Transport of Drugs and Endogenous Compounds Mediated by Human OCT1: Studies in Single- and Double-Transfected Cell Models

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Organic Cation Transporter 1 (OCT1, gene symbol: SLC22A1) is predominately expressed in human liver, localized in the basolateral membrane of hepatocytes and facilitates the uptake of endogenous compounds (e.g. serotonin, acetylcholine, thiamine), and widely prescribed drugs (e.g. metformin, fenoterol, morphine). Furthermore, exogenous compounds such as MPP+, ASP+ and Tetraethylammonium can be used as prototypic substrates to study the OCT1-mediated transport in vitro. Single-transfected cell lines recombinantly overexpressing OCT1 (e.g., HEK-OCT1) were established to study OCT1-mediated uptake and to evaluate transporter-mediated drug-drug interactions in vitro. Furthermore, double-transfected cell models simultaneously overexpressing basolaterally localized OCT1 together with an apically localized export protein have been established. Most of these cell models are based on polarized grown MDCK cells and can be used to analyze transcellular transport, mimicking the transport processes e.g. during the hepatobiliary elimination of drugs. Multidrug and toxin extrusion protein 1 (MATE1, gene symbol: SLC47A1) and the ATP-driven efflux pump P-glycoprotein (P-gp, gene symbol: ABCB1) are both expressed in the canalicular membrane of human hepatocytes and are described as transporters of organic cations. OCT1 and MATE1 have an overlapping substrate spectrum, indicating an important interplay of both transport proteins during the hepatobiliary elimination of drugs. Due to the important role of OCT1 for the transport of endogenous compounds and drugs, in vitro cell systems are important for the determination of the substrate spectrum of OCT1, the understanding of the molecular mechanisms of polarized transport, and the investigation of potential drug-drug interactions. Therefore, the aim of this review article is to summarize the current knowledge on cell systems recombinantly overexpressing human OCT1.

Keywords: HEK 293, double-transfected cell line, single-transfected cell line, P-glycoprotein, MATE1, OCT1, SLC22A1 (OCT1), MDCK cell line
| Drug/Compound | Cell model | \(K_m\) [µM] | Concentration* [µM] | Reference |
|---------------|------------|---------------|---------------------|-----------|
| 1-(2-phenoxyethyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-(3-phenylpropyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-(4-Phenyl-butyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-((m-phenoxyphenyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-((p-chlorophenethyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-((p-chlorophenyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-((p-methoxybenzyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-(p-methyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 1-((p-(p-phenoxy)phenyl)-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 131I-labeled \(m\)-iodobenzylguanidine | HEK293 | 37 kBq | Kobayashi et al. (2020) |
| 1-methyl-4-phenylpyridinium (MPP+) | Xenopus oocytes | 14.6 ± 4.39 | Zhang et al. (1997) |
| 1-methyl-4-phenylpyridinium (MPP+) | HEK293 | 32 | Gründemann et al. (2003) |
| 1-methyl-4-phenylpyridinium (MPP+) | HEK293 | 25.0 | Umehara et al. (2007) |
| 1-methyl-4-phenylpyridinium (MPP+) | HEK293 | 100 | Obianom et al. (2017) |
| 1-((m)-pentylbiguanide | HEK293 | 100 | Obianom et al. (2017) |
| 2-(2,4-dichlorophenyl)ethyl-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 2-(4-biphenyl)ethyl-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 2,2-diphenylethyl-biguanide | HEK293 | 14 ± 2.8 | Obianom et al. (2017) |
| 2,3-dihydro-1H-inden-2-yl-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) | HEK293 | 1 | Campbell et al. (2015) |
| 3-methoxymorphinan | HEK293 | 0.05–0.5 | Meyer et al. (2019) |
| 4-4-dimethylaminostyryl-N-methylpyridinium (ASP+) | HEK293 | 2.32 ± 0.29 | Ahlin et al. (2008) |
| 4-4-dimethylaminostyryl-N-methylpyridinium (ASP+) | HEK293 | 21.2 | Chen et al. (2017a) |
| 4H-1-benzopyran-4-one-biguanide | HEK293 | 100 | Obianom et al. (2017) |
| Acebutol-(R) | HEK293 | 19.9 ± 5.7 | Jensen et al. (2020b) |
| Acebutol-(S) | HEK293 | 201.9 ± 33.1 | Jensen et al. (2020b) |
| Acetylcholine | Xenopus oocytes | 5 | Jensen et al. (2020b) |
| Acetoclor | S2 | 151.2 ± 22.1 | Campbell et al. (2015) |
| Aflatoxin B1 | S2 | 0.1 | Tachampa et al. (2008) |
| Albuterol | HEK293 | 2.5 | Hendrickx et al. (2013) |
| Amifampridine | HEK293 | 506.1 ± 247.3 | Hendrickx et al. (2013) |
| Amlodidine | HEK293 | 2.5 | Hendrickx et al. (2013) |
| Amisulpride | HEK293 | 31.3 ± 4.3 | Hendrickx et al. (2013) |
| Anisodine | HEK293 | 1–5 | Hendrickx et al. (2013) |
| AR-H067637 | HEK293 | 26 | Hendrickx et al. (2013) |
| AR-H069927 | HEK293 | 116 | Hendrickx et al. (2013) |
| Atenolol | MDCK | 3080 | Hendrickx et al. (2013) |
| Atenolol racemate | HEK293 | 2.5 | Hendrickx et al. (2013) |
| Atenolol-(R) | HEK293 | 2.5 | Hendrickx et al. (2013) |
| Atenolol-(S) | HEK293 | 201.9 ± 33.1 | Hendrickx et al. (2013) |
| Atopane | HEK293 | 5.9 ± 1.4 | Hendrickx et al. (2013) |
| Azidoprocainamide | Xenopus oocytes | 100.9 ± 43.0 | Hendrickx et al. (2013) |
| Benzyltriethylammonium | MDCK | 148 ± 3.3 | Hendrickx et al. (2013) |
| Berberrubine | MDCK | 1.27 ± 0.23 | Hendrickx et al. (2013) |
| Bromosulphophthalein | HEK293 | 13.6 ± 2.6 | Hendrickx et al. (2013) |
| Butylscopolamine | HEK293 | 23.4 ± 2.3 | Hendrickx et al. (2013) |
| Cimetidine | HEK293 | 2.5 | Hendrickx et al. (2013) |
| cis-Diammine (pyr-idine)chloroplatinum(II) (cDPCP) | MDCK | 10 | Hendrickx et al. (2013) |
| Cisplatin | HEK293 | 1000 | Hendrickx et al. (2013) |
| Cilnidine | HEK293 | 2.5 | Hendrickx et al. (2013) |
| Coptisine | MDCK | 5.80 ± 1.0 | Hendrickx et al. (2013) |
| Cyclo(His-pro) | HEK293 | 655 ± 191 | Hendrickx et al. (2013) |
| Cycloguanil | HEK293 | 18.3 | Hendrickx et al. (2013) |
| DAPI | MDCK | 8.94 ± 1.26 | Hendrickx et al. (2013) |
| Debrisoquine | HEK293 | 5.9 ± 1.5 | Hendrickx et al. (2013) |
| Debrisoquine | HEK293 | 24.2 ± 1.3 | Hendrickx et al. (2013) |
| Dextrorphan | MDCK | 11.29 ± 3.3 | Hendrickx et al. (2013) |
| Dextromethorphan | HEK293 | 12.6 ± 1.0 | Hendrickx et al. (2013) |
| Dimethylphenylpiperazinium | HEK293 | 62.0 ± 23.3 | Hendrickx et al. (2013) |

(Continued on following page)
| Drug/Compound                   | Cell model | K_m [µM] | Concentration* [µM] | Reference                  |
|--------------------------------|------------|----------|---------------------|----------------------------|
| Dobutamine                     | HEK293     | 28.4 ± 16.8 |                      | Jensen et al. (2021)       |
| Dopamine                       | HEK293     | 2.9       |                      | Morse et al. (2020)        |
| Edrophonium                     | HEK293     | 26.4 ± 9.1 |                      | Jensen et al. (2021)       |
| Epiberberine                    | MDCK       | 526 ± 15.6 |                      | Perez et al. (2018)        |
| Ethambutol                      | HEK293     | 686       |                      | te Brake et al. (2016)     |
| Ethidium                       | CHO and HEK293 | 0.8 ± 0.2 |                      | Lee et al. (2009)          |
| Etilefrine-(R)                 | HEK293     | 232.9 ± 29.8 |                | Jensen et al. (2020b)      |
| Etilefrine-(S)                 | HEK293     | 214.0 ± 24.9 |                | Jensen et al. (2020b)      |
| Famotidine                      | HEK293     | 35.7 ± 7.3 |                      | Jensen et al. (2021)       |
| Fenoterol                      | HEK293     | 2.5       |                      | Hendrickx et al. (2013)    |
| Fenoterol-(R,R)                | HEK293     | 1.7 ± 0.3 |                      | Jensen et al. (2020b)      |
| Fenoterol-(S,S)                | HEK293     | 0.8 ± 0.2 |                      | Jensen et al. (2020b)      |
| Fendamidine                     | CHO        | 6.1 ± 1.1 |                      | Ming et al. (2009)         |
| Glycopyrrolate                  | HEK293     | 8.6 ± 6.1 |                      | Hendrickx et al. (2013)    |
| Guanfacine                      | HEK293     | 13.6 ± 1.3 |                      | Jensen et al. (2021)       |
| Hydromorphone                   | HEK293     | 56.1 ± 19.1 |                  | Jensen et al. (2021)       |
| Imeglimin                       | HEK293     | 1130      |                      | Chevalier et al. (2020)    |
| Ipratropium                     | HEK293     | 2.5       |                      | Hendrickx et al. (2013)    |
| Jatrorrhizine                   | MDCK       | 4.46 ± 0.4 |                      | Li et al. (2016)           |
| Ketamine                        | MDCK       | 73.9 ± 15.2 |                  | Keiser et al. (2018)       |
| Lamivudine                      | CHO        | 1250 ± 100 |                      | Minuesa et al. (2009)      |
| Lamivudine                      | HEK293     | 249 ± 51  |                      | Jung et al. (2008)         |
| Lamivudine                      | HEK293     | 786 ± 84  |                      | Arimany-Nardi et al. (2016) |
| Lamotrigine                     | KCL22      | 1         |                      | Dickens et al. (2012)      |
| Manzolol                        | HEK293     | 2.5       |                      | Hendrickx et al. (2013)    |
| Metformin                       | HEK293     | 1470 ± 190 |                      | Kimura et al. (2005)       |
| Metformin                       | CHO        | 2160 ± 360 |                      | Nies et al. (2009)         |
| Methylnaltrexone                | HEK293     | 20.3 ± 5.6 |                      | Meyer et al. (2019)        |
| Methylscopolamine               | MDCK       | 23.4 ± 4.0 |                      | Jensen et al. (2021)       |
| Minocycline                     | HEK293     | 2.26 ± 1.43 |                 | Jensen et al. (2021)       |
| Monocrotaline                   | HEK293     | 1         |                      | Seitz et al. (2015)        |
| Norfentanyl                     | HEK293     | 7.7 ± 0.8 |                      | Meyer et al. (2019)        |
| Norlevorphanol                  | HEK293     | 0.05–0.5 |                      | Meyer et al. (2019)        |
| Noroxycodone                    | HEK293     | 20.05 ± 6.5 |                 | Meyer et al. (2019)        |
| Norphenylephrine                | HEK293     | 994.1 ± 316.5 |              | Jensen et al. (2021)       |

*(Continued on following page)*
| Drug/Compound                              | Cell model | K<sub>m</sub> [µM] | Concentration* [µM] | Reference                |
|--------------------------------------------|------------|--------------------|---------------------|--------------------------|
| Octopamine                                 | HEK293     | 388.6 ± 246.4      |                     | Jensen et al. (2021)     |
| O-desmethyl tramadol                       | HEK293     | 1                 |                     | Tzvetkov et al. (2011)   |
| Orciprenaline-(R)                          | HEK293     | 780.5 ± 285.9      |                     | Jensen et al. (2020b)    |
| Orciprenaline-(S)                          | HEK293     | 808.8 ± 292.6      |                     | Jensen et al. (2020b)    |
| Oxaliplatin                                | MDCK       | 10                |                     | Lovejoy et al. (2008)    |
| Oxaliplatin                                | HEK293     | 1000              |                     | Yonezawa et al. (2006)   |
| Oxibutynin                                 | HEK293     | 8.82 ± 0.44        |                     | Wenge et al. (2011)      |
| Oxophenomium                               | HEK293     | 2.5               |                     | Hendrickx et al. (2013)  |
| Oxymorphone                                | HEK293     | 0.05              |                     | Meyer et al. (2019)      |
| p-(3-Aminoguanidino)-benzoic acid          | HEK293     | 100               |                     | Obianom et al. (2017)    |
| para-Aminosalicylic acid                   | HEK293     | 20.3 ± 4.6         |                     | Parvez et al. (2017)     |
| para-Hydroxymethamphetamine               | HEK293     | 14.5 ± 8.7         |                     | Wagner et al. (2017)     |
| Prazopanib                                 | C6          | 3.47              |                     | Eliavatty et al. (2018)  |
| Pentamidine                                | CHO         | 36.4 ± 8.3         |                     | Ming et al. (2009)       |
| Phenformin                                 | HEK293     | 8.82 ± 0.44        |                     | Obianom et al. (2017)    |
| Phenylephrine                              | HEK293     | 221.2 ± 60.3       |                     | Jensen et al. (2021)     |
| Picospolin                                 | HEK293     | 10                |                     | More et al. (2010)       |
| Pirbuterol-(R)                             | HEK293     | 75.3 ± 11.4        |                     | Jensen et al. (2020b)    |
| Pirbuterol-(S)                             | HEK293     | 72.9 ± 12.3        |                     | Jensen et al. (2020b)    |
| Prenalterol                                | HEK293     | 13.3 ± 3.4         |                     | Jensen et al. (2021)     |
| Procainamide                               | HEK293     | 2.5               |                     | Hendrickx et al. (2013)  |
| Procaterol                                 | HEK293     | 2.5               |                     | Hendrickx et al. (2013)  |
| Proguanil                                  | HEK293     | 17.7              |                     | Matthaei et al. (2019)   |
| Proguanil                                  | HEK293     | 8.1 ± 1.6          |                     | van der Velden et al. (2017) |
| Prostaglandin F<sub>2</sub>α               | S2-24       | 0.66              |                     | Kimura et al. (2002)     |
| Prostaglandin F<sub>2</sub>α              | S2-15       | 0.48              |                     | Kimura et al. (2002)     |
| Prothionamide                              | HEK293     | 805.8 ± 23.4       |                     | Parvez et al. (2018)     |
| Quercetin                                  | HEK293     | 2.2 ± 0.2          |                     | GlAESER et al. (2014)    |
| Ractopamine                                | HEK293     | 2.1 ± 0.76         |                     | Jensen et al. (2021)     |
| Ranitidine                                 | HEK293     | 1                 |                     | Bi et al. (2019)         |
| Ranitidine                                 | HEK293     | 2.5               |                     | Hendrickx et al. (2013)  |
| Ranitidine                                 | HEK293     | 62.9 ± 4.32        |                     | Meyer et al. (2017)      |
| Ranitidine                                 | Xenopus oocytes | 70 ± 9            |                     | BOURDET et al. (2005)    |
| Retorsine                                  | MDCk       | 102               |                     | Tu et al. (2014)         |
| Rhodamine 123                              | HEK293     | 0.54 ± 0.21        |                     | Jounan et al. (2014)     |
| Ritodrine                                  | HEK293     | 1.67 ± 0.21        |                     | Jensen et al. (2021)     |
| Rizatriptan                                | HEK293     | 1000              |                     | Matthaei et al. (2016)   |
| Salbutamol                                 | HEK293     | 224.2 ± 18.4       |                     | Salomon et al. (2015)    |
| Salbutamol-(R)                             | HEK293     | 222.5 ± 20.5       |                     | Jensen et al. (2020b)    |
| Salbutamol-(S)                             | HEK293     | 440 ± 209          |                     | Taubert et al. (2007)    |
| Saracatinib                                | HEK293     | 10                |                     | Harach et al. (2017)     |
| Sematilide                                 | HEK293     | 102 ± 24.6         |                     | Jensen et al. (2021)     |
| Sorafenib                                  | CHO         | 197 ± 42           |                     | BOXBERGER et al. (2014)  |
| Sotalol                                    | HEK293     | 195.9 ± 72.1       |                     | Swift et al. (2013)      |
| Sparteine                                  | MDCK       | 27.2 ± 2.8         |                     | Swift et al. (2013)      |
| Sulpiride                                   | MDCK       | 259.7 ± 5.4        |                     | Jensen et al. (2021)     |
| Sulpiride                                   | HEK293     | 2.57 ± 0.64        |                     | Neuf et al. (2021)       |
| Sulpiride                                   | HEK293     | 2.5               |                     | Dos Santos Pereira et al. (2014) |
| Sumatriptan                                | HEK293     | 46                |                     | Takano et al. (2017)     |
| Sumatriptan                                | HEK293     | 55.4 ± 7.8         |                     | Hendrickx et al. (2013)  |
| Sumatriptan                                | HEK293     | 2.5               |                     | Hendrickx et al. (2013)  |
| Terbutaline                                 | HEK293     | 2.5               |                     | Hendrickx et al. (2013)  |
| Tetraethylammonium (TEA)                    | Xenopus oocytes | 100              |                     | Zhang et al. (1997)      |
| Tetraethylammonium (TEA)                    | HEK293     | 140              |                     | Hendrickx et al. (2013)  |
| Tetraethylammonium (TEA)                    | HeLa       | 164 ± 17.9         |                     | Bednarczyk et al. (2003) |
| Tetraethylammonium (TEA)                    | MDCK       | 1750 ± 70          |                     | Yasujima et al. (2011)   |
| Tetraethylammonium (TEA)                    | HeLa       | 229 ± 78.4         |                     | Zhang et al. (1998)      |
| Tetraethylammonium (TEA)                    | HEK293     | 69.2              |                     | Umehara et al. (2007)    |
| Thiamine                                   | HEK293     | 1                 |                     | Bi et al. (2019)         |
| Thiamine                                   | HEK293     | 0.025             |                     | Liang et al. (2018)      |
| Thiamine                                   | HEK293     | 780 ± 64           |                     | Chen et al. (2014)       |
| Thiamine                                   | HEK293     | 1997 ± 174         |                     | Jensen et al. (2020a)    |
| Tiotropium                                  | HEK293     | 2.5               |                     | Hendrickx et al. (2013)  |

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**INTRODUCTION**

Transport proteins located in different membrane domains are important for the uptake, distribution and excretion of endogenous substances and drugs (International Transporter Consortium et al., 2010; König et al., 2013; Müller et al., 2018a; Koepsell, 2020). Whereas members of the SLC (Solute Carrier) transporter superfamily generally mediate the uptake of substances from the extracellular space into cells, members of the ABC (ATP-binding cassette) transporter superfamily are export proteins responsible for the energy-dependent export of substrates out of cells. SLC and ABC family members are important for the transport of a variety of approved drugs. Therefore, it is important to characterize drugs or drug metabolites as substrates or transport inhibitors. In vitro cell models are useful tools for this characterization. The importance of in vitro cell models is also highlighted by the fact that they are recommended as tools to study transporter-mediated drug interactions in the guideline/guidance of FDA Food and Drug Administration (2020) and EMA European Medicines Agency (2012).

This article focuses on transport data of the SLC22 family member OCT1 (gene symbol SLC22A1) generated by different in vitro cell models. OCT1 is predominantly expressed in liver and localized in the basolateral membrane of human hepatocytes (Gorboulev et al., 1997; Nies et al., 2008). It mediates the uptake of several endogenous and exogenous compounds and drugs (Table 1). Single-transfected cell models (e.g., HEK-OCT1 cells) recombinantly overexpressing OCT1 were established to study OCT1-mediated transport, to calculate transport parameters (e.g., $K_m$ values), to investigate the impact of genetic variations and to evaluate OCT1-mediated drug-drug interactions in vitro (Figure 1A; Table 1). Since OCT1 has an overlapping substrate spectrum with the apically localized export proteins MATE1 [gene symbol SLC47A1 (Nies et al., 2011)] and P-glycoprotein [P-gp, MDR1; gene symbol ABCB1 (Nies et al., 2008; Misaka et al., 2016)], double-transfected cell models have been established (MDCK-OCT1-MATE1 or MDCK-OCT1-P-gp) for investigating the vectorial transport mediated by both proteins (Table 2). MATE1 and P-glycoprotein are both localized in the apical (canalicular) membrane of human hepatocytes and responsible for the export of substances out of the cells into bile (Thiebaut et al., 1987; Otsuka et al., 2005). When expressed together with OCT1 in MDCK cells grown as a monolayer, OCT1 localizes in the basolateral and MATE1 or P-gp in the apical membrane (Figure 1B). In this experimental setup, substrates of OCT1 and MATE1/P-gp applied to the basolateral compartment will be first taken up into the cells mediated by OCT1 and subsequently exported via MATE1 or P-gp into the apical compartment (Figure 1B). Therefore, these cell models can be used to study not only OCT1-mediated uptake into the cells, but also the vectorial transport of substances from the basolateral into the apical compartment mimicking the transport processes during the hepatobiliary elimination e.g. of drugs (Taghikhani et al., 2017). Moreover, the importance of uptake and efflux transporters for perpetrator disposition can be assessed (Müller et al., 2018b). In this review, we summarize transport data related to the hepatocellular uptake transporter OCT1 obtained by studies in different cell models. Furthermore, the advantages and disadvantages of these cell models will be addressed.

**ORGANIC CATION TRANSPORTER 1 AND RELATED EXPORT PROTEINS**

**Organic Cation Transporter 1**

The rodent orthologue of human OCT1 (rOCT1) was first isolated from a rat kidney library and expressed in Xenopus oocytes. This rOCT1 transporter showed inhibitable and potential-dependent Tetraethylammonium (TEA) and 1-methyl-4-phenylpyridinium (MPP+) uptake (Gründemann et al., 1994). Additionally, in situ
hybridization and northern blotting analysis demonstrated Oct1 expression in rat hepatocytes and enterocytes. In 1997, human OCT1 (gene symbol: SLC22A1) was cloned and characterized by two independent working groups (Gorboulev et al., 1997; Zhang et al., 1997). Although Gorboulev et al. amplified hOCT1 using kidney cDNA, northern blot analysis demonstrated OCT1 expression mainly in the liver (Gorboulev et al., 1997), which was in line with the findings of Zhang et al. using liver cDNA (Zhang et al., 1997). Later, OCT1 was localized at the basolateral membrane of human hepatocytes (Nies et al., 2008). OCT1 facilitates the uptake of organic cations or weak bases (Table 1), which comprises approximately 40–67.5% of all drugs (Comer and Tam, 2001; Neuhoff et al., 2003; Manallack, 2007), into human hepatocytes. In the 2018 recommendations of the International Transporter Consortium (ITC), the investigation of OCT1-mediated transport during drug development was added, based on clinically important OCT1-mediated drug-drug interactions (Zamek-Gliszczynski et al., 2018a; Zamek-Gliszczynski et al., 2018b).

**Multidrug and Toxin Extrusion Protein 1**

The existence of an organic cation-H\(^+\) antiporter was already postulated back in 1985 by studying the transport of N\(^1\)-methylnicotinamide by the use of membrane vesicles, derived from the brush border membrane of rabbit kidney (Wright, 1985; Inui et al., 2000). The multidrug and toxic compound extrusion family (MATE) was first characterized in bacteria (Pallen, 1999) and Otsuka et al. (Otsuka et al., 2005) identified human and mouse orthologues of the bacterial MATE protein by genomic databank screening. The human MATE family consists of two members, the more widely expressed MATE1 protein and the kidney-specific member MATE2-K. The MATE1 protein is localized in the apical membrane of kidney proximal tubule epithelial cells and in the canalicular membrane of human hepatocytes (Otsuka et al., 2005; Masuda et al., 2006). MATE1 substrates are cations or have a positively charge at physiological pH (Nies et al., 2010). MATE proteins have a strong substrate overlap with the SLC22 family members OCT1, OCT2 and OCT3, indicating an interplay between these transporters in the hepatobiliary and renal elimination of drugs and endogenous compounds. The ITC recommends *in vitro* uptake studies using MATE-transfected cells, if the new molecular entity (NME) shows renal secretion as route of elimination or if the NME is an inhibitor of MATE1/2-K or OCT2 (Hillgren et al., 2013). So far, no criteria are defined for the evaluation of hepatic elimination of drugs mediated by MATE1. Detailed lists of substrates and inhibitors are available in several reviews (Terada and Inui, 2008; Damme et al., 2011; Nies et al., 2011; Motohashi and Inui, 2013; Nies et al., 2016; Koepsell, 2020).

**P-glycoprotein**

P-glycoprotein (P-gp) is an ABC transporter and acts as an efflux pump for a variety of drugs such as digoxin, dabigatran etexilate and indinavir. P-gp is due to its ability of extruding drugs an limiting factor for drug bioavailability (Fromm, 2004). The substrate spectrum shows a strong overlap with the substrates of the Cytochrome P450 enzyme CYP3A4 and both proteins together protect the organism from xenobiotics (Kivistö et al., 2004; von Richter et al., 2004). P-gp is expressed in the apical membrane of several tissues such as small intestine, liver and kidney (Thiebaut et al., 1987). Additionally, P-gp plays an
important role at blood-tissue barriers such as the blood-brain barrier and placenta, protecting the central nervous system or the unborn child from drugs or other xenobiotics (Fromm, 2004). Furthermore, P-gp is overexpressed in several cancer tissues, leading to multidrug resistance (Gottesman et al., 2002; Leopoldo et al., 2019). Wang et al. (Wang et al., 2003) analyzed by structure activity relationship analysis (SAR) several substrates and inhibitors of P-gp. They postulated that a tertiary nitrogen atom could be beneficial for the binding to P-gp due to the stronger interaction of the formed cation with the binding sites of P-gp. These cationic properties of some P-gp substrates already indicate that there might be an interplay between the OCT1-mediated uptake and the P-gp-mediated efflux during hepatobiliary elimination. Based on the recommendations of the ITC and FDA (International Transporter Consortium et al., 2010; Food and Drug Administration, 2020), a NME should be tested as P-gp substrate using inside-out oriented membrane vesicles or by vectorial transport assays using polarized grown cell lines such as Caco-2 cells or cell lines (MDCK, LLC-PK1) recombinantly overexpressing P-gp.

### CELL MODELS TO STUDY ORGANIC CATION TRANSPORTER 1 TRANSPORT FUNCTION

#### Single-Transfected Cell Models for Investigating Organic Cation Transporter 1

Use of single-transfected cell models expressing the transporter of interest is often the first step to gain insights into the substrate spectrum. The transporter is either transiently or stably transfected into a suitable cell line. The most commonly used cell lines for uptake studies are Human Embryonic Kidney 293 cells (HEK293). HEK293 cells are easy to culture and have, due to their human origin, comparable posttranslational protein modification to human tissues (Hu et al., 2018). Additionally, after transfection HEK293 cells are capable of expressing a variety of different proteins (Thomas and Smart, 2005). To study transport proteins, uptake assays can be used to determine transport parameters (Kₘ or Cₘₐₓ) values of the selected substrate (Figure 1A) or to perform drug-interaction studies. One limitation of using HEK293 cells is the lack of polarized growth, which excludes them for the analysis of transcellular transport studies. Other frequently used cell lines for establishing single-transfected cell models with the expression of one transport protein are Madin-Darby Canine Kidney cells (MDCK), Chinese Hamster Ovary cells (CHO), Drosophila Schneider 2 cells (S2), HeLa cells and Xenopus oocytes. Xenopus oocytes are a robust cell model, which is derived from Xenopus laevis (Zeng et al., 2020). The exogenous mRNA encoding the transport protein of interest is injected into oocytes leading to a functional expression of the protein. However, because of their limited longevity Xenopus oocytes cannot be used to generate stable transfectants.

Pioneering work on the characterization of OCT1 was done by Zhang et al. (Zhang et al., 1997). They were the first to clone OCT1 from human liver and they used Xenopus oocytes to analyze OCT1-mediated transport. They calculated the first transport Kₘ and Vₘₐₓ parameters for the uptake of the organic cation MPP⁺ and measured the IC₅₀ values for the inhibition of OCT1-mediated transport of MPP⁺ by the cations decynium-22, vecuronium and TEA (Zhang et al., 1997). Furthermore, they extended their research by using transiently transfected HeLa cells and characterized the transport of TEA and obtained IC₅₀ values for 15 different compounds (Zhang et al., 1998). The first inhibitor analysis using a wide range of compounds was done by Bednarczyk et al. (Bednarczyk et al., 2003). They used OCT1-
transfected HeLa cells and calculated IC₅₀ values of 30 structurally diverse organic cations and established a model of inhibitor/OCT1 interaction (Bednarczyk et al., 2003). These findings of structural requirements for OCT1 inhibition were extended by Ahlin and coworkers and their analysis of the inhibitory effect of 191 compounds on the OCT1-mediated uptake of ASP⁺ (Ahlin et al., 2008). ASP⁺ [4-(4-(dimethylamino)styryl)-N-methylpyridinium] is a fluorescent cationic model substrate for OCT1, which enables the fast screening of drugs as inhibitors of OCT1-mediated transport by analyzing fluorescence uptake. They identified 62 of the investigated compounds as inhibitors (cutoff value ≥50% inhibition) of which 66% were cations, 32% were neutral and repaglinide was the only anionic compound. Therefore, they estimated that high lipophilicity and a cationic character are the two main physicochemical properties of potent OCT1 inhibitors (Ahlin et al., 2008). A detailed analysis of the 'structure-transport relationship' was missing until Hendrickx et al. analyzed the uptake of 354 (with 83 marketed drugs) compounds into stably transfected HEK293 cells expressing OCT1 using a LC-MS/MS approach (Hendrickx et al., 2013). TEA and ipratropium served as reference compounds. In this study, the molecular volume of a compound was identified as the best descriptor for OCT1 substrates and lipophilicity was identified to be not important (Hendrickx et al., 2013). Recent publications emphasized the use of in silico predictions and machine learning approaches for the identification of new OCT1 substrates and their molecular characteristics (Baidya et al., 2020; Jensen et al., 2021). The OCT1 substrate and/or inhibitor spectrum has intensively been studied by various groups [e.g., (Gorboulev et al., 1997; Ciarimboli et al., 2005; Wenge et al., 2011; Tzvetkov et al., 2013; Knop et al., 2015; Otter et al., 2017; Meyer et al., 2019; Jensen et al., 2020b; Koepsell, 2020)].

Single-transfected cell models have also been extensively used to study the influence of genetic polymorphisms in the SLC22A1 gene on kinetic parameters of the OCT1-mediated transport (Kerb et al., 2002; Shu et al., 2003; Tzvetkov et al., 2011; Tzvetkov et al., 2013; Dos Santos Pereira et al., 2014; Mattheai et al., 2015; Meyer et al., 2017; Jensen et al., 2020b). A detailed list about the in vitro analyzed effects of genetic polymorphisms in the SLC22A1 gene has been published by Koepsell (2020). Furthermore, comparisons of human OCT1 with the orthologues of rat or mouse Oct1 has been performed using single-transfected cell models to gain insights into our understanding of potential substrate binding sites or protein regions involved in substrate recognition (Egenberger et al., 2012; Floerl et al., 2020; Koepsell, 2020; Meyer et al., 2020).

Table 1 summarizes currently known OCT1 substrates. We included all data where a Kₘ-value was determined or where the uptake was ≥2-fold higher in the OCT1-expressing cells compared to the uptake into the respective control cell line. Potential substrates with uptake ratios between 1.5 and 2 are shown in Supplementary Table S1, together with publications that were not able to reproduce uptake experiments with controversial substrates (e.g., imatinib). OCT1 inhibitors are shown in Supplementary Table S2. We also included inhibition experiments, where no IC₅₀ values were calculated, if the inhibitor was able to reduce the uptake of the substrate to ≤50%. Nevertheless, these lists are not exhaustive.

**Double-Transfected Cell Lines**

In contrast to HEK293 cells, MDCK cells form confluent monolayers when seeded on permeable membranes, such as microplate thinserts, separating a basolateral from an apical compartment (Figure 1B). These cells can be transfected with two cDNAs, for example one cDNA encoding for a basolaterally localized uptake transporter and one cDNA for an apically localized export protein. This allows a more versatile experimental setup, because these culture conditions enable transcellular transport measurements in combination with the measurement of the intracellular accumulation of the substrates. Furthermore, substrates can be applied either to the basolateral or apical compartment mimicking both routes of substrate transport, the route of excretion with the uptake of substrates from blood across the basolateral membrane and the export across the apical membrane into bile or urine (basal to apical transport) or the route of reuptake of substances across the apical membrane and the export into the blood (apical to basolateral transport e.g., during renal reabsorption). Limitations of this cell line are the expression of endogenous canine transporters such as canine Mdr1, Mrp2 and Oct2, which may affect the transport studies. Additionally, it is absolutely necessary to investigate the tightness of the cell monolayer to avoid paracellular transport of substances (Volpe, 2011).

The first double-transfected MDCK cell line expressing human OCT1 as uptake transporter together with P-gp in the apical membrane was established by Nies et al. [MDCK-OCT1-P-gp, Table 2 (Nies et al., 2008)]. The protein expression was investigated by immunoblot and immunofluorescence analysis and for the functional testing, TEA and MPP⁺ served as prototypic substrates for OCT1. Subsequent to the identification of berberine, a quaternary isoquinoline alkaloid, as an OCT1 and OCT2 substrate, the authors used the MDCK-OCT1-P-gp cell line to analyze the transcellular transport of this substance. The transport of berberine from the basal to the apical compartment was 3-fold, 5-fold and 1-fold higher in MDCK-OCT1-P-gp cells compared to the vectorial transport measured with MDCK-OCT1 and MDCK-P-gp single-transfected cells and MCDK control cells, respectively. Furthermore, the addition of the P-gp inhibitor LY335979 resulted in a decrease of the transcellular transport to the level measured in MCDK control cells. Even though the transcellular transport could be inhibited, an increase of the intracellular berberine amount was observed in MDCK-OCT-P-gp cells, indicating that LY335979 specifically inhibits the P-gp mediated export. Misaka et al. also established a MDCK-OCT1-P-gp double-transfектant and this cell line also showed a significant basal to apical transcellular transport of berberine, which could not be measured in the respective single-transfectants (Misaka et al., 2016). They also investigated the transcellular transport of nadolol (10 µM) with and without the addition of 1 µM zosuquidar, a known P-gp inhibitor, demonstrating that zosuquidar was able to significantly inhibit the basal to apical transport of nadolol (Misaka et al., 2016).
Sato et al. (Sato et al., 2008) established an OCT1-MATE1 double-transfected MDCK cell line and investigated the expression and localization by immunofluorescence microscopy. They used TEA as prototypic substrate and measured the transcellular transport from the basolateral to apical (b→a) and from the apical to basolateral (a→b) compartment demonstrating that the cellular accumulation was 66-fold higher, when TEA was applied to the basolateral compartment. Additionally, they were able to reproduce the pH-dependency of MATE1-mediated transport by varying the apical pH and demonstrated that the transcellular transport showed maximal transport rates at extracellular pH 6.5. The addition of 10 mM MPP⁺ or 1 mM levofloxacain significantly decreased the basolateral to apical transport of TEA. To further analyze the transport of organic cations, Sato and coworkers measured the transcellular transport and cellular accumulation of MPP⁺, metformin, cinetidine, creatinine, guanidine, procainamide and quinidine and found significant vectorial transport rates for all substances, applied to the basolateral compartment. Unfortunately, they did not show a comparison between transcellular transport rates and the cellular uptake of substances into the MDCK-OCT1-MATE1 double-transfectant and into the corresponding single-transfectants (MDCK-OCT1 or MDCK-MATE1). The importance of the interplay of OCT1 and MATE1, studied in double-transfected cell lines could also be demonstrated by Sato et al. (Sato et al., 2008). Experiments using HEK293 cells transfected with OCT1 only showed slightly higher uptake rates of quinidine and procainamide (<2 fold) and the HEK-MATE1 cell line showed small uptake rates for quinidine (<2 fold) compared to the uptake into the vector control cell lines. This is contradictory to in vivo data that had already shown that quinidine (Notterman et al., 1986) and procainamide (Somogyi et al., 1983) are secreted renally. This underestimation of the role of OCT1 and MATE1 for the transport of both substrates was abolished by the use of double-transfected cell lines where significant transcellular transport rates could be measured for procainamide as well as for quinidine (Sato et al., 2008).

Our working group extended the investigations of Sato et al. by also establishing a MDCK-OCT1-MATE1 double-transfectant (König et al., 2011). The corresponding single-transfected cell lines (MDCK-OCT1 and MDCK-MATE1) were also used for transport assays. The cellular accumulation of MPP⁺ (10 and 50 µM) and metformin (10 and 50 µM) was highest in MDCK-OCT1 single-transfected cells. Interestingly, the lowest intracellular accumulation was measured in the MDCK-MATE1 single-transfected cells and not in the MDCK control cells. This can be explained by MATE1-mediated efflux of MPP⁺ or metformin taken up by an endogenous transporter or diffused passively into the cells when applied to the basolateral compartment. Intracellular accumulation in the MDCK-OCT1-MATE1 double-transfected cell line was also significantly higher compared to the accumulation in the MDCK control cell line demonstrating OCT1-mediated uptake. As expected, there was no significant difference in the transcellular transport of the single-transfected cell lines and the MDCK control cells. In contrast, the MDCK-OCT1-MATE1 double-transflectant showed significantly higher transcellular transport rates for both substrates (10-fold basal to apical over apical to basal transcellular transport of metformin after 60 min). In the following years, several publications used double-transfected OCT1-MATE1 cell models to gain more insights into vectorial transport of organic cations. Reznicek et al. (Reznicek et al., 2017) used emtricitabine as substrate for vectorial transport studies and demonstrated that the transcellular transport is independent of OCT1-mediated uptake. This transport was saturable at very high concentrations (1 mM), temperature- and pH-dependent (decreasing the apical pH significantly increased the b→a transcellular transport). Furthermore, the addition of cinetidine and ritonavir, both known MATE1 inhibitors, resulted in an inhibition of the transcellular transport of emtricitabine by 43 and 35% in the double-transfectant, whereas the intracellular accumulation increased to 143 and 135%, respectively.

Chen et al. (Chen et al., 2017b) demonstrated that the basal to apical transcellular transport of ipratropium (0.5 µM) was 9.9-fold higher in MDCK-OCT1-MATE1 double-transfected cells compared to control cells and Deutsch and colleagues (Deutsch et al., 2019) identified trospium as substrate for both transporters using the same transporter combination. The vectorial basal to apical transport of trospium (1 µM) was 24.5-fold higher compared to the vectorial transport in the control cell line. As expected, the transcellular transport was highest at extracellular pH 6.5, whereas intracellular accumulation was lowest at this pH, demonstrating that OCT1 and MATE1 play an important role in the transcellular transport of trospium.

Ceckova et al. (Ceckova et al., 2016) analyzed the transcellular transport and intracellular accumulation of lamivudine in MDCK-OCT1-MATE1 double-transfected cells and their respective control and single-transfected cell lines. The transcellular transport (b→a) measured in the MDCK-MATE1 and MDCK-OCT1-MATE1 cells was significantly higher in comparison to the MDCK control cells and to the MDCK-OCT1 single-transfectant, whereas the intracellular accumulation of lamivudine was the highest in the MDCK-OCT1 cell line. This transcellular transport could be inhibited by the simultaneous application of lamivudine and mitoxantrone (2 µM) to the basolateral compartment and was reduced to a level which was not significantly different to the MDCK control cells. The fact, that mitoxantrone inhibition led to an increase of the intracellular accumulation of lamivudine, underlines the importance of MATE1 on the transport of lamivudine. Later, Ceckova et al. (Ceckova et al., 2018) used the MDCK-OCT1-MATE1 double-transfectant to study the inhibition of the transcellular transport of 2 nM MPP⁺ and 10 nM lamivudine by adding efavirenz. In both cases, the presence of 10 µM efavirenz in the basolateral compartment reduces the basolateral to apical transport in all single- and double-transfected cell lines, except in the MDCK control cells. The intracellular accumulation of both substrates was decreased in the MDCK-OCT1 cells but increased in the MDCK-MATE1 cells, confirming the potential of efavirenz as an in vitro inhibitor of both transport proteins (Ceckova et al., 2018). Li et al. (Li et al., 2018) addressed a potential drug-drug interaction between
with the phase I drug metabolizing enzyme CYP3A4 (Tu et al., 2014). To investigate this, Tu et al. established a double-transfected cell line. At all investigated time points the basolateral compartment was unaltered by the addition of nuciferine. This demonstrates that nuciferine is an inhibitor of both OCT1 and MATE1. Remarkably, when applying the same experimental setup to the MDCK-OCT2-MATE1 double-transfectant, the transcellular transport of metformin was also decreased but the intracellular accumulation of metformin significantly increased in a concentration-dependent manner after addition of nuciferine. This indicates, that the inhibition of MATE1 is responsible for this effect and nuciferine inhibits OCT1, but not OCT2 (Li et al., 2018).

In an interesting experimental setup van der Velden et al. (van der Velden et al., 2017) were not using MDCK cells to establish double-transfectants. Instead, they used single-transfected HEK293 cells expressing OCT1 and cotransfected them with MATE1 or with MATE2-K and analyzed proguanil uptake. Because of the lack of polarized growth, vectorial transport studies cannot be performed with the double-transfected HEK293 cells. There was no significant difference in the uptake rate of HEK-OCT1 cells compared to HEK-OCT1-MATE1 cells, but the HEK-OCT1-MATE2-K cells showed a significant lower intracellular accumulation of proguanil, indicating an interplay between OCT1-mediated uptake and MATE2-K-mediated export (van der Velden et al., 2017).

Double-transfected cell models cannot only be used to study the interplay of uptake and efflux transporters, but also to investigate the interplay between transport proteins and metabolizing enzymes. To investigate this, Tu et al. established a double-transfected MDCK cell line, expressing OCT1 together with the phase 1 drug metabolizing enzyme CYP3A4 (Tu et al., 2014). This CYP enzyme is responsible for the metabolism of approx. 50% of all marketed drugs (Zhou, 2008). They validated the mRNA expression by RT-qPCR and confirmed the OCT1-mediated uptake by using the prototypical substrate ASP2 with or without the presence of TEA as transport inhibitor. The MDCK-OCT1 single-transfectant as well as the MDCK-OCT1-CYP3A4 double-transfectant showed significantly higher ASP2 uptake rates compared to the control cell line, which was strongly reduced by the addition of TEA. The CYP3A4 function in the MDCK-OCT1-CYP3A4 cells was confirmed by a CYP3A4 metabolism activity assay and was comparable to the values determined in MDCK-CYP3A4 single-transfected cells. Subsequently, they tested the cytotoxic activity of retrorsine, a hepatotoxic pyrrolizidine alkaloid, using all established MDCK cell lines. Prior experiments showed that the uptake of retrorsine is significantly higher in MDCK-OCT1 cells compared to the uptake into the MDCK control cells. Furthermore, Fu et al. demonstrated that pyrrolizidine alkaloids exhibit cytotoxicity only after bioactivation, which is mainly mediated by CYP3A4 (Fu et al., 2004). In line with these findings, the cytotoxicity of retrorsine was highest in the MDCK-OCT1-CYP3A4 cell line because of both uptake and bioactivation. There was no difference in the cytotoxicity between control cells and MDCK-OCT1 cells, due to the missing CYP-mediated activation. The MDCK-CYP3A4 single-transfectant also exhibit significantly higher retrorsine sensitivity, but still significantly lower compared to the double-transfectant (Tu et al., 2014).

Instead of MDCK cells, Iwai et al. used Lilly Laboratory Cancer Porcine Kidney 1 cells (LLC-PK1) to establish an OCT1-P-gp double-transfected cell line (Iwai et al., 2011). LLC-PK1 cells form tight monolayers and LLC-P-gp cells are recommended by the FDA as bidirectional transcellular transport system for identifying P-gp substrates and inhibitors (Food and Drug Administration, 2020). OCT1 function in these double-transfected cells was confirmed by using MPP7 as prototypical substrate and the transport function of P-gp was verified by using digoxin as substrate. The basal to apical transcellular transport of 1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d]imidazolium bromide (YM155, 1 µM), a survivin suppressant and known substrate of OCT1 (Iwai et al., 2009), was much higher in the LLC-OCT1-P-gp double-transfectant compared to LLC-control, LLC-OCT1 and LLC-P-gp single-transfected cell lines, demonstrated by the high basal to apical flux ratio of 16.6. This transcellular transport decreased by adding cyclosporine A or 1 mM MPP7, respectively, indicating that YM155 is a substrate of both OCT1 and P-gp. The relatively high basal to apical transcellular transport of 1 µM digoxin was unaffected by the addition of 100 µM YM155 but was reduced to the level of the apical to basal transport by adding 10 µM cyclosporine A, demonstrating that YM155 has a low inhibitory effect on P-gp-mediated transport even at higher concentrations. Table 2 gives an overview about the studies using OCT1 expressing double-transfected cell lines.

**DISCUSSION**

In vitro cell models expressing transport proteins are useful tools for studies of transporter function and for the identification of transporter substrates and/or inhibitors. Therefore, the FDA and EMA recommend the usage of such cell lines during preclinical drug development. The FDA considers an investigational drug as a drug if uptake is ≥ 2-fold of the drug uptake in empty vector-transfected cells and if a known inhibitor can decrease the drug uptake to ≤50% at a concentration at least 10 times that of the Ki or IC50. To test whether a drug is an inhibitor it is recommended to determine...
the inhibition potency (Kᵢ or IC₅₀) of the drug on the uptake of a known substrate (Food and Drug Administration, 2020). In this review we describe cell models for the investigation of the SLC22 family member OCT1. Using single-transfected cell lines expressing OCT1, several drugs could be identified as substrates and inhibitors of this transporter (Table 1; Supplementary Table S2). Interestingly, it has been demonstrated that OCT1 transport inhibition is substrate-dependent. For example, Boxberger et al. detected substrate-dependent inhibition for several drug (e.g., ranitidine and fluoxetine) by using MPP⁺, serotonin and TEA as probe substrates in competitive counterflow experiments (Boxberger et al., 2018). Therefore, the use of multiple probe substrates for in vitro testings of OCT1 seems reasonable and the use of substrates for the inhibition analysis in vitro that can also be used in the subsequent clinical studies as recommended by the FDA Food and Drug Administration (2020).

Despite the frequent use of single- and double-transfected cell lines, in vitro-in vivo extrapolations (IVIVE) have still limitations. Many drugs listed in Supplementary Table S2 only inhibit the transport of substrates at concentrations above their therapeutic plasma concentration or environmentally exposed concentration so that the inhibitory potential is more theoretically relevant (Chedik et al., 2019). In vitro studies that analyzed opioids as inhibitors of OCT1, Meyer et al. showed that the calculated maximal unbound plasma concentrations for most of the tested opioids are lower than the obtained IC₅₀ values for OCT1 mediated transport (Meyer et al., 2019). Only the maximal portal vein concentration of tapentadol was comparable to the obtained IC₅₀ value, indicating a potential drug-drug interaction in vivo (Meyer et al., 2019). Furthermore, the influence of endogenous expression of transport proteins in the different cell lines, the use of different cell models (e.g., Table 1: Kₘ, TEA determined in MDCK cells, HEK293 cells and HeLa cells) and the independent establishment of several stable transfectants by different working groups lead to interlaboratory variability in the gained Kₘ and IC₅₀ values and to a limited IVIVE. The use of primary human hepatocytes after the in vitro validation of drugs as substrates or inhibitors of OCT1, as recommended by Bi et al., could be helpful to gain better predictions of the hepatic clearance or to identify potential DDIs and could help to evaluate the contribution of the OCT1-mediated transport of potential substrates by using selective inhibitors (Bi et al., 2019; Jensen et al., 2020a). Interestingly, strong variations in the uptake of OCT1 substrates (MPP⁺ and ASP⁺) were detected comparing human hepatocytes from different donors (De Bruyn et al., 2011; Fattah et al., 2017) and the genetic characterization revealed strong genetic variations between the tested batches, where 13 of 27 tested hepatocyte batches showed at least 1 nonfunctional allele of the SLC22A1 gene (Fattah et al., 2017).

The identification of OCT1 as rate-limiting transporter in the hepatic uptake of clinical important drugs together with in vivo data on reported genetic effects led to the update of the ITC recommendations, where OCT1 is now mentioned as transporter of emerging clinical importance (Zamek-Gliszczynski et al., 2018b).

Double-transfected cell lines could lead to an even better understanding of vectorial transport processes during hepatobiliary and renal elimination. They allow the simultaneous measurement of more parameters and are helpful to identify the individual transport protein underlying clinically observed drug-drug interactions and to study the impact of the respective transporters on perpetrator disposition (Müller et al., 2018b). Important double-transfected cell models for investigating the role of OCT1 in the hepatobiliary elimination of drugs are MDCK-OCT1-MATE1 cells expressing OCT1 together with the apically localized export protein MATE1. Both proteins share an overlapping substrate spectrum (Nies et al., 2011) and the vectorial transport of drugs mediated by both transporters has been described (Table 2). Interestingly, only by using double-transfected cell models the direction of the MATE1-mediated transport in the double-transfected cell lines resembles the physiological direction (efflux of substrates into the apical compartment), whereas the use of MATE1-transfected HEK293 cells only allows uptake measurements into the cell. In the recent years, several working groups established double-transfected cell lines to analyze the molecular mechanisms underlying polarized transport of endogenous compounds and drugs. Moreover, they are very useful tools for the understanding of the molecular mechanisms underlying clinically relevant drug-drug interactions (Table 2).

**AUTHOR CONTRIBUTIONS**

BH reviewed the literature, BH and JK drafted the manuscript, MFF revised the manuscript.

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**SUPPLEMENTARY MATERIAL**

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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