probable cases (out of 36) in one study [18], but not in two other studies in tears and conjunctival scraping samples [19,20], triggering questions about the mechanism and details of identification of the virus in ocular tissues and fluids [21,22]. Two recent studies by Xia et al. [23] and Wu et al. [24], showed also a low rate of virus detection in conjunctiva (4%). A recent meta-analysis including 1,167 patients, indicates that frequency of conjunctivitis associated with SARS-CoV-2 infection (COVID-19) was generally low: ~1.1% (3% in severe and 0.7% in nonsevere COVID-19 patients) [25].

On the other hand, two recent reports showed expression of established SARS-CoV [26] and SARS-CoV-2 [27] receptors, the angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine subtype 2 (TMPRSS2) in human conjunctiva, limbus and cornea [28,29]. This finding indicates that ocular surface cells including in the conjunctiva could be susceptible to the virus and theoretically serve as a point of entry for the viral infection. Therefore, a question arises: if the receptors for the virus are present in ocular surface tissues, why the incidence of ocular surface infection is so low and isolating the virus from ocular surfaces presents such a challenge?

Hypothesis

Our hypothesis is that the standing potential of the eye interacts with microdroplets carrying the virus and prevents – either partially or in whole – microdroplets from landing and attaching to the ocular surface. Such an interaction would greatly reduce the probability of virus presence on, and in turn reduce infections in, ocular surface tissues.

The fact that the eye is electrically charged was first demonstrated in an animal eye by du Bois-Reymond in 1849 [30] and re-discovered by Frithiof Holmgren in 1865 in a fish eye [31]. The first rigorous confirmation of the standing potential of the eye as a corneo-retinal potential in humans was provided by Mowerer et al. in 1935 [32]. In the 1960s, detailed investigation of the magnitude of the standing potential under standardized light-adapted conditions showed that it is on average ~+0.7 mV (range 0.25 to 1.1 mV), with the cornea having a positive charge compared to the back of the eye [33] and it was shown that this potential is relatively stable on an hourly, daily and weekly basis, but shows some diurnal variation. It has to be noted that these measurements were made indirectly with electrodes placed on both sides of the eyeball, at the outer and inner canthus of the eye, and, therefore, the true electrical potential in front of the cornea is likely to be higher (+5 mV), which was suspected since the 1940s based on animal studies and confirmed in humans in the 1970s with more direct laboratory measurements [34]. It was estimated further that if the corneal potentials were measured relative to the skin on the forehead or cheek, the potential difference would be even higher at +10 to +15 mV [35]. This potential could be further enhanced by electrostatic charges on the eyelid skin. It is well-documented that human oily skin can become highly positively electrostatically charged, as it is usually listed near the top of the triboelectric series [36].

Discussion

Viral presence in air samples

Bioaerosols generated by human exhalation are considered a possible route for SARS-CoV-2 spread [37–40]. In support of this possibility, previous studies testing of air samples showed the presence of another coronavirus (MERS-CoV) have found the virus in 4 of 7 air samples [41]. Studies of the SARS-CoV virus also suggested airborne transmission [42] and infective droplet inhalation [43], although some uncertainty remains [44]. Influenza virus RNA was also recovered from air sampled in a hospital [45]. Finally, a recent study identified seasonal human coronaviruses, influenza viruses and rhinoviruses in exhaled breath and coughs of patients with respiratory disease [46].

Factors influencing the interaction between bioaerosol and ocular surface

bioaerosol particle size

Currently, it is generally accepted that bioaerosols as an infectious disease transmission medium should be divided into three groups based on aerodynamic droplet diameter: small particles (less than 10 μm), that can remain airborne, larger droplets (more than 20 μm), which settle relatively quickly to the ground and an intermediate size (10–20 μm) may either settle or remain airborne [47]. Apart from the consensus built around this reasonable classification, there is a great deal of variation within the range and distribution of particle size reported by different studies, which likely depends largely on the methodology used to count and classify the particles. Thus, the following discussion of this topic should not be treated as a comprehensive analysis of this subject, but rather as one specific perspective on the reported results in view of the matter discussed here.

The measurement of the size of exhaled particles in a 2011 study showed that more than 82% of all exhaled particles from three healthy and 16 human rhinovirus (HRV)-infected subjects were within 0.3–0.5 μm diameter range, placing it firmly in the small droplets category [48]. Given that the average diameter of the SARS-CoV-2 virus is ~0.12 μm [49,50] and assuming that the maximal viral concentration per droplet should be an occupancy of ~30% of the whole surface of the droplet[2], the maximal viral load per particle can be estimated between

[2] Higher spatial arrangement of the viruses may be prevented by electrostatic

8 and 21 virions/droplet[3]. Although this appears like a small load per droplet, the same study found that the droplet production varied dramatically between subjects, with some subjects (4 out of 17) producing ~3500 particles/liter (range ~1000 to ~7000), equivalent to ~28,000 droplets/min, with the potential to spread more than 500,000 viral particles/min via airborne particles. In contrast, the rest of the subjects produced on average ~7.4 particles/liter (equivalent to ~52 droplets/min with some participants generating 0 droplets), thus, supporting the hypothesis that some people could be "high spreaders" of the viral infection. It should be emphasized that this study estimated the droplet size and production under regular breathing. The result is likely different when sneezing. A study estimating the droplet sizes from sneezing found almost exclusively large droplets with diameter larger than 50 μm (and up to 1,000 μm) [51]. For comparison, cough in healthy volunteers appeared to generate particles with less than 1 μm in diameter [52]. Some studies suggest that large droplets only originate from the oral cavity [53]. Although studies with coronavirus are not available, one study measured influenza virus in cough samples and found that 35% of the influenza RNA was isolated from particles > 4 μm diameter, while 23% of influenza RNA was isolated from particles 1 to 4 μm diameter, and 42% in particles < 1 μm [54]. High spreaders for the influenza virus were confirmed in another study [55]. The size and viral load have potential importance for the probability of landing on ocular surfaces. Thus, larger droplets, loaded with more viral particles are expected to have poorer aerodynamic characteristics and be more susceptible to gravity forces and quicker landing. Given lower airflow speed under normal breathing conditions, the main type of viral airborne spread seems to be small droplets, which can stay afloat for many minutes to hours, but carry relatively little viral load per droplet. As the intensity of exhaled airflow increases, as in loud talking, singing, coughing and sneezing, the droplet formation becomes more and more dominated by larger and larger droplets with high viral load per droplet and high initial velocity, but shorter airborne time.

Bioaerosol composition

Water. Although it is assumed that bioaerosol contains some amount of water, the exact water content of bioaerosol microdroplets is difficult to determine and probably varies a lot, depending on the relative humidity and temperature of ambient air. Most physiological models of human breathing assume 100% relative humidity and 36-37° C in the air of the lower respiratory tract, in accordance with experimental data [56,57]. With expiration (tidal breathing and room temperature air), some of this water content is reabsorbed in the upper respiratory tract, where the temperature is assumed to be ~32 °C below the glottis [57] and vary in the naso-pharyngeal cavity, from ~25 °C in the nasal vestibule, to ~30 °C in the internal nasal valve area and ~32 °C in the middle turbinate[58,59], with some differences (~2°C) between the air and nasal mucosa, allowing for an efficient water reabsorption during exhaling, but also providing favorable conditions for condensation and droplet formation. These values change with change in ambient air temperature, as inhalation of cold air lowers the temperature in both the upper [60] and lower respiratory tract [57], thus further improving the conditions for water condensation during exhalation. Once the bioaerosol leaves the human body, several factors will affect the water content, probably the most important one being change in droplet size, influenced by the rate of evaporation[61], interaction with each other (coalescence or fragmentation[62]) and relative position in relation to the center of airflow[63]. These effects are complex and still insufficiently understood in the case of exhaled aerosol.

Organic components. The main organic component of exhaled human air is an airway lining fluid component, which is highly diluted in water, with a consensus estimate for dilution between 2,000 to 10,000 times[64,65]. The airway lining fluid likely contains various types of lipids and electrolytes. Thus, a recent study identified 75 glycerophospholipids, 13 sphingolipids, 5 sterol lipids and 12 neutral glycerolipids in samples from healthy volunteers[66], with a large variation in composition between subjects, underscoring the complexity of organic content of exhaled air condensate.

Environmental conditions

Within this context, another phenomenon should be pointed out, namely the effect of temperature and relative humidity. Two aspects should be mentioned. First, natural evaporation from the lungs depends on temperature and humidity – at relatively low ambient air temperature (~2° C), water loss depends very little on relative humidity (2.0–2.2 ml H_2O per L/breath/min), but at higher temperature (~27° C), the range increases dramatically (0.5–2.1 ml H_2O per L/breath/min) with much less water loss in more humid air [67]. The second aspect to be mentioned is the water loss at low relative humidity, which is substantial [68]. Both factors would apply to most modern air-conditioned indoor environments, where the temperature is kept typically in the range of 20-23° C and humidity at ~40%.

Natural and artificial airflow restrictions

The electrical potential value of the eye does not seem very strong as a generator of an electrostatic repelling force (Coulombic force), but it has to be kept in mind that the volume of space to be protected from particles in front of the cornea is not large, particles need only be deflected less than 1 cm in front of the corneal surface, as the thickness of the eyelid margins is only about 2 mm [69]. Considering that ocular surface area exposed to air is typically 1.25–1.75 cm^2 [70–72] (although it can reach intermittently up to 3 cm^2, depending on the visual task), the "protected volume of air"[4] in front of the cornea is probably less than 0.4 cm^3 and does not exceed 0.75 cm^3.

Furthermore, human eyelashes act as passive dust controlling system and reduce evaporation and particle deposition up to 50% [73], further facilitating the defense against foreign particles (including microdroplets). What is the effect of blinking on the airflow within the "protected volume of air" is currently unknown, however, it can be speculated that it would create some change in low-velocity airflow, as it was estimated that blinking increases corneal temperature by 1.3° C [74] and this could create convection air microcurrents away from the corneal surface. The speed and direction of local airflow can be further modified by artificial objects in eye vicinity. For example, it is highly likely that even regular eyeglasses can restrict the airflow around the eyes, although we are not aware of a quantitative evaluation of this phenomenon.

Gravitational force

Another factor to be considered under the scenario of direct, close human-to-human communication is that the majority of microdroplets would not reach the eye. A recent study using a 3D-printed realistic face and airway model, with an outlet of a particle jet centered at the nose of

(*footnote continued*)
forces repelling them from each other within the droplet.

[3] This scenario reflects only surface distribution the virus; with larger droplets, virions can reside within the droplets (and it is often assumed, without direct evidence, that this is the main form of viral positioning in a droplet) but it has to be kept in mind that, as with surface distribution, electrostatic repelling forces would put an upper limit of how many viruses would reside within a droplet.

[4] The "protected volume of air" in this context can be defined as the volume of air enclosed in the space between the upper and lower eyelids when the eyelids are open, and the gaze is directed forward in primary position.

the model at a distance of 20 cm, found that more 80% of the generated aerosol particles with an initial velocity of 0.94 m/s was deposited on the lips rather than on the eyes [75].

Electrostatic force

Electrostatic (Coulombic) forces can play considerable part in aerosol deposition [76–79], especially for particle size range 0.01 μm to 5 μm [80], which, as discussed below is the most important range of water microdroplet particles carrying viruses. Additionally, it was shown that even relatively low voltage (12 V) ionizer device effectively captured airborne transmitted calicivirus, rotavirus and influenza virus and prevented airborne transmitted influenza A between animals [81]. However, it needs to be emphasized that, according to Hoque (2010): "No specific distribution has been identified in the literature to describe charge distribution in bioaerosols" [82], and, thus further work is needed to clarify the role of electrical charge of bioaerosols for deposition on human tissue in the real world.

Bioaerosol electrical charge

To the best of our knowledge, direct measurements of the electrical charge of bioaerosols generated by human exhalation have not been published to date. Therefore, we would advance some theoretical considerations supported by some indirect experimental data to explore what would be the more likely overall change of the droplets comprising the bioaerosol generated by exhalation and will focus on bioaerosol containing mostly coronaviruses. One such consideration is that the pH of exhaled breath condensate is slightly alkaline, e.g. in normative database from 404 subjects, the mean pH was 7.83, and the median pH was 8.0 [83] and similar results were obtained in another report and a meta-analysis [84,85]. However, the role of bioaerosol deaeration with CO_2-free gas to determine pH is unclear. Therefore, it is possible that the net charge of bioaerosol droplets generated by breathing and other activities is positive. If this would be the case, it would explain the low probability of airborne particles to land in sufficient numbers to the ocular surfaces and remain there for long enough time to attach to receptors and cause inflammation. Of note, the overall isoelectric point of coronaviruses has not been reported in the literature.

Limitations

Most of the factors discussed above would apply to relatively similar indoor controlled environments, including air conditioning, central heating, etc., with relatively slow airflow and limited temperature and humidity range. The production rate, spread and infective potential of bioaerosol from exhaled air would be very different in outdoor environments. It is likely that the outdoor infectious potential of bioaerosol would be much more dependent on environmental factors, such as temperature, humidity, wind speed and direction, air ionization, solar irradiance, etc. Currently, most people in industrialized nations spend most of their time indoors. A large (n = 9196), 2-year probability-based telephone survey in the US (1992–1994) found that the respondents reported spending an average of ~87% of their time in enclosed buildings and ~7.6% outdoors, confirming similar findings from earlier surveys in industrialized countries [86]. However, the exact proportion of infectious events for viral respiratory diseases occurring in indoor environments vs. outdoors is unknown and it is likely to be different for different types of viruses.

Testing the hypothesis

This hypothesis could be tested by measuring the electrical charge of bioaerosol generated by normal breathing in healthy subjects and in patients with viral infections caused by different viruses, causing respiratory infections or with suspected aerosol transmission pathway. An established reliable isoelectric point for all studied viruses would be very helpful in modeling the relationship between virion electrical charge and droplet electrical charge.

Conclusion

The discrepancy between the theoretically expected high rate of concurrence of viral ocular surface inflammation and its observed relatively low occurrence in COVID-19 is surprising. Natural protective factors related to anatomical and physiological properties of the eyes could prevent the deposition of large number of virus-loaded particles on the ocular surface and play a protective role. It is possible that the standing potential of the eye plays an important role in repelling aerosol particles (microdroplets) near the surface of the eye and serve as major contributing factor in securing either complete protection or fast elimination of certain type of bioaerosols containing viruses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110082.

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