Interactions of anti-COVID-19 drug candidates with hepatic transporters may cause liver toxicity and affect drug metabolism

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Abstract

Transporters in the human liver play a major role in the metabolism of endo- and xenobiotics. Apical (canalicular) transporters extrude compounds to the bile, while basolateral hepatocyte transporters promote the uptake or expel various compounds into the venous blood stream. In the present work we have examined the *in vitro* interactions of some key repurposed drugs advocated to treat COVID-19 (lopinavir, ritonavir, ivermectin, remdesivir and favipiravir), with the relevant key transporters in the hepatocytes. These transporters included the ABCB11/BSEP, ABCC2/MRP2, and MATE1 in the canalicular membrane, as well as ABCC3/MRP3, ABCC4/MRP4, OCT1, OATP1B1, OATP1B3, and NTCP, residing in the basolateral membrane. Lopinavir and ritonavir in low micromolar concentrations inhibited the ABCB11/BSEP and MATE1 exporters, as well as the OATP1B1/1B3 uptake transporters. Ritonavir had a similar inhibitory pattern, also inhibiting OCT1. Remdesivir strongly inhibited ABCC4/MRP4, OATP1B1/1B3, MATE1 and OCT1. Thus, these agents may cause severe drug-drug interactions and drug-induced liver injury. Favipiravir had no significant effect on any of these transporters. Since both general drug metabolism and drug-induced liver toxicity are strongly dependent on the functioning of these transporters, the variable interactions reported here may have important clinical relevance in the drug treatment of this viral disease and the existing co-morbidities.

Introduction

COVID-19 is a devastating new viral disease, with numerous patients requiring intensive care treatment and still showing a fatality rate about 2-3%. Several repurposed antiviral drugs have been proposed to have both *in vitro* and *in vivo* therapeutic effects against Sars-CoV-2, and currently the most promising such agents include ivermectin (IVE), ritonavir (RIT), lopinavir (LOP), favipiravir (FAV) and remdesivir (REM)[1-9]. Both pharmacokinetic and pharmacodynamic characteristics, as well as toxic side effects of pharmaceutical agents are greatly influenced by membrane transporters, especially in the tissue barriers and in the central organ for drug metabolism, in the liver. While the transporter-related pharmacology and toxicology properties of LOP, RIT and IVE have been already examined in several studies, there are no such detailed data available for REM and FAV. Previously we have studied the interactions of these repurposed anti-COVID-19 drugs with tissue barrier membrane transporters [10], while in the current work we have examined their interactions with the key hepatocellular transporters involved in drug metabolism and potential toxicities.

In the apical (canalicular) membranes of the human hepatocytes the key ATP-dependent endo- and xenobiotic extrusion pumps ("ABC efflux transporters") are ABCC2/MRP2, ABCG2/BCRP, and ABCB1/MDR1/ Pgp. Among these multispecific transporters ABCB1/Pgp mostly transports hydrophobic drugs, while both ABCC2 and ABCG2 are also transporting partially detoxified amphiphilic compounds – in fact, ABCC2 is the key transporter for bilirubin conjugates. ABCB11 (bile salt export pump, BSEP), has a restricted function for extruding bile salts into the canaliculi, but numerous drugs inhibit this transporter, leading to cholestasis and drug-induced liver injury (DILI). An important SLC-type transporter in the canalicular membrane, excreting both endogenous and exogenous toxic compounds, is the MATE1 (SLC47A1 - multidrug and toxin extrusion protein 1), mainly transporting cationic drugs, but also some zwitterionic and anionic molecules [11,12].

In the basolateral membranes of the hepatocytes the major ABC-type efflux transporters are ABCC3/MRP3, and ABCC4/MRP4, with variable affinities and specificities for elimination of

endo-and xenobiotics. The major function of these efflux pumps is to defend the hepatocytes from any potential overaccumulation of toxic agents and drugs by delivery to the venous blood [13].

The key SLC-type ("uptake") drug transporters in connection with the sinusoidal blood stream include OCT1/SLC22A1**,** preferentially supporting the hepatocellular entry of cationic agents, thus working in a coordinated fashion with the apical MATE1 in the bile delivery of such compounds through the hepatocytes. NTCP/SLC10A1 is primarily performing a sodium-dependent uptake of bile acids in the basolateral membrane of hepatocytes, thus NTCP, together with BSEP, is a major player in the enterohepatic circulation of bile acids. However, NTCP also promotes the hepatocyte uptake of several drugs, including statins, and this protein is a recognized risk factor for hepatic drug-drug interactions [14-16].

In the basolateral membrane of the hepatocytes OATP1B1 and OATP1B3 (formerly SLCO1B1 and SLCO1B3) are the key organic anion uptake transporters. Although with somewhat different selectivities and specificities, these transporters are responsible for the hepatocellular uptake and elimination of a wide variety of drugs and toxic compounds from the blood stream. Inhibition of these transporters causes an increase in blood retention and general toxicity of many clinically applied agents [17-20] and **Figure 1.**

In the present study we focused on examining repurposed anti-COVID-19 drug interactions with the above transporters by using specific *in vitro* assays. In the case of the ABC transporters, we employed isolated membranes in which the drug-modulation of the model substrates could be quantitatively examined. In the case of SLC-type transporters we used whole-cell assays, in which the specific modulation of the selected model substrate uptake by the overexpressed transporters could be directly studied. Since in our recently published work, focusing on barrier-resident transporters, we have already provided detailed studies for the ABCB1/Pgp and ABCG2/BCRP interactions of these compounds [10], we do not include these experiments here, while refer to them in the Discussion section.

Results

Hepatic transporter interactions of potential antiviral compounds, applied in anti-COVID clinical trials, have been investigated in *in vitro* assays developed for specific, individual testing of these transporters. In the assays for ABC type transporters, we have used vesicular transport

assays, properly reflecting the potential intracellular interactions of the tested drugs. In the case of SLC-type transporters we have applied transporter overexpressing model cells. The investigated drugs, favipiravir (FAV), ritonavir (RIT), lopinavir (LOP), remdesivir (REM) and ivermectin (IVE), were tested in a wide range, up to 50 μM concentrations, to reveal any potential interactions with the given transporters.

- **Vesicular transport assays for ABC transporters**

MRP2/ABCC2 is the key apical membrane efflux transporter of hepatocytes, responsible for the release of bilirubin and other metabolite- or drug-conjugates into the bile canaliculi. MRP2 drug interactions were characterized here by following the inhibitory effects of drugs on ATP- and benzbromarone-sensitive CDCF uptake into inverted membrane vesicles (Fig 2A). We found that IVE strongly decreased the transport rate of the probe substrate at higher than 10 μM concentration, while LOP and FAV had no effect. Interestingly, RIT and REM increased the probe substrate transport rate at high concentrations, possibly by an allosteric effect [21,22]. Since reduced glutathione (GSH) may affect MRP2-dependent substrate transport or drug interactions, we have also examined these transport processes in the presence of 2 mM GSH, but neither the vesicular uptake of CDCF, nor its inhibition by the drugs examined were affected by GSH (data not shown).

MRP3/ABCC3 is mostly a "safety lock" type conjugated metabolite transporter in the basolateral membrane of the hepatocytes transporting its drug substrates into the venous blood [23]. In this work MRP3 drug interactions were characterized by measuring ATP- and benzbromarone sensitive CDCF uptake into inverted HEK membrane vesicles overexpressing MRP3 (Fig 2B). In the case of MRP3, only RIT inhibited the vesicular uptake function at less than 10 μM concentration, whereas LOP and IVE were effective only at high concentrations (see Table 1), and their inhibitory effects were around 50% even at the highest concentrations applied.

MRP4/ABCC4 is an ABC efflux transporter present in numerous tissues, and has a major role in the liver, blood-brain barrier and kidney to extrude various metabolites and drugs from the cells. Interestingly, while MRP4 is localized to the apical membrane of kidney tubules and brain capillaries, it is a basolateral transporter in the hepatocytes [24-28]. MRP4 drug interactions were characterized by measuring ATP-sensitive DHEAS uptake into inverted HEK membrane vesicles, overexpressing MRP4 (Fig 2C). We found that most of the drugs examined had no significant inhibitory effect of MRP4-dependent substrate transport (a maximum of 10-30%

inhibition was observed by up to 50 μM of RIT, LOP, IVE or FAV), while REM had a strong inhibitory action on this transporter, with an IC_{50} of about 2.3 μM (Figure 2C).

BSEP/ABCB11 is the key apical membrane transporter in hepatocytes responsible for bile salt export into the canaliculi [29]. BSEP drug interactions were characterized here by measuring the effects of drugs on ATP- and glyburide sensitive taurocholate (TC) uptake into inverted HEK cell membrane vesicles overexpressing BSEP (Fig. 2D). We found that LOP and RIT strongly inhibited TC transport at low concentrations (see Table 1 for IC_{50} values) whereas IVE and REM had smaller but still significant effect at 50 μM. Favipiravir did not modify TC transport.

- **Cellular assays for SLC type transporters**

OATP1B1 and **OATP1B3** are important drug and metabolite uptake transporters localized in the basolateral membranes of hepatocytes. These proteins are involved in hepatic bilirubin and bile salt uptake, and are also responsible for the uptake of various statins which may also act as inhibitors of these transporters [18]. In this work we have studied OATP1B1 (Figure 3A) and OATP1B3 (Figure 3B) drug inhibition by measuring the fluorescent model substrate, pyranine, into A431 cells overexpressing the related transporters.

As shown in Figure 3, the drug inhibition panel for the two OATPs examined was somewhat different: IVE was less inhibitory for OATP1B1 than for OATP1B3, and RIT, LOP and REM had strong inhibitory effect but with slightly different IC_{50} values for both transporters (see Table 1). Favipiravir had no inhibitory effect on these OATPs.

MATE1 (SLC47A1) is localized to the apical membranes of the hepatocytes and has an important function of the release of endo- and xenobiotics, mostly organic cations (e.g. metformin) into the bile canaliculi. MATE1 is working in a coordinated fashion with the OCT1 basolateral uptake transporter (see below) in the hepatocytes, having a combined role in the metabolism and excretion of organic acid type drugs. MATE1 is an SLC-type proton counter-transporter, capable for working in either direction in the plasma membrane [12], and in this study, we have measured metformin uptake in MDCKII cells overexpressing MATE1. We compared this transport activity to that seen in mock-transfected MDCKII cells. As shown in Figure 4A, LOP, RIT and REM had a strong inhibitory action on this transporter, while IVE and FAV showed practically no inhibition of the MATE1 transport activity (see also Table 1).

OCT1 (SLC22A1*)* is the basolateral drug- and metabolite counterpart of the MATE1

transporter in the hepatocytes [11]. In many cases drugs are entering the liver cells from the blood stream through OCT1, and then leaving the cells into the bile by the transport activity of MATE1 (see above). In the present work, we have measured metformin uptake in HEK cells overexpressing OCT1 and compared this transport activity to that seen in mock-transfected HEK cells. As shown in Figure 4B, RIT and REM had a strong inhibitory action on this transporter, while LOP was less inhibitory and IVE and FAV had no significant inhibitory action on OCT1 transport (see also Table 1).

NTCP/SLC10A1 is the major basolateral bile acid uptake transporter in the hepatocytes, working in coordination with the apical bile efflux transporter BSEP [14]. In this work we have measured taurocholate (TC) uptake in HEK cells overexpressing NTCP, and compared this transport activity to that seen in mock-transfected HEK cells. As shown in Figure 4C, the repurposed anti-COVID agents had relatively weak inhibitory action on this uptake transporter: RIT, LOP and REM had IC_{50} values at or above 50 μM, and IVE and FAV showed little inhibition and only at high concentrations.

Discussion

In the present work we have studied the transporter interactions of several repurposed, potential anti-COVID-19 agents. Here we focused on key membrane transporters involved in the liver metabolism of endo- and xenobiotics and applied well documented assay techniques to characterize these interactions. We selected lopinavir, ritonavir, ivermectin, remdesivir and favipiravir, as promising repurposed anti-COVID-19 agents for these studies. Some of the data presented here reinforce previously indicated transporter-drug interactions, while the comparative study of the hepatocyte transporters, working in a network to allow combined drug uptake and excretion should help to decipher both potential drug-induced liver injury (DILI) and alterations of general hepatic drug metabolism. In addition, in case of the clinically most promising remdesivir and favipiravir, there are only limited data available for specific transporter interactions.

IVE is a widely applied anti-parasitic drug, especially effective in various tropical diseases. IVE has also been indicated to inhibit the cellular replication of the SARS-CoV-2 virus [1], while clinical trials showed no convincing anti-viral efficacy in this disease [30,31]. LOP and RIT are efficient HIV protease inhibitors and were shown to also have *in vitro* efficacy against SARS-

CoV-2 replication [6,32]. However, clinical studies performed until now do not support their anti-COVID-19 efficiency, either when applied separately, or in combination with the name Kaletra [5]. Currently none of these compounds are promoted to be used in COVID-19 outside selected clinical trials [33].

The effects of both IVE, LOP, and RIT on membrane transporters have already been examined in numerous *in vitro* and *in vivo* studies. IVE is known to be strongly neurotoxic, and the ABCB1/Pgp and ABCG2/BCRP efflux transporters are responsible for the reduced human IVE toxicity, by restricting the absorption of this drug in the intestine and protecting the brain passage of IVE through the blood-brain barrier [34-37]. Several transporters have been reported to be strongly inhibited by these drugs, and as shown in the literature [29,38-44] and in our previous experiments (employing a wide array of *in vitro* assays), IVE, LOP, and RIT inhibited the ABCB1/Pgp transporter in submicromolar, while the ABCG2/BCRP transporter in low micromolar concentrations ([10], see also Table 1C). Thus IVE, LOP, and RIT, by seriously impairing ABCB1- and ABCG2-dependent drug extrusion from hepatocytes into the bile canaliculi, may cause severe drug-drug interactions (DDI) and DILI.

As documented in the results section, IVE has relatively little effect on most of the hepatic transporters examined here, while this drug strongly inhibits especially the OATP1B3 uptake transporter. A preferential inhibition of this transporter has already been indicated by [45], by using different assay systems. We found that LOP has relatively little inhibitory effect on the ABCC2/MRP2, ABCC3/MRP3 or ABCC4/MRP4 efflux transporters, or the NTCP and OCT1 uptake transporters. In contrast, LOP in low micromolar concentrations strongly inhibits the ABCB1/Pgp, ABCG2/BCRP and ABCB11/BSEP and the MATE1 exporter proteins, as well as the OATP1B1 and OATP1B3 uptake transporters (this latter effect has already been noted by [45]. RIT has a similar inhibitory pattern regarding these transporters, although RIT also inhibits OCT1 (see Table 1). As a combined effect on the liver transporters, LOP (and/or RIT) probably has the strongest effect on the canalicular drug and bile salt extrusion, as well as the hepatocellular uptake of bilirubin, bile salts and certain pharmacological agents. OATP1B transported drugs, potentially affected by LOP and RIT, include statins, repaglinide, olmesartan and valsartan. [18,46,47].

The BSEP/ABCB11 inhibition by LOP found in this study raises the probability that the use of LOP and drugs often applied in treating co-morbidities in COVID-19 (e.g. statins, Cyclosporin

A, rifampicin, or glyburide), may result in the accumulation of bile salts in the liver, leading to cholestasis and DILI (see US FDA web site, [29]). In case of MATE1, verified substrate drugs, the transport of which may be inhibited by LOP include metformin, some antivirals like acyclovir or ganciclovir, and anticancer drugs like oxaliplatin or topotecan[12].

Since REM is a most promising repurposed drug in treating COVID-19, in this work we carefully assessed potential transporter inhibition by this compound in various assay system. We found that OATP1B1, OATP1B3 and OCT1 uptake transporters, present in the basolateral membrane, and the MATE1 exporter in the canalicular membrane are strongly inhibited by REM. This combined inhibitory action may significantly affect the uptake and the biliary extrusion of both anionic and cationic drugs, thus significantly affect general drug metabolism.

An interesting finding in this work was that among the ABC exporters REM almost uniquely inhibited the ABCB4/MRP4 protein. The MRP4 multispecific pump in the liver extrudes endogenous metabolites, including bile acids, urate, and conjugated steroid hormones into the bile canaliculi. When treating co-morbidities in COVID-19, clinically important MRP4 drug substrates include cephalosporin antibiotics, diuretics like furosemide or hydrochlorothiazide, olmesartan, and anticancer drugs, including methotrexate or topotecan [13,24-26,28,48-51]. In addition, MRP4 inhibitors include non-steroidal anti-inflammatory and cardiovascular drugs.

It should be noted that while in this work we focused on liver transporters, MRP4 is also highly expressed in the basolateral renal tubular membranes and here this protein has an important role in the renal excretion of uric acid and anionic drugs. In addition, MRP4 in the intestinal epithelia is involved in the oral absorption of various drugs, while in the BBB it has a protective role of the CNS from drugs [27,50]. Thus, REM inhibition of MRP4 may have multiple effects on general uric acid and drug metabolism.

As a summary (also depicted in the visual abstract and detailed in Table 1), we found that the examined repurposed anti-COVID-19 agents, with the exception of FAV have multiple inhibitory effects on hepatic membrane transporters. The results of this study may significantly help to decipher the effects of these transporter-drug interactions on general drug metabolism and drug-induced liver toxicity. In addition, the variable interactions quantitatively assessed here should be considered in the clinical drug treatment strategies of the COVID-19 disease and the existing co-morbidities.

Methods

The source of materials is provided in the detailed descriptions of the Methods applied. Lopinavir, ritonavir and ivermectin was obtained from Sigma Aldrich Inc., favipiravir (T-705) from MedChem Express, remdesivir was kind gift of Lajos Szente, Cyclolab Ltd, Budapest, Hungary.

For the ***ABC-type transporter assays*** we have used isolated membrane vesicle-based functional assays. ABCC2/MRP2 vesicles were prepared from Sf9 cells infected by human MRP2 coding baculovirus by a previously established method [22]. ABCB11/BSEP, ABCC3/MRP3 and ABCC4/MRP4 membranes were prepared from transporter expressing HEK293 cells by Solvo Biotechnology (Budapest, Hungary). Vesicular uptake of transporter specific substrates was measured, and transporter related uptake was defined as the difference between the uptake with and without ATP (in the presence of AMP). Transporter specific inhibitors were also applied to control specificity of transporters. For BSEP, radiolabeled taurocholate (1.5 μCi/sample of radiolabeled compound plus 2 μM unlabeled TC) (PerkinElmer Co), for MRP2 and MRP3, CDCF (5(6)-Carboxy-2′,7′-dichlorofluorescein, Sigma) (5 μM), for MRP4 radiolabeled dehydroepiandrosterone sulfate (DHEAS) (0.2 μCi radiolabeled compound plus 0.5 μM unlabeled DHEAS) (PerkinElmer Co) substrates were used. Applied specific inhibitors were 100 μM glyburide for BSEP, 40 μM benzbromarone for MRP2 and MRP3, 50 μM MK571 for MRP4. 30 μg (MRP2, MRP3) or 50 μg (BSEP, MRP4) protein/sample was incubated for 10 or for 1.5 minutes (MRP4) with specific substrate at 37^{o}C or at 32°C (MRP4) in the presence of test drugs (added in 1 μl of DMSO) and incubation was started by pipetting 4 mM Mg-ATP or Mg-AMP to the samples. DMSO was added to the controls as well. Sample volume was 75 μl. After the incubation, samples were filtrated and washed rapidly on Millipore manifold vacuum filter in MSFBN6B10 (Millipore, Burlington, MA, US) filter plate. After the plates were dried, Optiphase HiSafe (PerkinElmer) scintillation cocktail was added to each well for detection of radiolabeled compounds. CDCF was dissolved from the filter by 10% SDS and as a fluorescence stabilizer 0.1N NaOH was added. The amount of radiolabeled substrates inside the filtered vesicles was determined by liquid scintillation counting by a MicroBeta Scintillation Counter (PerkinElmer, Waltham, MA, US). CDCF fluorescence was measured by VictorX (PerkinElmer Perkin-Elmer, Waltham, MA, US) plate reader at 492/635 nm.

*For the **SLC type transporter assays*** we have used whole cell assays, by employing cell lines specifically expressing the investigated hepatic transporters and the relevant control, mock-transfected cell lines [52].

*For the **OATP1B1 and OATP1B3 transporter assays**,* the interaction between potential anti-COVID-19 agents and OATPs was investigated in a microplate-based indirect assay employing pyranine as described [53]. Briefly, A431 cells (ATCC) overexpressing the OATPs [53] and mock transfected A431 cells as control were seeded on 96-well plates in a density of $8x10^4$ cells /well and cultured for 24 h at 37 °C. Next day, cell culture media was removed and the cells were preincubated in uptake buffer in the presence or absence of anti-COVID-19 agents for 5 min at 37 °C. The reaction was started by the addition of uptake buffer containing a final concentration of 10 μM (OATP1B1) or 20 μM (OATP1B3) pyranine and cells were incubated at 37°C for 15 min (OATP1B1) or 30 min (OATP1B3). The reaction was stopped by removing the supernatant and the cells were washed with ice-cold PBS. Fluorescence of cells was determined using an Enspire fluorescent plate reader (PerkinElmer Co), Ex/Em: 460/510 nm. OATP-dependent transport was determined by extracting fluorescence measured in mock transfected cells.

MATE1 (SLC47A1), OCT1 (SLC22A1) and NTCP (SLC10A1) transporter inhibition assays were performed as follows: Overexpressing cell lines created by lentiviral method were used for these assays described in [54]. MATE1 was overexpressed in Madin-Darby canine kidney strain II (MDCKII) cells while OCT1 and NTCP in HEK293 cells. Cells were seeded into 96-well tissue culture plates at a cell density of $1x10^5$ cells per well. Experiments were performed 16-24 hours after seeding. Before the experiments, cells were pre-incubated at 37°C for 5 minutes in Krebs Henseleit buffer (KHB, pH 8.0) or Hank's balanced salt solution (HBSS, pH 7.4) containing the tested drug at increasing concentration, DMSO for solvent control and the reference inhibitor (RI) for reference inhibitor wells. After the pre-incubation step, the solutions were removed. Experiments were performed at 37°C in KHB or in HBSS containing the appropriate probe substrate and drug at increasing concentration, or solvent or RI. The organic solvent concentrations were equal in all wells. Cells overexpressing MATE1 and OCT1 were incubated with metformin (10 μM, including 0.2 or 0.1 μCi/mL ^{14}C-metformin) for 10 or 5 minutes, respectively, and the cells overexpressing NTCP were incubated with TC (2 μM, including 2 μCi/mL ^{3}H-TC) for 2 min at 37°C. MDCKII or HEK293-Mock (empty vector transduced) cells were used as control. After the experiment, cells were washed twice with 100 μL of cold HBSS

and lysed with 50 µL of 0.1 M NaOH and then analyzed by liquid scintillation.

Data and Statistical Analysis

All experiments were performed in duplicates and repeated in three biological replicates, thus each data point reflects six independent measurements. The group sizes were equal in all experiments. Normalization for the per cent (%) of control values was performed after subtracting the background levels as specified in the assay descriptions above for avoiding the inherent differences of the transporter activities and to properly reflect the inhibitory potential of each drug examined. IC_{50} values were calculated by nonlinear regression analysis using GraphPad prism software (version 5.01, GraphPad, La Jolla, CA, US).

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authorship contribution

Participated in research design (Cs.A., É.B., Cs. Ö.-L., Á.T., B.S.), conducted experiments (Cs.A., É.B., Á.T.), performed data analysis (Cs.A., É.B., Á.T.), wrote or contributed to the writing manuscript (Cs. Ö.-L., B.S.)

Competing Interests Statement

The authors declare no conflict of interest. Cs.A. was an employee of SOLVO Biotechnology, a Charles River Company, when the work was performed.

Figure Legends and Figures

Figure 1.

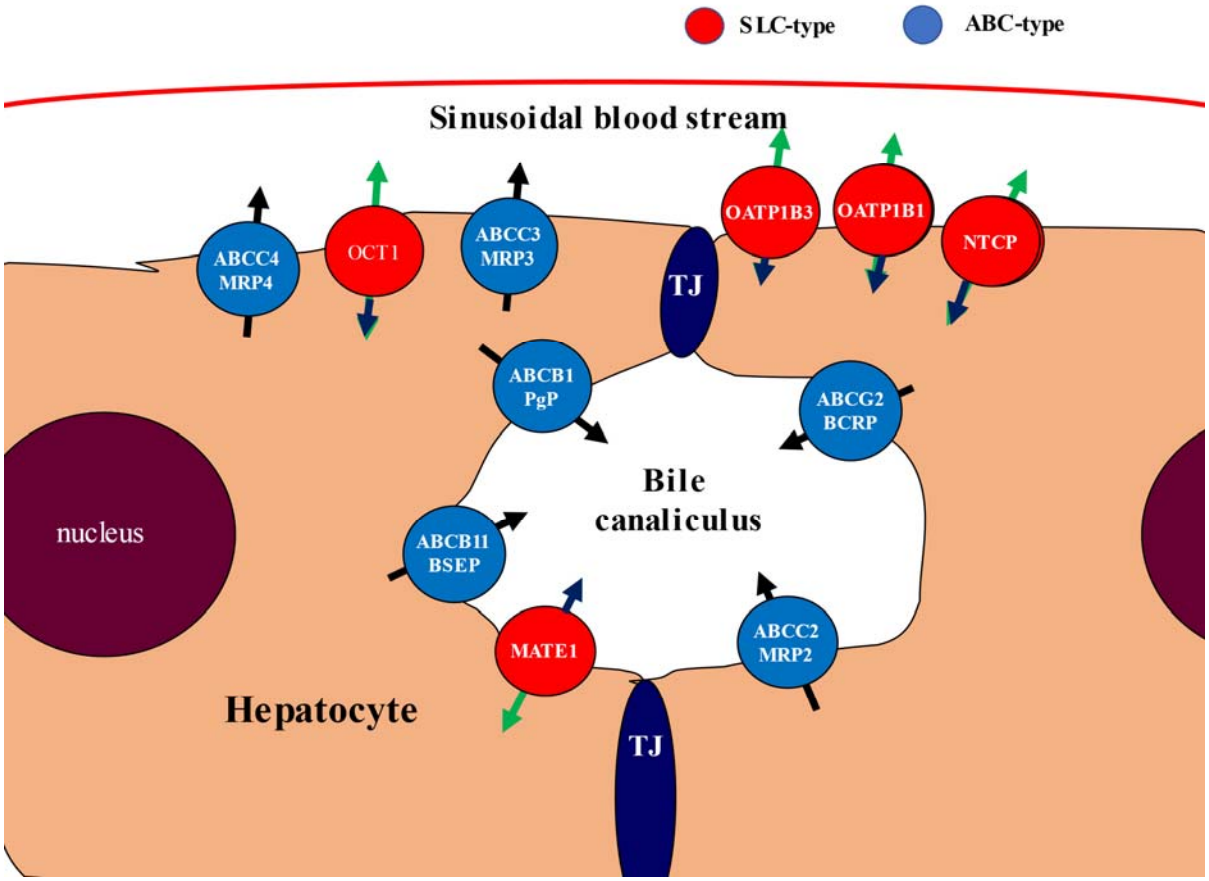

Figure 1. *Schematic presentation of the localization and transport directions of the hepatocyte transporters examined in the present work.* SLC type transporters are colored red, ABC type transporters are blue, TJ: tight junction.

Figure 2.

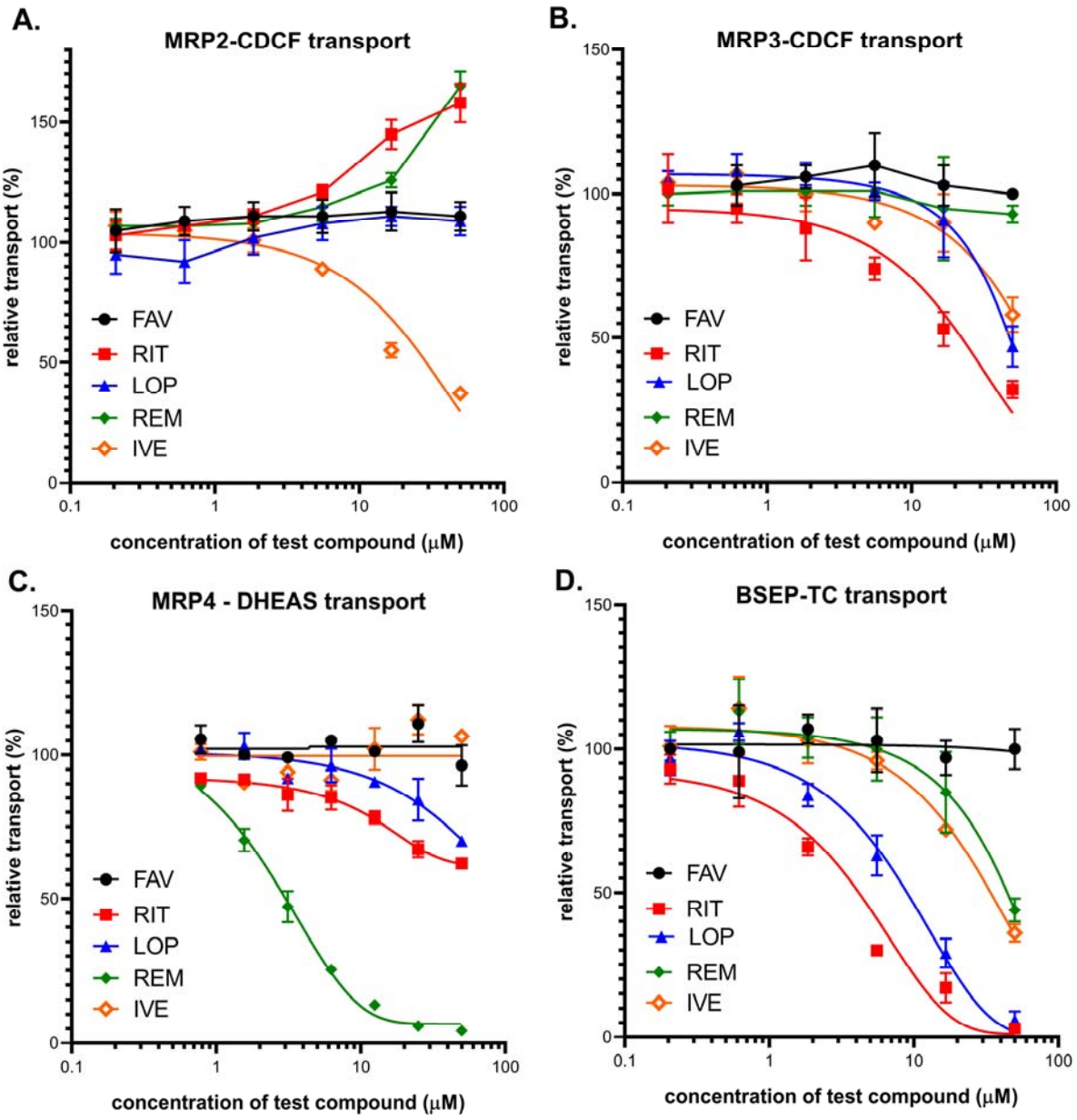
A.
MRP2-CDCF transport
relative transport (%)
concentration of test compound (μM)
FAV
RIT
LOP
REM
IVE
B.
MRP3-CDCF transport
relative transport (%)
concentration of test compound (μM)
FAV
RIT
LOP
REM
IVE
C.
MRP4 - DHEAS transport
relative transport (%)
concentration of test compound (μM)
FAV
RIT
LOP
REM
IVE
D.
BSEP-TC transport
relative transport (%)
concentration of test compound (μM)
FAV
RIT
LOP
REM
IVE

Figure 2. *Inhibition of vesicular uptake of transporter-specific substrates by FAV, RIT, LOP, REM and IVE. Panel A. Inhibition of ATP-dependent vesicular uptake of CDCF in Sf9 cell membrane vesicles overexpressing MRP2.* 30 μg membrane vesicle protein was incubated with 5 μM CDCF at 37°C for 10 minutes. Average values of 3 independent experiments +/- SE are shown. *Panel B. Inhibition of ATP- and benzbromarone sensitive CDCF uptake into inverted HEK cell membrane vesicles overexpressing MRP3.* 30 μg membrane vesicle protein was incubated with 5 μM CDCF at 37°C for 10 minutes. Average values of 3 independent experiments +/- SE are shown. *Panel C. Inhibition of ATP- dependent DHEAS uptake into inverted HEK cell membrane vesicles overexpressing MRP4.* 50 μg membrane vesicle protein was incubated with 0.5 μM DHEAS at 32°C for 1.5 minutes. Average values of 3 independent experiments +/- SE are shown. *Panel D. Inhibition of ATP- and glyburide-sensitive taurocholate (TC) uptake into inverted HEK cell membrane vesicles, overexpressing BSEP.* 50 μg membrane vesicle protein was incubated with 2 μM TC at 37°C for 10 minutes. Average values of 3 independent experiments +/- SE are shown.

Figure 3.

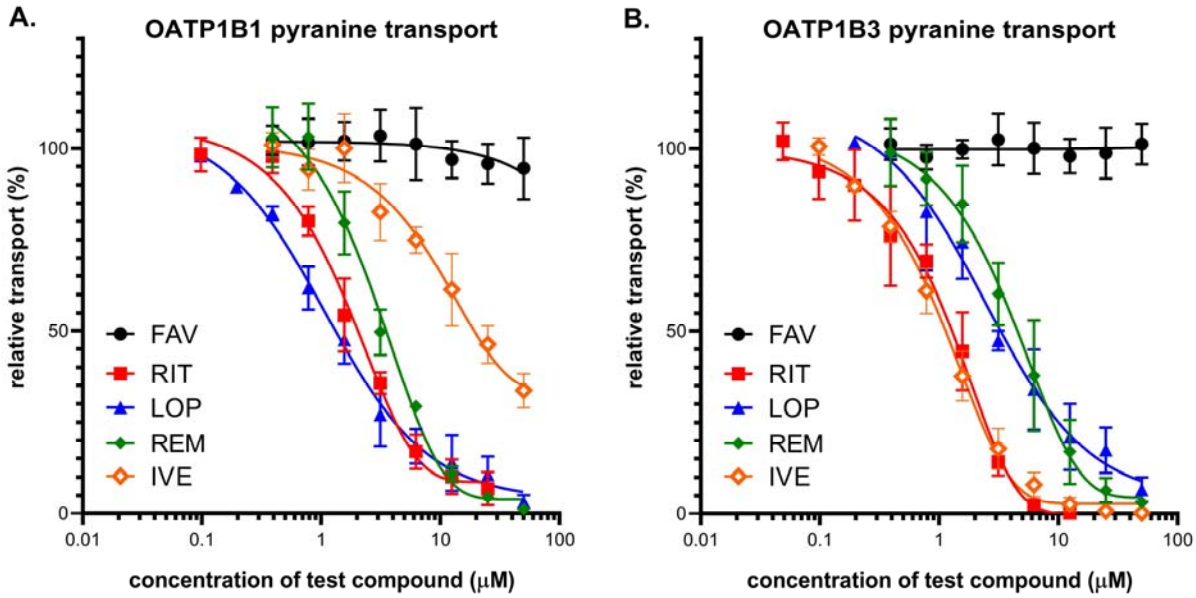

Figure 3*. Inhibition of OATP1B1 (Panel A) and OATP1B3 (Panel B) dependent pyranine uptake in transporter overexpressing A431 cells by repurposed anti-COVID agents.* Cells were incubated with 10 μM (OATP1B1) or 20 μM (OATP1B3) pyranine in the presence or absence of increasing concentrations of anti-COVID agents at 37°C for 15 or 30 minutes (OATP1B1 or OATP1B3, respectively). Average values of 3 independent experiments +/- SD are shown.

Figure 4.

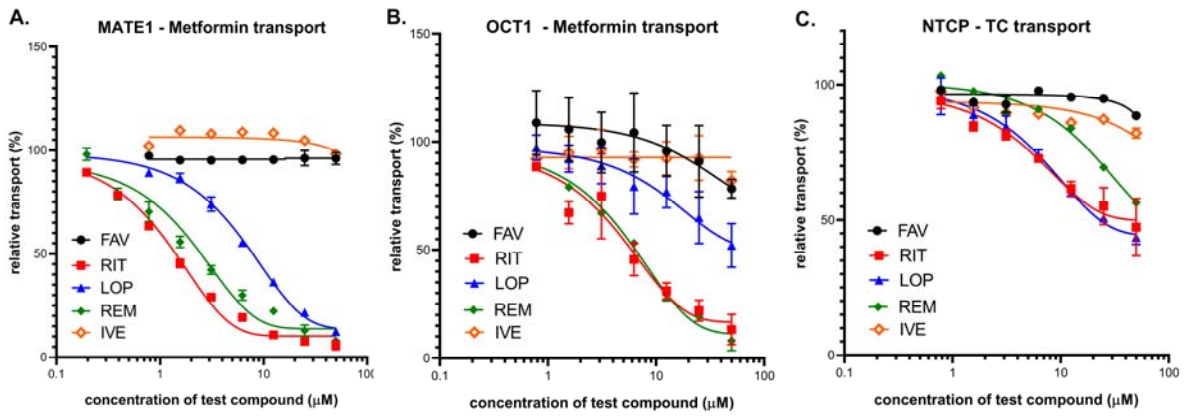

Figure 4. *Inhibition of the cellular uptake of transporter-specific substrates by the repurposed anti-COVID agents FAV, RIT, LOP, REM and IVE. Panel A. Inhibition of MATE1 dependent metformin uptake in MATE1-overexpressing MDCKII cells.* Uptake was measured by 10 μM metformin for 10 min at 37°C. Average values of 3 independent experiments +/- SE are shown. *Panel B. Inhibition of OCT1-dependent metformin uptake in OCT1-overexpressing HEK cells.* Uptake was measured by 10 μM metformin for 5 min at 37°C. Average values of 3 independent experiments +/- SE are shown. *Panel C. Inhibition of NTCP-dependent taurocholate uptake in NTCP-overexpressing HEK cells.* Uptake was measured by 2 μM taurocholate for 2 min at 37°C. Average values of 3 independent experiments +/- SE are shown.

TABLE 1. Summary of the transporter modulation properties of the repurposed anti-COVID-19 drugs examined in transport assays. Approximate IC_{50} (μM) values were determined by nonlinear regression analysis of the data shown in the results section, using GraphPad prism software (version 5.01, GraphPad, La Jolla, CA, USA). Favipiravir (not listed) had no effect on any of the transporters examined.

TABLE 1/A. Effects of drugs on hepatocyte exporters (basolateral: MRP3 and MRP4, apical: BSEP, MRP2 and MATE1).

Hepatocyte exporters	ABCC3/ MRP3		ABCC4/ MRP4		ABCB11/ BSEP		ABCC2/ MRP2		MATE1/ SLC47A1	
	type	IC_{50} μM	type	IC_{50} μM	type	IC_{50} μM	type	IC_{50} μM	type	IC_{50} μM
LOP	inh	>40	no effect	-	inh	7.8	no effect	-	inh	7.7
RIT	inh	17	no effect	-	inh	3.2	increase	-	inh	1.4
IVE	inh	>50	no effect	-	inh	≥30	inh	≥20	no effect	-
REM	no effect	-	inh	2.9	inh	≥40	increase	-	inh	2.3

TABLE 1/B. Effects of drugs on hepatocyte importers (uptake transporters).

Hepatocyte importers	OATP1B1		OATP1B3		NTCP/ SLC10A1		OCT1/ SLC22A1	
	type	IC_{50} μM	type	IC_{50} μM	type	IC_{50} μM	type	IC_{50} μM
LOP	inh	1.1	inh	2.6	inh	≥50	inh	>50
RIT	inh	1.4	inh	1.5	inh	≥50	inh	5.9
IVE	inh	≥20	inh	1.4	no	-	no effect	-
REM	inh	2.9	inh	4.3	inh	≥50	inh	6.1

TABLE 1/C. Effects of drugs on the hepatocyte exporters ABCB1/Pgp and ABCG2/BCRP, as measured previously in similar vesicular transporter assay systems (Telbisz et al, 2021).

Hepatocyte exporters	ABCB1/Pgp		ABCG2/BCRP	
	type	IC_{50} μM	type	IC_{50} μM
LOP	inh	0.6	inh	4.2
RIT	inh	0.3	inh	7.5
IVE	inh	0.3	inh	1.1
REM	inh	>20	inh	>50