Could near-infrared light cure COVID-19?

Photobiomodulation therapy for the treatment of neurological complications of COVID-19

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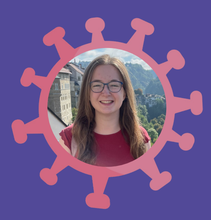

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Introduction

As of April 2023, there have been **750+ million** cases of COVID-19 worldwide.[1] COVID-19 can result in a range of severe complications, such as the pro-inflammatory cytokine storm, acute respiratory distress syndrome, thrombosis and neurological issues. **Photobiomodulation therapy** (PBMT), using near-infrared light with the wavelength **1068 nm**, could be used as a **non-invasive, drug-free treatment** for COVID-19.

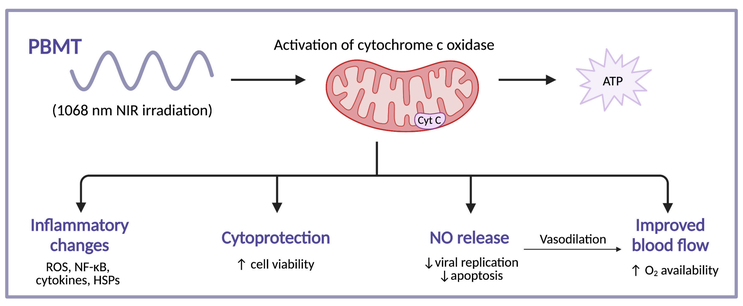

Figure 1: Cellular benefits of PBMT

It is thought that these effects will benefit the respiratory system and other organs targeted by SARS-CoV-2. This project focuses on cells of the nervous system, since **33.6%** of COVID-19 patients receive a **neurological** (e.g. anosmia, insomnia, stroke, delirium) or psychiatric diagnosis in the 6 months following COVID diagnosis.[2]

Methods

Human **SH-SY5Y neuronal** and rat **C6 glioma** cells were grown for at least 24h in DMEM/F12 media supplemented with 10% FBS. Starved cells were instead grown in serum free media (SFM) containing 0% FBS. After 24h, the below protocol was followed:

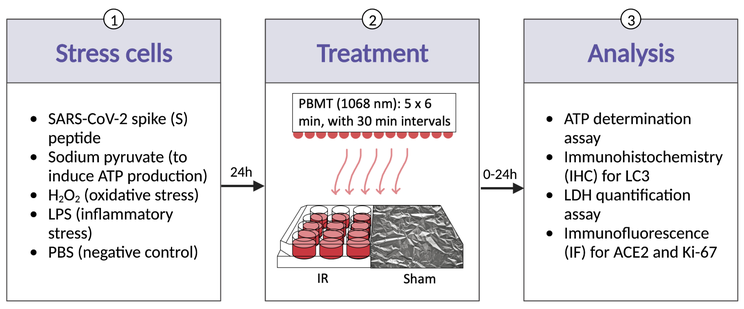

Figure 2: Protocol for the treatment of cells

SPSS and Microsoft Excel were used to perform statistical analysis with t-tests and one-way ANOVAs (where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$). ImageJ's automatic 'Analyze particles' feature was used to count cells.

Conclusions

PBMT shows promising effects against SARS-CoV-2 infection and neurological complications, including boosting **mitochondrial metabolism**, increasing **proliferation** and **reducing viral attachment** by reversing ACE2 membrane localisation.

In **future experiments**, similar methods will be used to study PBMT with HMC3 cells, as microglia are thought to cause COVID-19's high prevalence of neurological symptoms. Further research on all cells will involve ATP quantification assays, calcium imaging and IF to study the localisation of CD147 and TMPRSS2, which are involved in SARS-CoV-2 binding.

Results

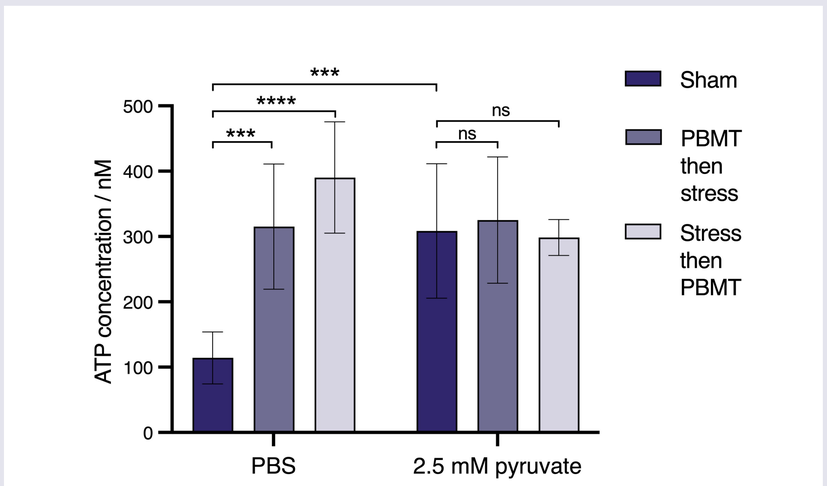

Figure 3: Quantification of ATP using Molecular Probes ATP determination kit (A22066) from SH-SY5Y cell lysates. Single-factor ANOVAs were performed, and bars represent mean ± SD for n = 4-8 lysates.

⇦ **ATP production increased** significantly in neuronal cells treated with PBMT both before and after the addition of the control PBS (figure 3). This obeys the cytochrome *c* oxidase theory of activation, as NIR irradiation is boosting mitochondrial activity.

PBMT has also shown benefits against cellular stress in the form of starvation. Incubation with serum free media (SFM) reduced the number of glia and neuronal cells, and PBMT was able to **increase the cell number** back to control levels (figure 4) ⇩

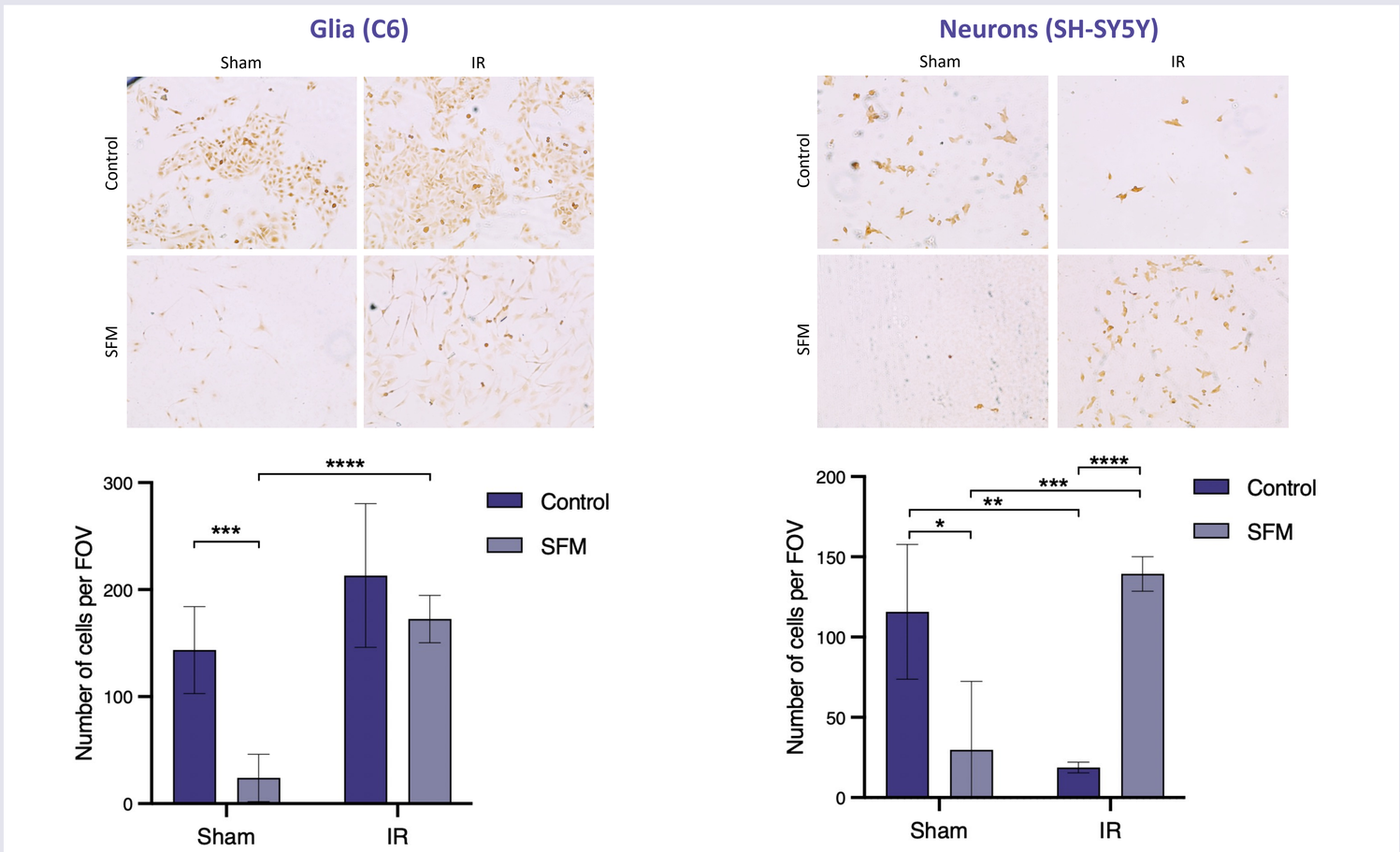

Figure 4: Immunohistochemical (IHC) staining for the autophagosome marker LC3 in cells grown with control (10% FBS) or SFM, followed by PBMT. When normalised to cell area, there were no significant changes to autophagy, but cell number differed significantly. Cells were counted automatically with ImageJ software, and graphs show mean ± SD for n = 4-7 images.

The reason for the cell number increase (figure 4) was explored; PBMT had no effect on the cytotoxicity marker LDH under any stressor (data not shown) but did increase the percentage of **proliferating cells expressing Ki-67** in starved glial cells (figure 5). ⇨

In glial cells, the **S-peptide-induced translocation** of the main SARS-CoV-2 receptor, ACE2, to the cell membrane is reversed by PBMT (figure 6), which may be useful in reducing viral attachment. ⇩

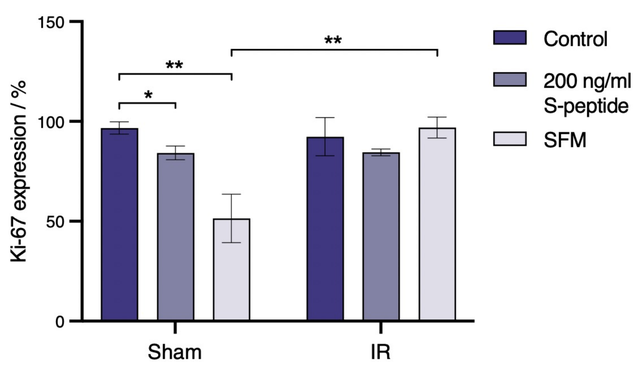

Figure 5: Percentage expression of the proliferation marker Ki-67, counted from immunofluorescence images of C6 glial cells, using DAPI nuclear staining to give total cell number. Bars represent mean ± SD for n = 3 images.

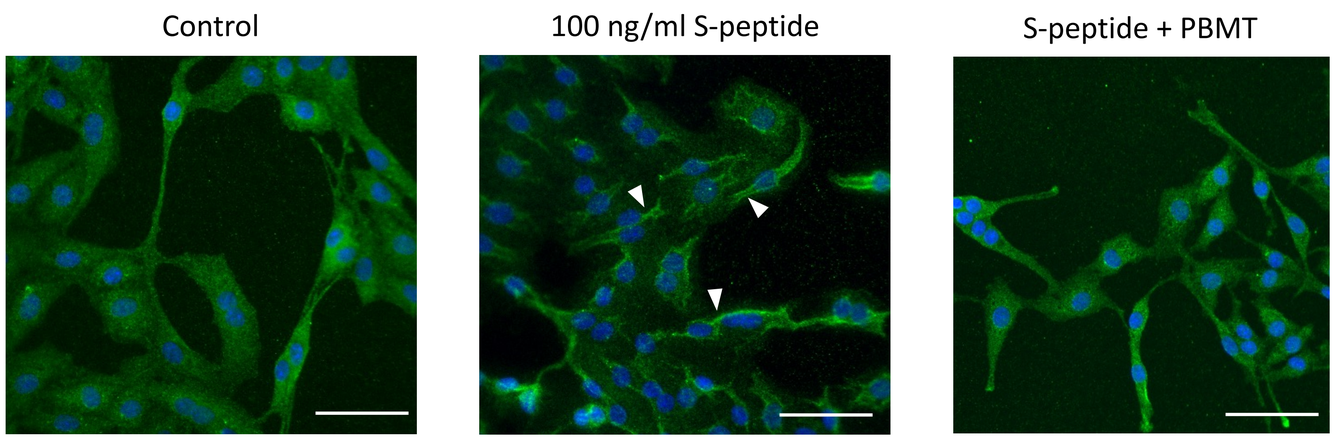

Figure 6: Immunofluorescence (IF) staining of C6 cells with DAPI (blue) and anti-angiotensin-converting enzyme 2 (anti-ACE2, shown in green / Alexa Fluor 488.) Scale bar represents 50 µm, and arrowheads show examples of membrane localisation.

[1] World Health Organization, https://covid19.who.int/, accessed 05/04/23; [2] Taquet *et al*. (2021), Lancet Psychiatry 8; 416-427