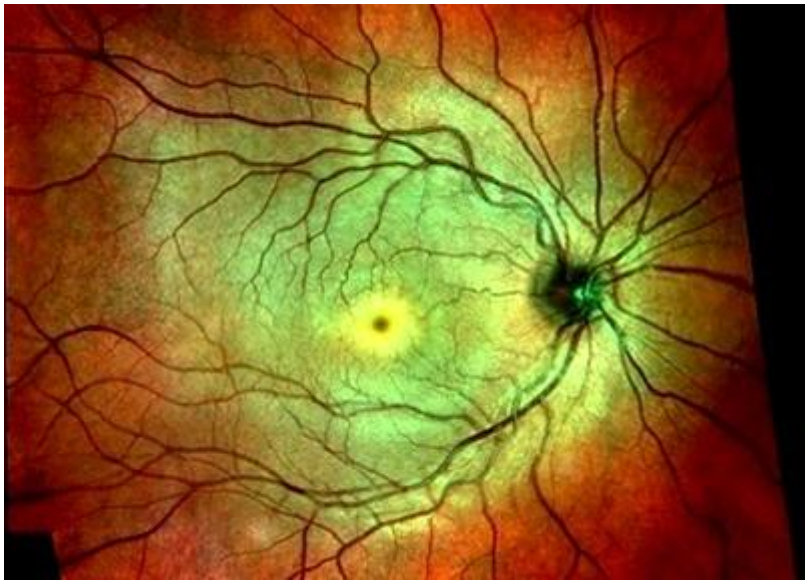
The eye is an immune-privileged organ protected by a blood-retinal barrier (BRB), which consists of an outer barrier of retinal pigment epithelium (RPE) and an inner barrier of retinal vascular endothelium (RvEC). Several respiratory and neurotropic viruses, such as influenza, coronaviruses, Ebola, Zika, and West Nile viruses, have been shown to breach the BRB and cause ocular complications. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is known to cause ocular manifestations. Furthermore, viral RNA and proteins were detected in various ocular tissues and fluids. In this study, the authors from the United States investigated the possible ocular transmission and tropism of SARS-CoV-2 and its interaction with cells lining the BRB in K18-hACE2 transgenic mice expressing human ACE2 (hACE2). The results showed retinal inflammation and microvascular abnormalities in mice exposed to SARS-CoV-2 *via* the intranasal route.

Prior studies reported ocular manifestations in 11.03% of COVID-19 patients, manifested as conjunctivitis, keratoconjunctivitis, episcleritis, chemosis, epiphora, dry eye or foreign body sensation, eye redness, tearing, itching, ocular pain, cotton wool spots, hyperreflective lesions at the level of ganglion cell and inner plexiform layers, retinal artery and vein occlusion, retinal hemorrhage, vascular sheathing, macular neuroretinopathy, optic nerve infarction, optic nerve edema, optic neuropathies, cerebral vein thrombosis, uveitis, and glaucoma.
https://discovermednews.com/reduced-corneal-innervation-increased-dendritic-cell-density-long-covid-patients/

It is believed that SARS-CoV-2 may be further disseminated through the ocular surface *via* nasolacrimal ducts which link the ocular surface to the respiratory tract.



About the study

The authors investigated the transmission and tropism of SARS-CoV-2 in K18-hACE2 transgenic mice expressing hACE2. The animals were infected with 0.4 x 103, 0.4 x 104, and 0.4 x 105 plaque-forming units (PFUs) of SARS-CoV-2 *via* the intranasal or ocular routes. Immunofluorescence staining was used to detect the presence of viral spike (S) antigen in eye globes, lungs, and brains 7-, 14-, and 21- days post-infection (DPI). Quantitative PCR (qPCR) was used to measure the viral RNA copies and confirm the spreading of SARS-CoV-2 to the eye, lungs, and brain after intranasal or ocular exposure.

The innate/inflammatory response generated in the retina and anterior segment tissues was examined by the expression of pattern-recognition receptors, such as Toll-like receptor-3 (TLR3), and melanoma differentiation-associated protein-5 (MDA5), inflammatory cytokines/chemokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β), C-X-C motif chemokine ligand-2 (CXCL-2), interferon (IFN)-γ, and IFN-stimulated genes such as MX dynamin-like GTPase-1 (MX1) and 2'-5'-oligoadenylate synthetase-2 (OAS2).

Since the eye is an immune-privileged organ protected by a BRB, the researchers investigated *in vitro* interaction of SARS-CoV-2 with the outer barrier of the retinal pigment epithelium (RPE) and the inner barrier of the retinal vascular endothelium (HRvEC), as well as the expression of the viral entry receptors angiotensin-converting enzyme 2 (ACE2),

transmembrane serine protease 2 (TMPRSS2), and tyrosine-protein kinase receptor UFO (AXL).

To validate the permissivity of BRB in comorbid conditions, BRB cells were acclimatized to normal and hyperglycemic conditions before infection with SARS-CoV-2. Cell death in these *in vitro* experiments was determined by the TUNEL assay.

Lastly, the long-term effects of SARS-CoV-2 protein on the overall retinal health were investigated through intravitreal injection of the S protein (100 ng/eye) in C57BL/6J mice (n = 5). Thirty days after the injection, the eyes were tested using fundus imaging and fluorescence angiography.

Results

After intranasal or ocular exposure to SARS-CoV-2, dose-dependent mortality was observed in intranasal-exposed K18-hACE2 mice by day 9, whereas the ocular route did not cause weight loss or moribund illness. Mice intranasally exposed to SARS-CoV-2 had a substantial presence of viral S antigens in the lungs and brain. Also, the lungs and brain showed the presence of viral RNA copies only after intranasal exposure. In contrast, ocular exposure did not result in lung infection and moribund illness.

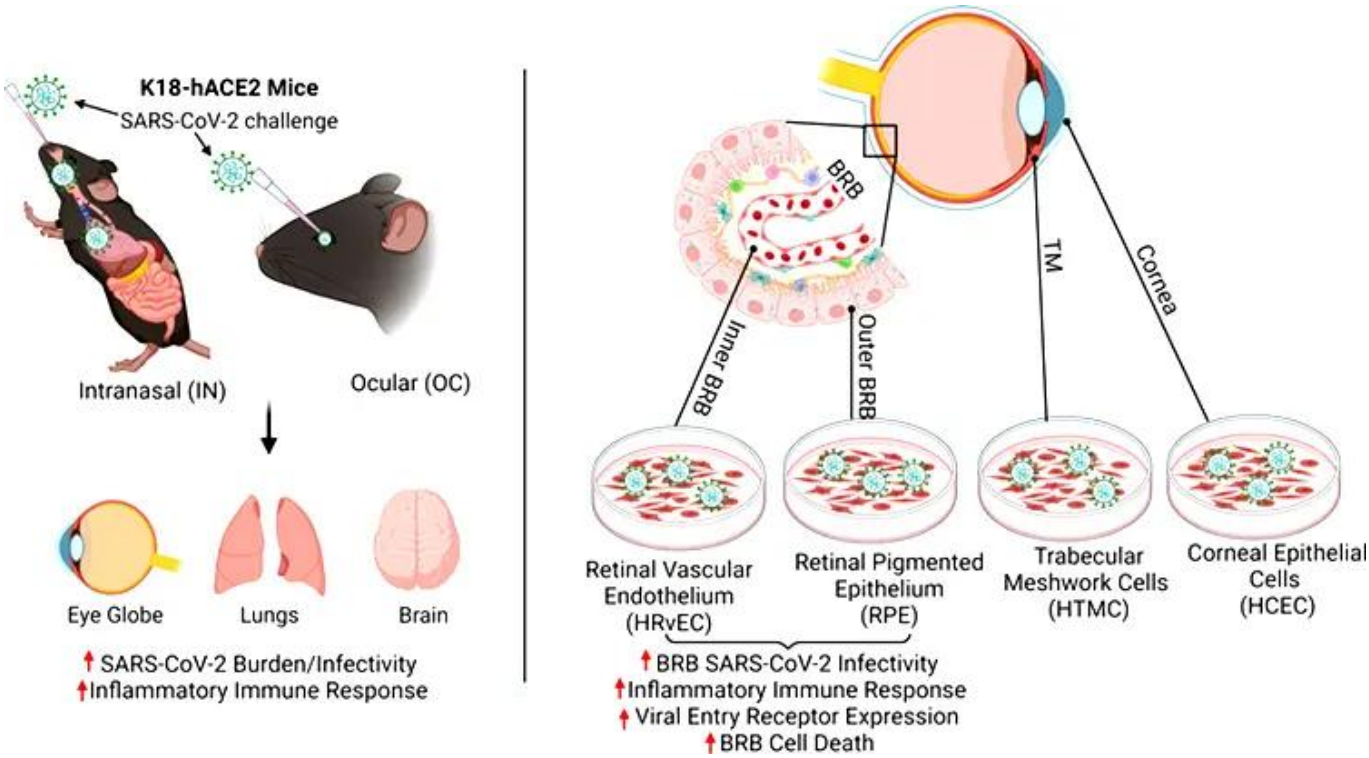
In both intranasal- and ocular-exposed mice, immunofluorescence staining revealed the substantial presence of viral S antigens in various parts of the eye from days 7 to 21, with a peak at day 14 post-infection. Viral S antigens were detected in both anterior and posterior eye segments, including retinal layers, iridocorneal section, and ciliary bodies, but not in the corneal tissue in either intranasal- or ocular-exposed groups. This indicates that corneal epithelium unexpectedly exhibited resistance to SARS-CoV-2 infection. Also, viral RNA was present in the eye globes in both intranasal- and ocular-exposed groups.

Exposure to SARS-CoV-2 elicited an innate inflammatory/antiviral response in different ocular tissues, as evidenced by the induced expression of PRRs: TLR3, MDA5, inflammatory cytokines/chemokines: TNF-α, IL-1β, CXCL-2, and antiviral genes (IFNγ, MX1, and OAS2). The mice exposed to SARS-CoV-2 *via* the intranasal route had remarkably higher inflammatory (TNF-α) and antiviral (IFN-γ, MX1, and OAS2) responses in retinal tissue than ocular-exposed groups, indicating that retinal hyperinflammatory immune response was triggered by intranasal exposure to SARS-CoV-2. The innate response of the anterior segment was comparable in both groups.

In vitro study of the BRB demonstrated that SARS-CoV-2 infected and replicated in primary human RPE and HRvEC cells, as evidenced by a time-dependent increase in the S antigen positivity. SARS-CoV-2 infection significantly enhanced the expression of TLR3, MDA5, TNF-α, IL-6, CXCL-2, IFNγ, MX1, and OAS2 genes in both RPE and HRvEC cells. Most of these genes showed a time-dependent induction in BRB cells. Surprisingly, human corneal epithelial cells (HCEC) were resistant to SARS-CoV-2 infection.

Additionally, SARS-CoV-2 induced cell death in RPE and HRvEC cells, as evidenced by the increase in TUNEL-positive cells compared to uninfected controls. HCEC and HTMC cells were found to be comparatively resistant to SARS-CoV-2-induced cell death, which may be attributed to their reduced permissivity.

As SARS-CoV-2 has shown tropism to the anterior eye segment, causing uveitis and glaucoma, the infectivity of human trabecular meshwork cells (HTMCs) has also been tested. The findings indicated that HTMCs were susceptible to SARS-CoV-2 infection.



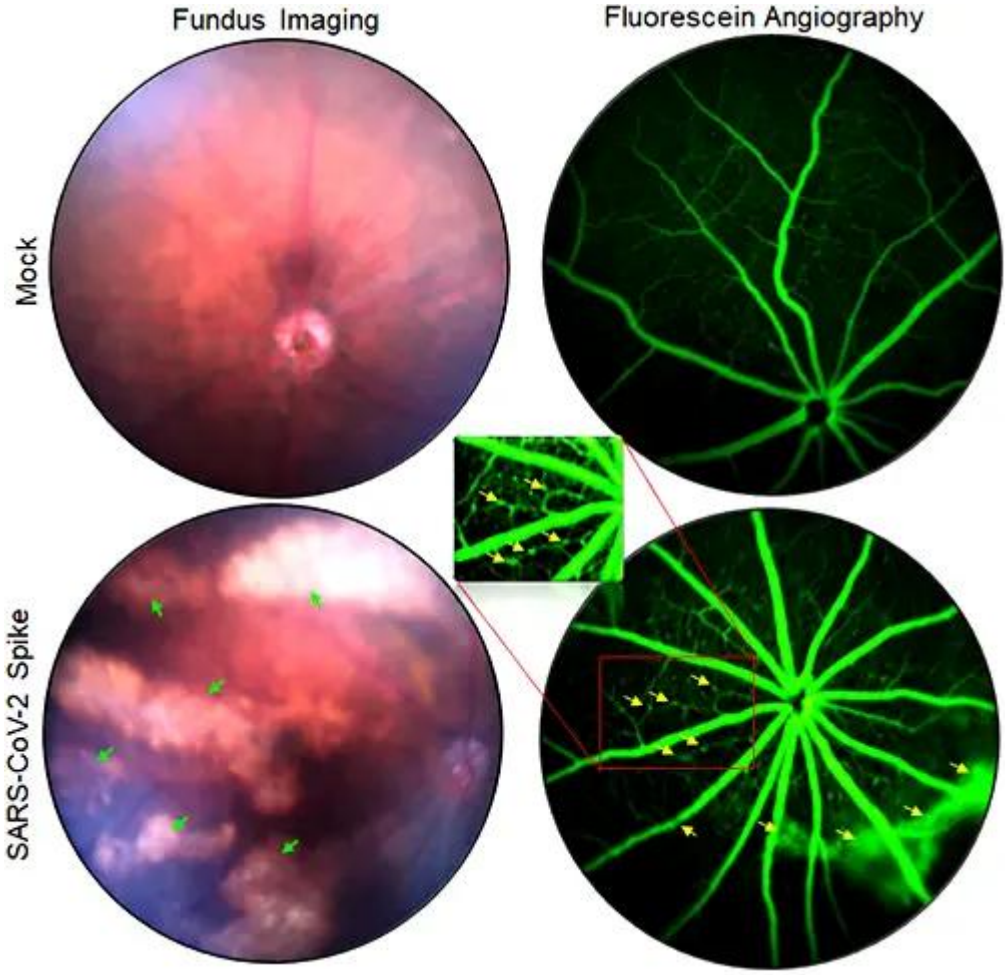
Original figure from the article by Monu M, et al. A schematic illustration of SARS-CoV-2 ocular tropism via cells lining the blood-retinal barrier (BRB). Cells lining the BRB, outer BRB: RPE, and inner BRB: HRvEC are highly permissive to SARS-CoV-2

infection, whereas corneal epithelial cells are comparatively resistant to infection.

Compared with the mock control, SARS-CoV-2 significantly enhanced the expression of all three receptors, ACE2, TMPRSS2, and AXL in tested cells, as evidenced by increased fluorescence positivity for these receptors. These findings show that the virus may use ACE2, TMPRSS2, or AXL receptors for eye entry *via* BRB.

Hyperglycemia increased the susceptibility of RPE, HRvEC, and HTMC to SARS-CoV-2 infection, whereas HCEC appeared less permissive to SARS-CoV-2. Hyperglycemia also increased the expression of ACE2, TMPRSS2, and AXL receptors on BRB cells and increased virus-induced cell death in these ocular cells.

Importantly, the prolonged presence of SARS-CoV-2 S protein in ocular tissues (thirty days after the intravitreal injection) resulted in microaneurysms, retinal atrophy, and vein occlusion in the retina compared to the mock-treated animals. According to the authors, these findings of retinal long-term pathologies and vascular changes seen after prolonged presence of the S antigen may be related to long COVID symptoms.



Original figure from the article by Monu M, et al. The fundus image and fluorescein angiography after the long-term exposure to SARS-CoV-2 S protein show retinal atrophy (indicated with green arrows), microaneurysm, vein occlusion, and vascular leakage (marked with yellow arrows) in the eyes.

Conclusion

This study demonstrated that the intranasal infection of K18-hACE2 mice with SARS-CoV-2 resulted in viral positivity in various parts of the eye, including the retina and the anterior segment tissue, and elevated viral titers in the lungs and brain. SARS-CoV-2 also induced a hyperinflammatory immune and antiviral response in the retina. The cells lining the BRB, the outer and inner barriers, were highly permissive to SARS-CoV-2 replication. They showed enhanced expression of viral entry receptors and increased susceptibility to SARS-CoV-2-induced cell death. Unexpectedly, primary human corneal epithelial cells were resistant to SARS-CoV-2 infection. Comorbidity (hyperglycemia) exacerbated the ocular

manifestations induced by SARS-CoV-2.

Long-term exposure to SARS-CoV-2 spike antigen resulted in retinal pathologies, including microaneurysm, retinal atrophy, and vein occlusion in mouse eyes. The authors concluded that further studies are needed to determine the long-term consequences of SARS-CoV-2 infection on retinal health.

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Journal Reference

Monu M, et al. SARS-CoV-2 infects cells lining the blood-retinal barrier and induces a hyperinflammatory immune response in the retina via systemic exposure. PLoS Pathog 2024; 20(4): e1012156. (Open Access) https://doi.org/10.1371/journal.ppat.1012156.g00