Photobiomodulation reduces the cytokine storm syndrome associated with Covid-19 in the zebrafish model

Supplementary files

Histopathological Analysis

Histopathological analyses were performed on adult zebrafish males (n = 24/4 per group) subjected to the different treatments (1) PBM (control), (2) rSpike, (3) rSpike+laser collected over two periods (after 6 and 24 h). For this purpose, individuals from different experimental groups were fixed. The material was then dehydrated in an increasing series of alcohol (70% for 4 h; 95% for 4 h), infiltrated, and embedded in paraffin (Leica). Histological sections 5 µm thick were obtained and stained with Hematoxylin and Eosin for observation of general cellular structures. The material was documented using a microscope with a JVC camera and a computer with Leica QWIN software.

Methodology details are in a supplementary file Table 1. Summary of histopathological findings in different organs of zebrafish injected with rSpike and treated with low-level laser. The number of males with histopathological changes out of the total number of males injected. Adult males were subjected to control group (n = 4), injected with rSpike (n = 4), and injected with rSpike plus subjected to a low-power laser (n = 4).

Sistema	Órgãos	Modificações/ Patologias	Controle	SARS-COV2 rSpike 6h	SARS-COV2 rSpike 24h	SARS-COV2 rSpike + PBM 6h	SARS-COV2 rSpike + PBM 24h
Circulatório	Fígado	-Hepatócitos turgidos;	0/4	3/4	4/4	2/4	4/4
		-Hiperemia de vasos	0/4	4/4	4/4	4/4	4/4
		-Hiperemia de vasos sinusoides;	0/4	3/4	4/4	3/4	0/4
		-Necrose	0/4	0/4	2/4	1/4	1/4
Digestivo	Intestino	- Quiliferos	0/4	02/4	4/4	3/4	0/4
		- Lifangectasia	0/4	2/4	4/4	3/4	0/4
		Linfócitos nas vilosidades intestinais	0/4	4/4	4/4	3/4	0/4
Reprodutivo	Testículo	-	0/4	0/4	0/4	0/4	0/4
Nervoso	Cérebro	-	0/4	0/4	0/4	0/4	0/4

Herein, the inflammatory response is characterized by the enlightening of the increased metabolic differences caused by r-Spike, followed by the gene overexpression and the production of proteins associated with the inflammatory process, gene expression modulates various metabolic pathways,

We observed PBM tissue repairing effect in a zebrafish model COVID-19-associated cytokine storm syndrome. After inoculation of r-Spike, the most widespread findings were lesions in the circulatory and digestive systems. (a Supplementary Discussion and Supplementary References)

Regarding the intestinal tissue, histopathological analyses showed lymphangiectasia, with dilatation of the chylifers and activation of the immune response with the occurrence of lymphocytic cells present in the intestinal villi. An equivalent response profile was also observed in patients with ischemic enteritis due to impaired microvascular blood flow by SARS-CoV-2 infection [2, 3, 4]. The increased intestinal chylomicrosis observed may also indicate white blood cell obstruction triggered by inflammation.

Multifactorial causes can lead to liver damage during COVID-19 infection, analogous to what was observed, such as inflammation, intrahepatic immune activation, and disruption of the liver-intestine axis [5, 6, 7].

Other clinical studies in patients with COVID-19-induced liver injury also resemble our results, including sinusoidal dilatation, coagulopathy under continuous stimulation of pro-inflammatory cytokines, hepatocyte cell membrane dysfunction, and liver necrosis [8, 9, 2, 10]. Our data suggest that the vessel obstruction and the observed hepatocyte turgidity may indicate circulatory alteration of blood cells and the cytopathic effect of hepatocytes due to inflammation generated by rSpike.

Gastrointestinal (GI) and hepatic manifestations promoted by COVID-19 have also been documented and associated with worsening disease and increased mortality rates in intensive care unit (ICU) patients [11, 12, 13, 14]. In the present study, we also observed the mortality of individuals injected with rSpike compared to healthy individuals. These data suggest the hypothesis that intestinal and liver tissue compromise in individuals with SARS-COV-2 may also be related to elevated mortality rates in these individuals.

1. Referências bibliograficas

[2] Su S, Shen J, Zhu L, et al.: Involvement of digestive system in COVID-19: manifestations, pathology, management and challenges. Therap Adv Gastroenterol. **13**:1756284820934626. 10.1177/1756284820934626 (2020).

[3] Papa A, Covino M, Pizzolante F, Miele L, Lopetuso LR, Bove V, et al. . Gastrointestinal symptoms and digestive comorbidities in an Italian cohort of patients with COVID-19. *Eur Rev Med Pharmacol Sci*. **24**:7506–11. 10.26355/eurrev_202007_21923 (2020).

[4] Redd WD, Zhou JC, Hathorn KE, McCarty TR, Bazarbashi AN, Thompson CC, et al. . Prevalence and characteristics of gastrointestinal symptoms in patients with severe acute respiratory syndrome coronavirus 2 infection in the United States: a multicenter cohort study. *Gastroenterology*. **159**:765–7.e2. 10.1053/j.gastro.2020.04.045 (2020).

[5] Morgan, K., Samuel, K., Vandeputte, M., Hayes, P. C., & Plevris, J. N. SARS-CoV-2 infection and the liver. *Pathogens*, **9**(6), 430 (2020).

[6] Sonzogni A, Previtali G, Seghezzi M, Grazia Alessio M, Gianatti A, Licini L, Morotti D, Zerbi P, Carsana L, Rossi R, Lauri E, Pellegrinelli A, Nebuloni M. Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations. *Liver Int.*;**40**:2110–2116 (2020)

[7] Boettler T, Marjot T, Newsome PN, Mondelli MU, Maticic M, Cordero E, Jalan R, Moreau R, Cornberg M, Berg T. Impact of COVID-19 on the care of patients with liver disease: EASL-ESCMID position paper after 6 mo of the pandemic. *JHEP Rep.* **2**:100169 (2020).

[8] Fan, Z., Chen, L., Li, J., Cheng, X., Yang, J., Tian, C., ... & Cheng, J. Clinical features of COVID-19-related liver functional abnormality. *Clinical Gastroenterology and Hepatology*, **18**(7), 1561-1566 (2020).

[9] Tian, S, Xiong, Y, Liu, H, et al. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. Mod Pathol; 1–8 (2020)

[10] Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y., & Wang, C. Remdesivir in adults with severe COVID-19: a randomized, double-blind, placebo-controlled, multicentre trial. *The lancet*, **395**(10236), 1569-1578 (2020).

[11] Wan Y, Li J, Shen L, Zou Y, Hou L, Zhu L, et al. Enteric involvement in hospitalized patients with COVID-19 outside Wuhan. *Lancet Gastroenterol. Hepatol.* **5**:534–5.10.1016/S2468-1253(20)30118-7 (2020).

[12] Chen R, Yu YL, Li W, Liu Y, Lu JX, Chen F, et al. Gastrointestinal symptoms associated with unfavorable prognosis of COVID-19 patients: a retrospective study. *Front Med.* 7:608259. 10.3389/fmed.2020.608259 (2020).

[13] Chen, H., Tong, Z., Ma, Z., Luo, L., Tang, Y., Teng, Y., ... & Qi, X. (Gastrointestinal Bleeding, but Not Other Gastrointestinal Symptoms, Is Associated With Worse Outcomes in COVID-19 Patients. *Frontiers in Medicine*, **8**. (2021).

[14] Wu, D., Hacking, S., & Lee, L. A Series of COVID-19 Cases With Findings in the Gastrointestinal and Hepatobiliary System. *Cureus*, **14**(2) (2022).