Article
https://doi.org/**10.61900/SPJVS.2023.03.14**

CARDIOVASCULAR CONSEQUENCES AND COVID-19 INFECTION: ESTABLISH THE MODEL

Serban MOROSAN[1,2*], Andreea COZMA[3], Anca Dascalu[3]

Department of Public Health, Iasi University of Life Sciences, Romania[1]
Department of Exact Sciences, Iasi University of Life Sciences, Romania[3]
UMS28, Sorbonne Université/INSERM, Paris, France[2]

[*]E-mail: serban.morosan@uaiasi.ro

Abstract

Recent studies have highlighted that the risks for developing cardiovascular alterations are significantly increased in patients who previously suffered from Covid-19. This study aims at determining the functional and structural long-term effects of Covid-19 disease on the cardiovascular system using a specific and original humanized mouse model recapitulating the endogenous cardiovascular expression of the SARS-CoV-2 main receptor ACE2 (Angiotensin Converting Enzyme 2). We will focus on studying the systemic and pulmonary vessels and the cardiac tissue to understand how SARS-CoV-2 infection leads to cardiac and vascular tissue remodelling and function alteration.

Keywords: virus, cardiovascular, SARS-CoV-2 infection

INTRODUCTION

Covid-19 is a multifaceted disease which often combines a pneumonia with anomalies in the function of other organs including the cardiovascular system. In patients with a severe form, the infection causes microangiopathy (microvascular thrombosis) and endothelial inflammation at the pulmonary, cardiac, hepatic and cerebral levels in patients. Recent studies have highlighted important long-term cardiovascular consequences after SARS-CoV-2 infection even without hospitalization with increased risk of myocardial ischemia, stroke, myopericarditis, arrhythmias, atrial fibrillation or pulmonary fibrotic lesions (1). This risk depends on the severity of the disease but it remains significant in patients with a less severe form. At the pulmonary level, clinical reports observe that, following infection, pulmonary vascular wall is thickened and that several patients developed pulmonary hypertension. Systemic vessels are also modified after Covid-19 as patients show persistent endothelial dysfunction and increased arterial stiffness several months after infection (14). Hence some studies suggest that infection could increase the risk of developing hypertension.

Different mechanisms were proposed to explain the cardiovascular effects of the virus. First, the infection-induced massive immune response (cytokine storm) can activate endothelial cells leading to endothelial dysfunction, permeability and thrombi formation (2). SARS-CoV-2 could also directly infect cardiovascular cells through ACE2 binding. This was observed in vitro (3) but is difficult to observe in patients (4,5). At the vascular level, recent publications and our own preliminary observations suggest a very low endothelial expression of ACE2 and a high expression by smooth muscle cells (SMC) and pericytes (6,7). At the cardiac level, ACE2 is expressed by some cardiomyocytes (8) and we also observed expression at the epicardial level in mice. The consequences of virus infection of vascular and cardiac cells are still poorly understood. It was recently suggested that it could induce myofibrogenic transition of pericytes (9) and reduce expression of contractile proteins in cardiomyocytes (10,11). ACE2 hydrolyses angiotensin (Ang) I and II leading to Ang-(1-7) formation. Interestingly, virus binding could drive ACE2 internalization (12) leading to decreased vascular ACE2 activity, increased Ang II levels (pro-hypertensive and pro-inflammatory) and decreased Ang-(1-7) levels (anti-hypertensive and anti-inflammatory), thereby promoting local inflammation, vasoconstriction and vessel wall pressure increase. Most observations of SARS-

CoV-2 infection-induced tissue effects have been made on patients post-mortem specimens, which does not allow to understand the mechanisms involved as well as the temporality of the lesions and their fate over time.

MATERIAL AND METHODS

In this study we used hACE2-KI transgenic mice, and epithelial cells-expressing hACE2-K18 mice in order to differentiate the effects due to the immune and inflammatory reaction (K18 mice) from the effects related to the infection of cardiovascular cells (KI mice). We used higher virus dose (20000 pfu/mouse) leads to a more severe disease in hACE2-KI mice to determine the sub-lethal dose of virus to be used for the project.

Mice (hACE2-KI, 2 to 5 months of age) infected with a sub-lethal virus dose (administered intranasally under anaesthesia) will be studied early after at 5 and 10 days in order to determine if the virus is present in the different organs studied (lung, heart, aorta, small resistance mesenteric vessels) and to identify infected cells. The study was performed by RT-PCR and immunofluorescence assays. We used markers of the different cell types (endothelial vWF, SMC α-SMA, cardiomyocytes α-actinin, epicardial progenitors WT1 (13), fibroblasts, and vascular progenitors PDGFRα (14)) to detect co-labeling with SARS-CoV-2 Spike protein. To assess infected mice susceptibility to pulmonary hypertension, hACE2-KI mice infected with a sub-lethal virus dose was followed for 1 and 3 months. Infection was confirmed by RT-PCR detection of viral RNA in the mice stool 2, 5 and 8 days after infection. Cardiac function was studied by echo-Doppler and ECG-TUNNEL for the analysis of the electrical signal, in particular the duration of P waves, and for the detection of rhythm abnormalities and the quantification of extrasystoles. Systemic arterial pressure (tail cuff and carotid catheter) and right ventricular pressure (RVSP, reflecting pulmonary arterial pressure) was measured to detect the presence of systemic or pulmonary hypertension. Heart will be either processed for immunohistological analysis to assess tissue cardiac remodeling or will be separated to weigh the 2 ventricles and assess right ventricular hypertrophy.

hACE2-KI mice was infected or not with a sub-lethal dose and kept for 1 and 3 months (estimated from the results obtained in the previous experiments) to determine their susceptibility to pulmonary hypertension by subjecting the mice to chronic hypoxia for 21 days. The mice was subjected to echocardiography and measurement of RVSP and right ventricular hypertrophy.

In all groups, we assessed the remodelling of tissues of interest: mainly lung, systemic vessels, ventricles, and right atrium. Using immunofluorescence, histological staining, RT-PCR, we measureed fibrosis, cardiomyocyte size, pulmonary arterial muscularization, systemic and pulmonary vascular wall thickness, inflammation, proliferation and apoptosis of the different cell types (cardiomyocytes, endothelial cells, SMC, pericytes, fibroblasts/interstitial cells, progenitors (13, 14).

RESULTS AND DISCUSSION

SARS-CoV-2 does not bind to the murine receptor mACE2. We have set up and compared the effects of SARS-CoV-2 infection in two mouse models expressing the human receptor hACE2: the widely used K18-hACE2 transgenic mouse model, expressing hACE2 in keratin 18 (K18)-expressing epithelial cells, and the novel hACE2-KI (knock-in) model (from Cyagen) where the hACE2 expression is controlled by the endogenous mACE2 promoter and completely comparable to the endogenous mACE2 (our observations). hACE-K18 mice infected with a low virus dose (5 pfu/mouse) show a high mortality rate (60%) with severe pneumonia and weight loss. In comparison, hACE2-KI mice response to higher virus doses (50 to 5000 pfu/mouse) is very attenuated, with small weight loss and 100% survival. Our main novel finding is that, although hACE2-KI mice develop only mild pneumonia following SARS-CoV-2 infection, they display after 3 weeks a significant late vascular remodeling with neomuscularization of small pulmonary vessels. Importantly, such a vascular remodeling was absent in K18-hACE2 mice lungs, despite a more severe pulmonary disease with major inflammation, suggesting that it depends on ACE2 expression in pulmonary vascular cells (e.g. pericytes or SMC).

CONCLUSIONS

Our hypothesis is that infection of ACEE2-expressing vascular cells could lead to long-term vascular remodeling, muscularization of the pulmonary vessels and pulmonary fibrosis and could increase the susceptibility to the development of pulmonary hypertension.

REFERENCES

1. Xie at al, Nat Med. 2022 28(3):583-590.
2. Gu et al. Nat Rev Cardiol (2021) 18 : 194–209.
3. Suzuki et al. Vasc Pharmacol (2021) 137: 106823.
4. Bulfamante et al, Biomedicines (2020) 8: 626.
5. Bussani et al. EBioMedicine (2020) 61: 103104
6. Muhl et al. Stem Cell Reports (2022) 17:1089-1104
7. McCracken et al. Circulation (2021) 143:865-868.
8. Bargehr et al. ESC Heart Failure (2021) 8: 4119-4129.

9. Avolio et al. Clin Sci (Lond). (2021) 135(24):2667-2689.
10. Perez-Bermejo et al. Sci. Transl. Med. (2021) 13: eabf7872.
11. Siddiq et al. J Virol. (2022)96(2):e0106321.
12. Portales et al. Life Sci. (2022) 293:120284.
13. Suffee et al. Circ Res (2020) 126(10):1330-1342.
14. Dierick et al. Circ Res. (2016) 118(5):822-33.