

Hypertension Highlights

Cardiovascular medications and regulation of COVID-19 receptors expression



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ABSTRACT

Introduction: Emerging epidemiological studies suggested that Renin–Angiotensin–Aldosterone system (RAAS) inhibitors may increase infectivity and severity of COVID-19 by modulating the expression of ACE2.

Methods: In silico analysis was conducted to compare the blood expression levels of SARS-CoV-2 entry genes between age and gender matched cohort of hypertensive patients versus control, and to determine the effect of common cardiovascular medications on the expression of COVID-19 receptors in vitro using primary human hepatocytes.

Results: The transcriptomic analysis revealed a significant increase of ACE2 and TMPRSS2 in the blood of patients with hypertension. Treatment of primary human hepatocytes with captopril, but not enalapril, significantly increased ACE2 expression. A similar pattern of ACE2 expression was found following the in vitro treatments of rat primary cells with captopril and enalapril. Telmisartan, a second class RAAS inhibitors, did not affect ACE2 levels. We have also tested other cardiovascular medications that may be used alone, or in combination with RAAS inhibitors. Some of these medications increased TMPRSS2, while others, like furosemide, significantly reduced COVID-19 receptors.

Conclusions: The increase in ACE2 expression levels could be due to chronic use of RAAS inhibitors or alternatively caused by other hypertension-related factors or presence of other comorbidities. Treatment of common comorbidities often require chronic use of multiple medications, which may result in an additive increase in the expression of ACE2 and TMPRSS2. Our data suggest that more research is needed to determine the effect of different medications, as well as medication combinations, on COVID-19 receptors.

1. Introduction

Over the last two decades, three waves of coronavirus outbreaks among humans has irrupted, Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002, Middle East respiratory syndrome (MERS) in 2012, and the latest novel SARS-CoV-2 in December 2019. The new SARS-CoV-2 was found to share 79.6% sequence identity with SARS-CoV (8). Besides, the virus uses the same cell receptor as SARS-CoV, Angiotensin Converting Enzyme 2 (ACE2) to enter the cell. It was also found to need Transmembrane Serine Protease 2 (TMPRSS2) for priming of the

viral spike protein [1]. The level of expression of the viral receptor (ACE2) and TMPRSS2, may be critical for the ability of the virus to transmit and replicate.

Patients with hypertension are at high risk of developing severe symptoms following COVID-19 infection [2]. Vaduganathan et al. speculated that this could be due to an upregulation of ACE2, based mostly on contradicting animal studies [3]. The assumption that Renin–Angiotensin–Aldosterone system (RAAS) inhibitors may increase infectivity and severity of COVID-19 by modulating the expression of ACE2 was recently extensively discussed [3]. The ability of these

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medications to potentially upregulate the expression of ACE2 was evaluated based on reported literature. Given the lack of human studies and the conflicting results from animal investigations, it was recommended that these evidence-based medications should be continued in all clinically stable patients even if they are infected with COVID-19 [3].

Therefore, using publicly available transcriptomic data, we have tested the ability of common cardiovascular medications to regulate COVID-19 receptors expression in vitro.

2. Methods

Bioinformatic analyses were conducted to evaluate the effect of different cardiovascular medications on expression levels of ACE2 and TMPRSS2 gene signatures in primary human cells. Publicly available gene expression datasets deposited in Open TG-GATEs (Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System), National Center for Biotechnology Information Gene Expression Omnibus (NCIB GEO, <http://www.ncbi.nlm.nih.gov/geo>) and the European Bioinformatics Institute (EMBL-EBI, <https://www.ebi.ac.uk>) were used. Cardiovascular medication treatments were extracted from TG-GATEs database and GSE42808 dataset, using the high and middle concentrations. All the selected studies used the Affymetrix microarray platforms (Supplementary Table 1). The details for the in vitro treatments and the Open TG-GATEs project are publicly available and previously published (<https://toxico.nibiohn.go.jp/>). To confirm the observed effect of angiotensin-converting enzyme inhibitors, captopril and enalapril on primary human hepatocytes, the expression of ACE2 was also analyzed in primary rat hepatocytes treated with these medications (Supplementary Fig. 1).

The comparison between peripheral blood mononuclear cell (PBMC) from healthy versus hypertension patients was carried out using

GSE24752, GSE70528, and GSE42057 datasets conducted using GPL570 Affymetrix chip.

Before data preprocessing, all the data were evaluated for quality control (QC), and all poor-quality data was removed. The raw Affymetrix data was normalized and log transformed. Microarray data (CEL files) were pre-processed with Robust Multi-Array Average (RMA) technique using R software. Log-transformed normalized intensities were used in the final analyses where differentially expressed genes between treated and control were carried out using LIMMA analyses (Linear Models for MicroArray data). Statistical analyses were performed using R software (v 3.0.2) and Prism (v8; GraphPad Software). For all analyses, P-values <0.05 were considered significant.

3. Results and discussion

In silico analysis was conducted to compare the blood expression levels of SARS-CoV-2 entry genes between age and gender matched cohort of hypertensive patients versus control (Supplementary Table 2). Here, we are clearly reporting a significant increase of ACE2 and TMPRSS2 in the blood of patients with hypertension (Fig. 1A). This increase in ACE2 expression levels could be due to chronic use of RAAS inhibitors or alternatively caused by other hypertension-related factors or presence of other comorbidities. Moreover, these patients are often on multiple drug therapy for the control of blood pressure, and the observed regulation of receptors could be contributed by the net effect of all of these medications rather than one.

In addition, the effect of common cardiovascular medications on the expression of COVID-19 receptors was determined in vitro using primary human hepatocytes, which are known to express these receptors at a comparable level to bronchial lung tissue (GTEX Portal, <https://gtexpal.org>) (Fig. 1B). Within the RAAS inhibitors, we observed a dose

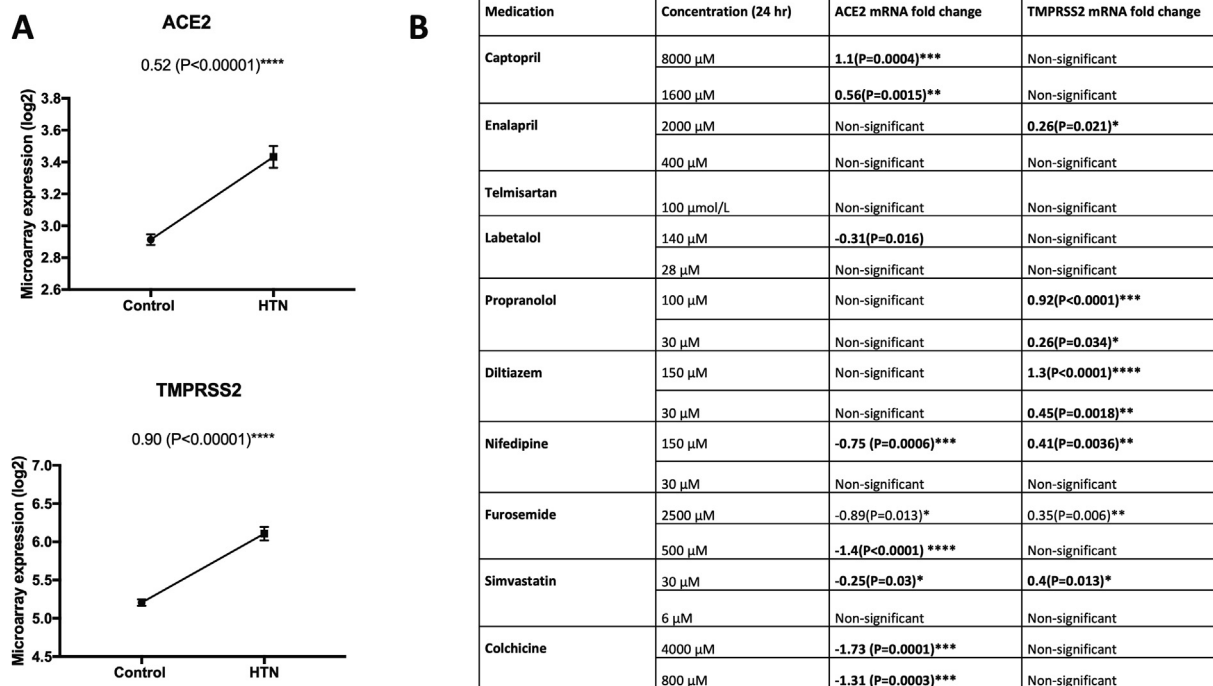


Fig. 1. Microarray expression levels of ACE2 and TMPRSS2 in peripheral blood mononuclear cells (PBMC) and treated primary human hepatocytes. Panel A shows the significant increase in expression levels of ACE2 and TMPRSS2 in PBMCs of hypertensive versus healthy controls (n=22 healthy versus n=11 HTN). The gene expression data for controls were taken from GSE42057 data set, and for hypertensive patients were extracted from GSE24752 and GSE70528. Panel B shows the effect of common cardiovascular medications on expression levels of COVID-19 receptors of ACE2 and TMPRSS2 in primary human hepatocytes (n=2 per group). Gene expression data for cardiovascular medications were extracted from TG-GATEs database and for Telmisartan were extracted from GSE42808 dataset. In GSE42808, Human umbilical vein endothelial cells (HUVEC) were treated with Telmisartan (n=2 per group). Fold change (P-value) are given for Panel A and B. HTN, Hypertension.

dependent increase of ACE2 expression with captopril treatment, while no significant change in expression was detected with enalapril. In addition, similar pattern of ACE2 expression was found following the in vitro treatments of rat primary cells with captopril and enalapril (Supplementary Fig. 1). Treatment with Telmisartan, a second class RAAS inhibitors, also did not result in any increase of ACE2 expression (Fig. 1B). In fact, Telmisartan protected human aortic smooth muscle cells against angiotensin-II induced reduction in ACE2 protein expression [4].

Medications such as propranolol and diltiazem upregulated TMPRSS2. It could be suspected that co-prescription of these medications with RAAS inhibitors increase infectivity and severity of COVID-19. The chronic use of other medications such as furosemide, labetalol, and nifedipine was found to reduce ACE2 expression and hence may decrease disease infectivity. Colchicine, which is used for treatment of pericarditis and have also recently shown to lower ischemic cardiovascular events post myocardial infarction [5], decreased the levels of ACE2 expressions significantly (Fig. 1B). This medication is undergoing clinical trial for treatment of COVID-19 (<https://ClinicalTrials.gov/show/NCT04322682>).

Given the fact that SARS-CoV-2 infection does not increase expressions of ACE2 and TMPRSS2, medications induced upregulation of these receptors in tissues with low baseline expression, such as nerve tissue, could increase exposure of these sites to viral infection. Therefore, although the increased risk of developing severe COVID-19 infections should not be correlated solely to the use of RAAS inhibitors, data presented here suggest that we should be very vigilant about the potential medication effects and therefore more elaborative research is needed to guide proper usage of these medications and suggest safer alternatives.

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Authors contribution

R.H., N.S.A., F.S.A., S.A., Q.H., and T.K. conceived and designed the experiments; N.S.A., F.S.A, R.H, analyzed the data. R.H. Revised the manuscript. All authors contributed to writing and revision of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijchy.2020.100034>.

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