Effect of common medications on the expression of SARS-CoV-2 entry receptors in liver tissue

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Received: 26 April 2020 / Accepted: 12 August 2020 / Published online: 17 August 2020
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Abstract
Besides lung drastic involvement, SARS-CoV-2 severely affected other systems including liver. Emerging epidemiological studies brought the attentions towards liver injury and impairment as a potential outcome of COVID19. Angiotensin-converting enzyme 2 (ACE2) and Transmembrane serine protease (TMPRSS2) are the main cell entry receptors of SARS-CoV-2. We have tested the ability of medications to regulate expression of SARS-CoV-2 receptors. Understanding that may reflect how such medications may affect the level of infectivity and permissibility of the liver following COVID-19. Using transcriptomic datasets, Toxicogenomic Project-Genomics Assisted Toxicity Evaluation System (Open TG-GATEs) and GSE30351, we have tested the ability of ninety common medications to regulate COVID-19 receptors expression in human primary hepatocytes. Most medications displayed a dose-dependent change in expression of receptors which could hint at a potentially more pronounced change with chronic use. The expression level of TMPRSS2 was increased noticeably with a number of medications such as metformin. Within the analgesics, acetaminophen revealed a dose-dependent reduction in expression of ACE2, while non-steroidal anti-inflammatory drugs had mixed effect on receptors expression. To confirm the observed effects on primary human hepatocytes, rat hepatocyte treatments data was obtained from DrugMatrix toxicogenomic database (GSE57805), which showed a similar ACE2 and TMPRSS2 expression pattern. Treatment of common co-morbidities often require chronic use of multiple medications, which may result in an additive increase in the expression of ACE2 and TMPRSS2. More research is needed to determine the effect of different medications on COVID-19 receptors.

Keywords ACE2 · TMPRSS2 · SARS-CoV-2 · COVID-19 · Liver · Medications · Metformin · Acetaminophen · Nsaids · Hepatocyte
Introduction

Besides the respiratory tissue, the SARS-CoV-2 infection has affected other systems such as gastrointestinal tract and liver. Emerging epidemiological studies have brought the attentions towards liver injury and liver impairment as a potential outcome of COVID19 infection. Liver impairment was reported in the previous coronavirus infections (Alsaad et al. 2018) affecting up to 60% percent of patients with SARS-COV-1 (Chau et al. 2004). Similarly, in COVID-19, more than half of patients developed abnormal levels of alanine aminotransferase and aspartate aminotransferase (AST) (Zhang et al. 2020). This liver abnormalities were not detected in pre-clinical stage of disease, and only started appearing with disease progression and appearance of infection symptoms. Moreover, higher liver dysfunction was observed with more severe presentations of COVID-19 and more admission to intensive care units (Guan et al. 2020; Huang et al. 2020). In the COVID-19, the resulting hepatic dysfunction is accompanied by abnormal coagulation and fibrinolytic pathways leading to worse outcomes (Tang et al. 2020; Thachil et al. 2020).

Liver impairment during COVID-19 infection could be due to several factors including the direct infection of liver cells with SARS-CoV-2 (Wang et al. 2020; Zhao et al. 2020), cytokine storm and inflammation, and drug-induced toxicity. To understand that, liver impairment needs to be correlated clinically with the course of the disease. Liver enzymes abnormalities early during infection could mostly be attributed to direct infection of liver cells; while late impairments could mostly be due to failure of other organs. Angiotensin-converting enzyme 2 (ACE2) and Transmembrane serine protease (TMPRSS2) are the main cell entry receptors of SARS-CoV-1 and SARS-CoV-2. Recently, several studies have shown the direct link between the number of receptors and the level of SARS-CoV-2 viral infection (Matsuyama et al. 2020; Monteil et al. 2020; Yang et al. 2007). We have tested the ability of ninety commonly used medications to regulate expression of SARS-CoV-2 receptors in liver cells. Understanding that may reflect how such medications may affect the level of infectivity and permissibility of the liver following COVID-19 infection (Boeckmans et al. 2020).

Results and discussion

The effect of common medications on the expression of SARS-CoV-2 receptors was determined in vitro using human primary hepatocytes (Fig. 1a, b), which are known to express these receptors at a comparable level to bronchial lung tissue (Fig. 1c). Two doses, a high and a low of each medication, were tested. Most medications displayed a dose-dependent change in expression of entry genes which could hint at a potentially more pronounced change with chronic...
use of these medications. To confirm the observed effect of the medications on the expression of SARS-CoV-2 receptors in primary human hepatocytes, we have determined their expression in primary rat hepatocytes treated with part of the medications presented in Fig. 1b. Not all medications present in Fig. 1b were available in this dataset. A similar pattern of ACE2 and TMPRSS2 expression was observed following treatment of rat primary cells with all medications tested (Supplementary Fig. 1).

The expression level of TMPRSS2 was increased noticeably with a number of medications such as metformin, theophylline, and omeprazole (Fig. 1b). To our knowledge, this observed effect of these common medications on the liver expression of TMPRSS2 has not been reported previously. The increase in expression of TMPRSS2 will lead to an increase in priming of ACE2 and the virus spike protein and hence may increase the levels of infectivity (Wambier and Goren 2020). This transmembrane serine protease is known to be essential for activation of other viral infections with hepatic involvement such as hepatitis C (Esumi et al. 2015). This effect should, therefore, be considered when these medications are prescribed for individuals at high risk of hepatic infections.

Within the analgesics, acetaminophen revealed a dose-dependent reduction in expression level of ACE2, while the effect of non-steroidal anti-inflammatory drugs (NSAIDs) ranged between no expression change with ibuprofen and meloxicam and mixed effect of decreased ACE2 and increased TMPRSS2 expressions with diclofenac, naproxen, and nimesulide (Fig. 1b; Supplementary Table 1).

As for infection medications, griseofulvin decreased ACE2 expression, while nitrofurantoin caused dose-dependent increase in TMPRSS2. Interestingly, among medications used for COVID-19 infection, both erythromycin, which comes under the macrolide group of azithromycin, and hydroxychloroquine derivative displayed no change in expression of ACE2 and TMPRSS2. On the other hand, treatment with controversial corticosteroid medications like dexamethasone caused a slight decrease in ACE2 and an increase in TMPRSS2 expression (Supplementary Table 1).

Another interesting group of medications was anti-diabetics. In this group, metformin caused a dose-dependent increase in both of ACE2 and TMPRSS2; while rosiglitazone showed a decrease in ACE2 and an increase in TMPRSS2 (Fig. 1b; Supplementary Table 1).

Metformin, a first-line treatment for type 2 diabetes, enhances the insulin secretion in response to glucose level elevation via the activation of SIRT1 (Yamamoto and Takahashi 2018). SIRT1 was shown to regulate ACE2 expression (Clarke et al. 2014), and hence the observed upregulation of ACE2 following metformin treatment could be due to Metformin induced SIRT1 expression (Fig. 2a). Another noticeable effect of metformin treatment was the increased expression of androgen-regulated gene TMPRSS2 by more than two-fold, with no significant change in the expression of androgen receptor. Therefore, metformin may increase TMPRSS2 through an androgen-independent mechanism (Fig. 2b).

Treatment of common co-morbidities such as cardiovascular and metabolic disorders often requires chronic use of multiple medications, which may result in an additive increase in the expression of ACE2 and TMPRSS2. For example, metformin is often prescribed along with one or more other medications such as gemfibrozil and amitriptyline; both medications were found to increase ACE2 and TMPRSS2 expression (Supplementary Table 1).

Our results are based on public gene expression datasets and thus they may or may not reflect changes in protein expression. Therefore, confirmatory experiments at the mRNA and protein levels are needed to support our findings. Given the fact that SARS-CoV-2 infection does not increase expressions of ACE2 and TMPRSS2 (Hoffmann

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**Fig. 2** Metformin caused a dose-dependent effect on gene expression of SARS-CoV-2 entry genes in primary human hepatocytes. **a** Treatment with 1000 μM metformin for 24 h significantly increased expression of both ACE2 and SIRT1 (n=2/group). **b** Metformin caused a significant dose-dependent increase in mRNA levels of TMPRSS2, but it did not change expression of androgen receptor (AR). All treatments (n=2/group) were conducted for 24 h.
et al. 2020; Saheb Sharif-Askari et al. 2020), the combined
effect of chronic use of these medications could affect liver
susceptibility to the SARS-CoV-2 infection. Although the
increased risk of developing severe COVID-19 infections
should not be correlated solely with the use of medications,
data presented here suggest that we should be vigilant about
the potential medication effects. Obviously, more elaborative
research is needed to guide proper usage of these medica-
tions and suggest safer alternatives.

Methods
Bioinformatic analyses were conducted to evaluate the effect
of different groups of medications on expression levels of
ACE2 and TMPRSS2 gene signatures in primary human
hepatocytes. Publicly available gene expression datasets
available via Toxilogometrics Project-Genomics Assisted
Toxicity Evaluation System (Open TG-GATEs) (Igarashi
et al. 2015), National Center for Biotechnology Information
Gene Expression Omnibus (NCIB GEO, https://www.ncbi.
nlm.nih.gov/geo) and the European Bioinformatics Institute
(EMBL-EBI, https://www.ebi.ac.uk) were used. All the
selected studies used the Affymetrix microarray platforms.
Medication treatments were extracted from TG-GATEs
database using the high and middle concentrations. The
data for chloroquine derivative medication were extracted
from the GSE30351 dataset. The expression of ACE2 and
TMPRSS2 was also analyzed in medication treated primary
rat hepatocytes (Supplementary Fig. 1). The rat in invitro
treatments were obtained from DrugMatrix toxicogenomic
database (GSE57805). Hybridization to the whole genome
was performed for all included samples using the GPL570
human and RG230_2.0 rat GeneChip (Affymetrix, CA). The
details for the in vitro treatments, the Open TG-GATEs and
DrugMatrix project are publicly available and previously
published (Gusenleitner et al. 2014; Igarashi et al. 2015).
Further, the information on dose, duration and number of
treatments are provided in the Supplementary Table 1 and
Supplementary Fig. 1.

Orthogonal data were extracted using single value
decomposition (SVD) (Gusenleitner et al. 2014; Igarashi
et al. 2015; Kossenkov and Ochs 2010; Uehara et al. 2010).
All the included data were evaluated for quality control
(QC). The QC was performed at each step of GeneChip
analysis and covered details on background signals, corner
signals, and expression of housekeeping genes (Gusenleit-
nier et al. 2014; Igarashi et al. 2015; Kossenkov and Ochs
2010; Uehara et al. 2010). The raw Affymetrix data were
normalized and log transformed. Microarray data (CEL
files) were pre-processed with Robust Multi-Array Average
(RMA) technique using R software (Hughes and Butte
2015). Log-transformed normalized intensities were used
in Linear Models for MicroArray data (LIMMA) analyses
to identify differentially expressed genes between treated
and control hepatocytes. The number of RMA normalized
gene array included was 20,606 genes. We used the default
Benjamini-Horchberg correction for multiple testing (Dudoit
et al. 2002; Smyth Gordon 2004). Statistical analyses were
performed using R software (v 3.0.2) and Prism (v8; Graph-
Pad Software). For all analyses, p values < 0.05 were con-
sidered significant.

Author contributions RH, NSA, FSA, QH, and TK conceived and
designed the experiments; NSA, FSA, BAM, EA, analyzed the data.
MA, SA, RH, and SAH revised the manuscript. All authors contributed
to writing and revision of the manuscript.

Funding This research has been financially supported by Tissue
Injury and Repair (TIR) group operational grant (Grant code: 150317);
COVID-19 research grant; seed grant (Grant code: 2001090275); and
by collaborative research grant (Grant code: 2001090278) to RH, Uni-
versity of Sharjah, UAE; and by a Sandoq Al Watan Applied Research
& Development grant to RH; and by Prince Abdullah Ben Khalid
Celiac Disease Research Chair, under the Vice Deanship of Research
Chairs, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Compliance with ethical standards
Conflict of interest The authors have no conflicts of interest to declare.

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