Can a Calcitonin Gene-Related Peptide (CGRP) receptor antagonist mitigate neuroinflammatory, hyper-immune, and nausea-like responses to SARS-CoV-2 infection in preclinical mouse models?

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Abstract: In December 2019, the coronavirus disease (COVID-19) caused by SARS CoV-2 was identified. COVID-19 causes a respiratory illness like the flu with symptoms such as fever, cough, headache, chills, and nausea. The FDA has approved Biohaven Pharmaceuticals to proceed to a clinical trial of its CGRP-receptor antagonist to treat patients with severe COVID-19, suggesting that the neuroinflammatory reaction that is initiated by CGRP in response to SARS-CoV-2 could be a therapeutic target for treating severe COVID-19. We were interested in testing if a CGRP receptor antagonist (olcegepant) would mitigate COVID-19 symptoms in mice. As a readout of SARS-CoV-2 infection symptoms, we have assessed weight loss, O_2 saturation, temperature in young and old mouse models with CGRP receptor antagonized by olcegepant (2 mg/kg/day/SQ). In ongoing experiments, we will be also monitoring the presence of a nausea-like state by assessing hypothermic responses to provocative motion. To date, we have determined that CGRP receptor antagonism is only protective in older C57B6 mice, as there was no significant difference between CGRP receptor antagonism and placebo controls in younger mice. Ongoing studies will determine if CGRP antagonism is similarly protective against nausea - like symptoms. Information gained from these studies will provide a direct assessment of whether a CGRP-receptor antagonist can mitigate both mild and severe symptoms associated with SARS-CoV-2 infection.

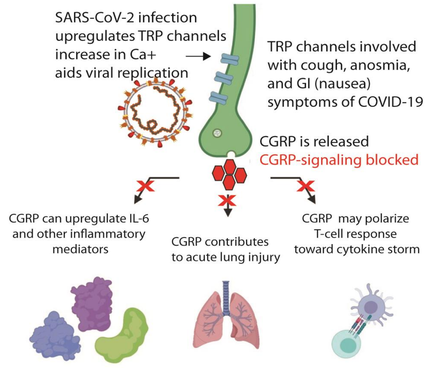


Fig. 1. Calcitonin Gene-Related Peptide (CGRP) is a 37 - amino acid peptide with strong vasodilating properties, that has a fundamental role in migraine pathophysiology. Its antagonism is effective in treating migraine and currently available therapies are the monoclonal antibodies and receptor antagonists "gepants". However, CGRP has several other functions in the body. CGRP-induced vasodilation modifies vascular permeability and potentially allows for the recruitment of inflammatory cells to the local area involved in tissue inflammation and sepsis. Depending on the situation, CGRP may promote or protect from inflammation through its ability to increase cAMP. At present, the world is facing the COVID-19 pandemic caused by the SARS-CoV-2 virus. SARS- CoV-2 infection causes CGRP's release from TRP channels leading to adverse inflammatory events. There is a sound premise supporting the testing of CGRP receptor antagonists to mitigate the neuroimmune consequences of COVID-19.

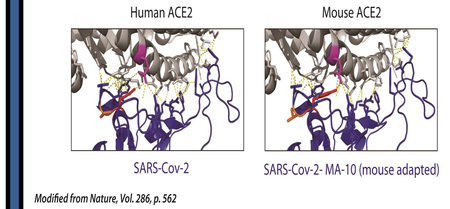
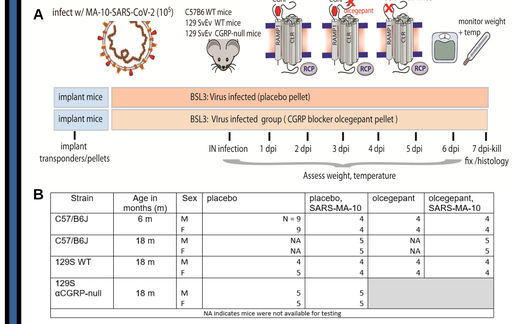


Fig. 2. SARS-CoV-2 cannot infect WT laboratory mice owing to insufficient interactions between viral spike protein and mouse orthologue of the angiotensin-converting enzyme-2 (ACE2) . Instead, we used a mouse-adapted SARS-CoV-2 virus for our studies, with permission from Baric's laboratory (Dinnon et al. 2020, Nature), which uses the native mouse ACE2 receptor for entry into cells. **A.** Table showing interacting residues with spike protein and hACE2 receptor. Amino acid positions are numbered relative to SAR-CoV (top row) and SARS-CoV-2 (bottom row). Green shading indicates contracts as determined by published crystal structures. SARS-CoV Urbani, SARS-CoV, MA15, WIV1, and SHC014 S proteins can use mouse ACE2 as a functional receptor, whereas SARS-CoV-2 cannot. Red outline indicates Q498 in SARS-CoV-2, which makes contact with human ACE2, and is divergent in SARS-CoV. **B.** Modeling of SARS-CoV-2 with human ACE2 receptor. **C.** Modeling of modified MA-10 SARS-CoV-2 with restored interaction for mouse ACE2.



B

Strain	Age in months (m)	Sex	placebo	placebo, SARS-MA-10	olcegepant	olcegepant, SARS-MA-10
C57/B6J	6 m	M	N = 9	4	4	4
		F	9	4	4	4
C57/B6J	18 m	M	NA	5	NA	5
		F	NA	5	NA	5
129S WT	18 m	M	4	4	4	4
		F	5	4	4	4
129S αCGRP-null	18 m	M	5	5		
		F	5	5		

NA indicates mice were not available for testing

Fig. 3. Study's timeline is depicted. **A.** Change in weight (%) and abdominal temperature (°C) were measured from 0 to 7 days after infection (dpi) with 10^5 pfu mouse-adapted SARS- CoV-2 (SARS-MA-10); in male and female mice in the presence of placebo or CGRP-receptor antagonist olcegepant slow release pellets [(olcegepant, BIBN4096, Tocris; 2 mg/kg/day/SQ) . Three mouse strains were tested: C57/B6J WT, 129S WT, and 129S αCGRP-null mice. Within each wildtype (WT) strain and sex, four groups of mice were tested: placebo only, olcegepant only, placebo with 10^5 MA-SARS-CoV-2, and olcegepant with 10^5 SARS-CoV-2 MA-10. Mice were given olcegepant in a dissolvable pellet form to achieve long-lasting CGRP-receptor antagonism. Mice not treated with olcegepant were given a placebo pellet. SARS-CoV-2 MA-10- infected mice were tested at Cornell University's ABSL-3 environment. **B.** Sample sizes are depicted across strain, age, sex, and treatment.

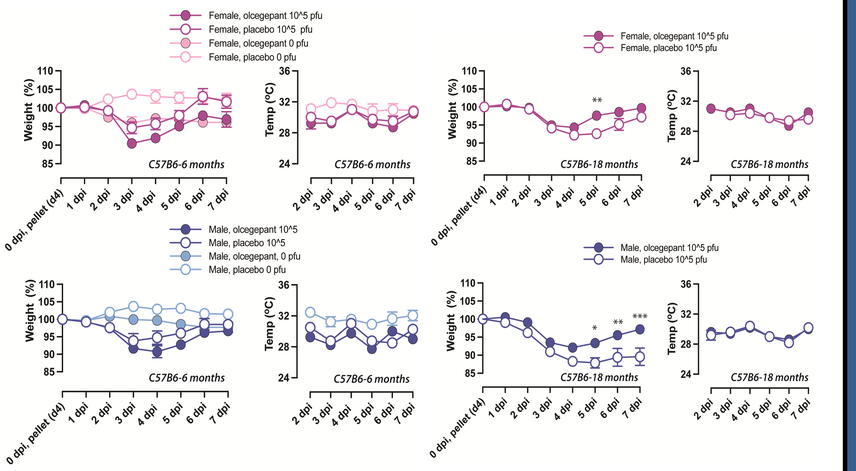


Fig. 4. <u>CGRP's antagonism by olcegepant significantly prevented weight loss in older C57/B6 mice but was not protective in younger (6-month) C57/B6J mice</u>. A 2-way mixed effects model was applied to virus-treated mice to compare olcegepant's effects as compared to placebo-treated animals. .**A.** In 6-month C57/B6J mice, no protective effects of olcegepant were observed in either sex. **B.** In 18-month C57/B6J mice treated with virus, mice treated with the CGRP-receptor antagonist olcegepant showed lower weight loss and faster recovery to baseline weight than compared to mice without olcegepant. Older females were less affected by virus, as a significant difference was seen at only 5 dpi (*adj p-val < 0.01*). Older males were more affected by virus and better protected by olcegepant, as a significant difference in weight was seen at and past 5 dpi (*5 dpi, adj p-value < 0.05; 6 dpi, adj p-value < 0.01; 7 dpi, p-value < 0.001*). No significant differences were observed in abdominal temperature readings for either age group or either sex, showing olcegepant only protected from weight loss and recovery.

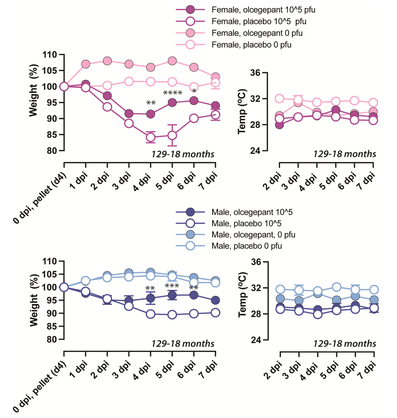


Fig. 5. <u>CGRP's antagonism by olcegepant significantly prevented weight loss in older 129S WT mice.</u> <u>A 2-way mixed effects model was applied to virus-treated mice to compare olcegepant' s effects as compared to placebo-treated mice.</u> All female and male mice treated with virus experienced gradual weight loss after infection of SARS-MA-10. In females, mice treated with olcegepant experienced lower weight loss and faster recovery to baseline than mice given virus only (*4 dpi, adj p-val < 0.01; 5 dpi, adj p-val < 0.0001; 6 dpi, adj p-val < 0.05).* Males showed a similar protective effect of olcegepant against weight loss from 4-6 dpi (*4 dpi, adj p-val < 0.01; 5 dpi, adj p-val < 0.001; 6 dpi, adj p-val < 0.01*). No differences were seen in abdominal temperature readings for either sex.

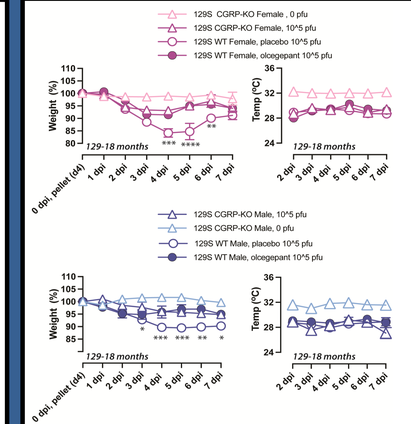


Fig 6. <u>αCGRP-KO mice exposed to SARS-CoV-2 MA-10 experienced weight changes (%) similar to wildtype mice given SARS-CoV-2 MA-10 and the CGRP-antagonist olcegepant</u>. A 2-way mixed effects (ME) model was used to compare virus treated αCGRP-null mice and virus treated 129S WT mice. After 3 dpi in either sex, αCGRP-null mice given virus experienced lesser weight loss than 129S WT mice given virus. A significant difference was seen in females from 4-6 dpi (*4 dpi, adj p-val < 0.0005; 5 dpi, adj p-val < 0.0001; 6 dpi, adj p-val < 0.01*). In males, a significant difference in weight (%) was seen from 3-7 dpi (*3 dpi, adj p-val < 0.05; 4 dpi, adj p-val < 0.001; 5 dpi, adj p-val < 0.001; 6 dpi, adj p-val < 0.01; 7 dpi, adj p-val < 0.05)*. A separate 2-way ME model compared αCGRP-KO mice given virus and 129S WT mice given virus and olcegepant. In the latter comparison for either sex, no significant differences were seen from 0-7 dpi, indicating αCGRP removal resembles olcegepant blockage of the CGRP receptor. No differences were seen in abdominal temperature readings between virus treated groups in either sex.

Discussion: Two other human studies have investigated the effect of CGRP antagonism on individuals infected with SARS-CoV-2. In one study from Spain, [*E. Caronna, V. Jose Gallardo, A. Alpuente et al., Safety of anti-CGRP monoclonal antibodies in patients with migraine during the COVID-19 pandemic: present and future implications, Neurologia, https://doi.org/10.1016/j.nrl.2021.03.003*] no deleterious effects of CGRP antagonism were found and no differences in symptom severity were found between CGRP antagonism to other treatment modalities in migraine subjects. In another study, which was a case report studying the effects of SARS-CoV-2 infection in a patient with chronic migraine treated with a CGRP monoclonal antibodies [*Grassine et al. 2021, Neurological Sciences, https://doi.org/10.1007/s10072-021-05329-5*], SARS-CoV-2 infection increased severity of migraine attacks. These increased attacks were treated with increase dosage of CGRP monoclonal antibodies. Finally, we are awaiting clinical findings from a phase 2 clinical trial from Biohaven Pharmaceuticals of its CGRP-receptor antagonist (zavegepant; currently in phase 3 trials for migraine) to treat patients with severe COVID-19 [*ClinicalTrials.gov Identifier: NCT04346615].* Based on our mouse studies, we predict that CGRP antagonism will be protective in their severely ill population.

We conclude that antagonizing the CGRP receptor may be a viable treatment option for the mitigation of COVID-19 symptoms, as we saw a protective effect of the CGRP-receptor antagonist olcegepant on minimizing weight loss after viral infection in older mice from two different mouse strains (C57B6 and 129S). We also saw that, through the αCGRP-null mice, αCGRP removal resembles CGRP receptor blockage by olcegepant. Our future work involves assessing for motion-induced nausea in these same mouse models in the presence of virus and olcegepant.

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