Evaluation of Reactive Oxygen Species (ROS) production in Endothelial cells (ECs) in response to COVID-19 patients serum.

Jankauskas Stanislovas.
Published online: 2023-10-31
https://firstapproval.io/publication/BBFDWYD

Oxidative stress and endothelial dysfunction have been shown to play crucial roles in the pathophysiology of COVID-19 (coronavirus disease 2019)(1,2,3,4). We hypothesized that oxidative stress and lipid peroxidation induced by COVID-19 in endothelial cells could be linked to the disease outcome. Thus, we collected serum from COVID-19 patients on hospital admission, and we incubated these sera with human endothelial cells, comparing the effects on the generation of reactive oxygen species (ROS) between patients who survived and patients who did not survive. We found that the serum from non-survivors significantly increased ROS production. Our data indicate that serum from patients who did not survive COVID-19 triggers ROS production in human endothelial cells.

1 folders & 12 files – 75.64 MB

Unique archive cryptographic hash: SHA-256: 48c4129c125507ffc555de69b1e844a42cbed780835cd457d9882ec16e58598a

Background & Aims

To find out if COVID-19 mortality correlates with increased ROS production in ECs. Serum from patients demised to COVID-19 will increase ROS production in ECs.

Materials and methods

Human umbilical vein endothelial cells.

Patients' samples

We obtained plasma samples of patients hospitalized with COVID-19 on the first day of hospital admission. Samples were divided into survivors (patients dismissed from the hospital) and non-survivors (N=22 and N=20, respectively). Mean age was 62±8.4 years and 74% were male in survivors' group and 63±14 years and 72% male in non-survivors' group. Mean time to death from blood sampling was 17.4±16.7 days in non-survival group. The study was approved by the Institutional Ethical Committee (IRB #202011756).

Cell Culture

Human umbilical vein endothelial cells (HUVECs) (Sigma, C-12205) were cultured in EGM-2 medium (Lonza, CC4147) and incubated at 37 °C and 5% CO2. Experiments on HUVECs were performed at passages 3-7. HUVECs were plated on glass bottom culture dishes (MatTek Corporation, P35GCOL-0-10-C). When 70-80% confluent, the cells were treated with 10% patients' serum for 24h under normal condition (37 °C and 5% CO2). To prevent clot formation 10,000 U/mL Heparin (Sigma, H3393-100KU) was added to serum before the experiment. Reactive oxygen species (ROS) assay

ROS production was quantified 2'-7'-dichlorofluorescin diacetate (H2DCF-DA, InvitrogenTM, D399), as described previously (PMID: 20884348). Incubation for both fluorescent probes, as well as washing and imaging were done in a Krebs-Ringer solution (NaCl 115mM, KCl 5mM, NaHCO3 10mM, MgCl2 2.5mM, CaCl2 2 mM, HEPES 20 mM) supplemented with 10mM glucose. After 24h of treatment with 10% patients' serum, HUVECs were incubated with 2.5 µg/mL Hoechst 33342, trihydrochloride, trihydrate (InvitrogenTM, H21492) for 30 min, in the dark, at room temperature (RT). Then, HUVECs were washed once and incubated with 10µM H2DCF-DA for another 15 min RT, in the dark. Then HUVECs were washed 3 times and incubated without any fluorescent probes for another 15 min, RT in the dark. Immediately after this, cells were imaged by Nikon CSU-W1 Spinning Disk confocal microscope using a 40x objective (Nikon Corporation). Cells were excited with a laser at wavelengths 405 nm and 488 nm for Hoechst and H2DCF-DA respectively. Light emission was detected using 455/50 and 520 /40 filters for Hoechst and H2DCF-DA respectively. The same settings (laser intensity, exposure time, pinhole width, etc) were used for imaging of both experimental groups. In order to prevent H2DCF-DA photodynamic reaction, fields of view search and focusing were performed using a Hoechst signal. Images were converted to .jpg format and quantification of H2DCF-DA fluorescence intensity was performed using ImageJ software (NIH).

Data description

File name consist of “ExperimentalGroup-Probe1Name-Probe2Name-ObejectiveMagnification-PictureID.format”
Pictures with the same PictureID but different formats are the same picture.

Software

Images were converted from .nd2 to .jpg format and quantification of H2DCF-DA fluorescence intensity was performed using ImageJ software (NIH).

Authors

Jankauskas Stanislovas Albert Einstein College of Medicine Wilf Family Cardiovascular Research Institute

Granting organizations

- The Santulli’s Lab is supported in part by the National Institutes of Health (NIH): National Heart, Lung, and Blood Institute (NHLBI: R01-HL159062, R01-HL164772, R01-HL146691, T32-HL144456), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK: R01-DK123259, R01-DK033823) (to G.S.), by the National Center for Advancing Translational Sciences (NCATS: UL1TR002556-06) (to G.S.), by the Diabetes Action Research and Education Foundation (to G.S.), and by the Monique Weill-Caulier and Irma T. Hirschl Trusts (to G.S.). S.S.J. is supported in part by a postdoctoral fellowship of the American Heart Association (AHA-21POST836407); U.K. is supported in part by a postdoctoral fellowship of the American Heart Association (AHA-23POST1026190); F.V. is supported in part by a postdoctoral fellowship of the American Heart Association (AHA-22POST995561); and J.G. is supported in part by a postdoctoral fellowship of the American Heart Association (AHA-20POST35211151).

Related articles

Primary articles (publications based on this dataset):
[https://doi.org/10.3390/antiox12020326]

1. Sardu C., Gambardella J., Morelli M.B., Wang X., Marfella R., Santulli G. Hypertension, Thrombosis, Kidney Failure, and Diabetes: Is COVID-19 an Endothelial Disease? A Comprehensive Evaluation of Clinical and Basic Evidence. J. Clin. Med. 2020;9:1417. doi: 10.3390/jcm9051417
2. Montiel V., Lobysheva I., Gérard L., Vermeersch M., Perez-Morga D., Castelein T., Mesland J.-B., Hantson P., Collienne C., Gruson D., et al. Oxidative stress-induced endothelial dysfunction and decreased vascular nitric oxide in COVID-19 patients. Ebiomedicine. 2022;77:103893. doi: 10.1016/j.ebiom.2022.103893.
3. Vardakas P., Skaperda Z., Tekos F., Kouretas D. ROS and COVID. Antioxidants. 2022;11:339. doi: 10.3390/antiox11020339.
4. Chernyak B.V., Popova E.N., Prikhodko A.S., Grebenchikov O.A., Zinovkina L.A., Zinovkin R.A. COVID-19 and Oxidative Stress. Biochem. (Moscow) 2020;85:1543–1553. doi: 10.1134/S0006297920120068