European Journal of Pharmaceutics and Biopharmaceutics 112 (2017) 187–195

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Effects of frequently used pharmaceutical excipients on the organic cation transporters 1–3 and peptide transporters 1/2 stably expressed in MDCKII cells

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ARTICLE INFO

Article history:
Received 9 June 2016
Revised 4 October 2016
Accepted in revised form 23 November 2016
Available online 27 November 2016

Keywords:
Pharmaceutical excipients
Solubilizing agents
Drug transporter
Uptake transport
Organic cation transporter (OCT)
Peptide transporter (PEPT)
Polyethylene glycol (PEG)
Hydroxypropyl-β-cyclodextrin
Solutol HS 15 (macrogol 15 hydroxystearate, polyoxyl 15 hydroxystearate)
Cremophor
Tween (polysorbate)
Kolliphor (poloxamer)
MDCK cells
In vitro
Cell model
Competition assay
Inhibition assay

ABSTRACT

There is ample evidence that pharmaceutical excipients, which are supposed to be pharmacologically inactive, have an impact on drug metabolism and efflux transport. So far, little is known whether they also modulate uptake transporter proteins. We have recently shown that commonly used solubilizing agents exert significant effects on the function of organic anion uptake transporting polypeptides. Therefore, we investigated in this study the influence of frequently used pharmaceutical excipients on the transport activity of organic cation transporters OCT1, OCT2 and OCT3 and the peptide transporters PEPT1 and PEPT2.

Inhibition of the OCTs and PEPTs by the excipients polyethylene glycol 400 (PEG), hydroxypropyl-β-cyclodextrin (HPCD), Solutol® HS15 (SOL), Cremophor® EL (CrEL), Tween® 20 (Tw20), Tween® 80 (Tw80), Kolliphor® P188 (P188) and Kolliphor® P407 (P407) was evaluated using stably transfected MDCKII cells with radio-labeled reference substrates and established inhibitors as controls. Intracellular accumulation of [3H]-1-methyl-4-phenylpyridinium (MPP^+) for the OCTs and [3H]-glycyl-sarcosine (Gly-Sar) for the PEPTs was measured by liquid scintillation counting after cell lysis.

Our studies revealed that PEG, HPCD, SOL, CrEL, Tw20 and Tw80 were potent inhibitors of OCT1-3 (e.g., Tw20 IC_{50} values < 0.04%). Cellular uptake of Gly-Sar by PEPT1 and PEPT2 was strongly inhibited by both Tw20 and Tw80. SOL was also a strong inhibitor of PEPT1 and PEPT2 (e.g., SOL IC_{50} values < 0.02%), while CrEL showed significantly inhibition of only PEPT2. The substantial inhibitory effects of certain solubilizing agents on OCTs and PEPTs should be considered if they are to be used in dosage forms for new chemical entities and registered drugs to avoid misinterpretation of pharmacokinetic data and undesired drug interactions.

1. Introduction

In the current drug development landscape, about 40% of all new chemical entities (NCE) are poorly water-soluble compounds [1]. Therefore, there is a need for excipients to solubilize such candidates in both the early preclinical and clinical evaluation, as well as for development of the marketed drug dosage forms [2,3]. Contrary to the expectation that pharmaceutical excipients are pharmacologically inactive, there is abundant evidence that they can influence drug metabolism and efflux transport. In this regard, solubilizing agents such as Cremophor EL® (CrEL) or Tween® 80 (Tw80) interact *in vitro* with cytochrome P450 (CYP) enzymes and the ATP-binding cassette (ABC) transporters ABCB1, ABCG2

Abbreviations: CYP, cytochrome P450; ABCB1, P-glycoprotein; ABCC2, multidrug resistance associated protein 2; ABCG2, breast cancer resistance protein 1; OATP, organic anion transporting polypeptide; NTCP, Na^+/taurocholate cotransporting polypeptide; PEPT1, peptide transporter 1; PEPT2, peptide transporter 2; MDCKII cells, Madin-Darby canine kidney cells; WT, wild-type (control); DAPI, 4′,6-diamidino-2-phenylindole; Gly-Sar, glycyl-sarcosine; MPP^+, 1-methyl-4-pyridinium; PEG, polyethylene glycol 400; HPCD, hydroxypropyl-β-cyclodextrin; SOL, Solutol HS 15; CrEL, Cremophor EL; Tw20, Tween 20; Tw80, Tween 80; P188, Kolliphor P188; P407, Kolliphor P407; NCE, new chemical entities; CMC, critical micelle concentration; IC_{50}, half-maximal inhibitory concentration; MIE, maximal inhibitory effect.

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http://dx.doi.org/10.1016/j.ejpb.2016.11.028
0939-6411/

and ABCC2 [4–8]. There is also evidence from clinical studies in man confirming excipient-mediated changes in pharmacokinetics of drugs attributed to metabolism (e.g., inhibition of acetaminophen oxidation via CYP2E1 by propylene glycol), efflux transport (e.g., increase in digoxin bioavailability after ABCB1 inhibition by Cremophor RH40) or a combination of both processes (e.g., multiple-fold increased saquinavir exposure by inhibition of ABCB1 and CYP3A in presence of CrEL) [9,10]. Therefore, thorough evaluation of potential intrinsic pharmacological effects of solubilizing excipients on drug metabolism and transport is necessary to design new dosage forms for preclinical and clinical evaluation of NCEs and the marketed products, and to understand the rationale behind unexpected interactions of dosage forms with disposition and, hence, therapeutic efficacy and safety of concomitantly prescribed drugs. The information from the literature on interactions of excipients with the pharmacokinetic processes, however, is still rather limited. Concerning their potential influence on the function of drug uptake transporters, we have recently shown that the solubilizing agents polyethylene glycol 400 (PEG), hydroxypropyl-β-cyclodextrin (HPCD), Solutol® HS 15 (SOL) and CrEL inhibit organic anion transporting polypeptides (OATPs) and the sodium-taurocholate cotransporting polypeptide (NTCP) *in vitro*, transporters with mostly anionic substrate specificity [11,12,12]. Effects on other uptake transporters of pharmacokinetic importance, such on members of the organic cation transporter (OCT) or peptide transporter (PEPT) families have not been sufficiently investigated [13,14]. OCTs and PEPTs mediate the cellular uptake of various endogenous and xenobiotic compounds with preferentially cationic (OCTs) or zwitterionic (PEPTs) chemical characteristics [15–18]. They are involved in the uptake of drugs into enterocytes (OCT1, OCT3, PEPT1), hepatocytes (OCT1, OCT3), proximal renal tubular cells (OCT2, OCT3, PEPT2) and the lungs (OCT1-3, PEPT2), and can significantly influence drug disposition, as has been convincingly confirmed for the antidiabetic drug metformin (OCT1) and for the β-lactam antibiotic cefadroxil (PEPT1) [16,19–23].

Pharmaceutical excipients with OCT and/or PEPT modulating activity might be additional confounders in the pharmacokinetics of and, in turn, efficacy and safety of substrate drugs. Therefore, we investigated the effects of the widely used excipients PEG, HPCD, SOL, CrEL, the polysorbates Tween® 20 (Tw20) and Tw80, as well as the poloxamers Kolliphor® P188 (P188) and Kolliphor® P407 (P407) on the function of OCT1-3 and PEPT1/2 stably expressed in MDCKII cells.

2. Material and methods

2.1. Chemicals

PEG and HPCD were obtained from Applichem (Darmstadt, Germany). CrEL, Tw20, Tw80, P188, P407, Glycyl-Sarcosine (Gly-Sar) and 1-methyl-4-pyridinium (MPP^+) were purchased from Sigma-Aldrich (Taufkirchen, Germany). SOL was a kind gift from Bayer Healthcare (Berlin, Germany). [3H]-Gly-Sar (29.4 Ci/mmol) was supplied by Hartmann Analytic (Braunschweig, Germany) and [3H]-MPP^+ (80 Ci/mmol) by American Radiolabeled Chemicals (Saint Louis, USA).

2.2. Cell culture

MDCKII cells were cultivated in the full growth Dulbecco's Modified Eagle Medium (DMEM, low glucose) supplemented with 10% fetal bovine serum, 4 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin (PAN Biotech, Aidenbach, Germany) at 37 °C, 95% humidity, and 5% carbon dioxide.

2.3. Characterization of stably transfected cell lines

Stably transfected MDCKII cells overexpressing OCT1, OCT2 or OCT3 were generated as previously described [24]. MDCKII-PEPT1 and MDCKII-PEPT2 cells were kindly provided by Pfizer (Groton, Connecticut, U.S.A.). All cells were characterized with regard to mRNA expression and protein content, localization as well as transport function of the respective transporter using real-time quantitative PCR, targeted quantitative proteomics by LC–MS/MS, immunofluorescence analysis and uptake assays using standard probe substrates.

2.4. Immunofluorescence staining and microscopy

The cells were seeded on cover slips in 24-well plates (Falcon, Heidelberg, Germany) in complete growth medium (w/o penicillin/streptomycin) and fixed with acetone/methanol (1:1) for 15 min at −20 °C. After permeabilization of the plasma membrane with 0.1% Triton X-100 for 5 min, cells were stained with mouse polyclonal antibody anti-OCT1 (ab167483, Abcam, Cambridge, UK), rabbit polyclonal antibody anti-PEPT1 (ab78020, Abcam, Cambridge, UK), anti-PEPT2 (251226, ABBIOTEC, San Diego, U.S.A) or mouse monoclonal antibodies anti-OCT2, anti-OCT3 (659801 and 653701, Biolegend, Fell, Germany), respectively. Goat-anti-rabbit or goat-anti-mouse conjugated to Alexa Fluor 488 (Life Technologies, Darmstadt, Germany) were used as secondary antibodies and the nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). Negative controls were performed by using the secondary antibodies only. Immunofluorescence microscopy analysis was performed using the laser scanning confocal microscopy system LSM780 (Carl Zeiss MicroImaging, Jena, Germany).

2.5. Gene expression and protein abundance

Gene expression of OCTs, PEPTs and the reference genes 18S and GAPDH were measured by real-time quantitative RT-PCR under standard conditions (2 min at 50 °C, 10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) using TaqMan® Gene Expression Assays (Life Technologies, Darmstadt, Germany) and a 7900 HT Sequence Detection System (Applied Biosystems, Weiterstadt, Germany). The data were analysed using the SDS 2.3 software (Applied Biosystems, Foster City, USA). The verification and quantification of the respective transporter protein in the membrane fraction of the stably transfected MDCKII cells was performed using targeted quantitative proteomics by LC–MS/MS as previously described [25].

2.6. Transport studies

The cells were seeded in 24-well plates in complete growth medium (w/o penicillin/streptomycin) at an initial density of 60.000 cells per well and cultivated for three days. Cells were washed once with incubation buffer (142 mM NaCl, 5 mM KCl, 1 mM K_2HPO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 5 mM glucose, 12.5 mM HEPES; pH 7.3, 37 °C) before starting the experiments. After incubation at 37 °C, the cells were washed three times with ice-cold incubation buffer and lysed with cell lysis buffer containing 0.5% Triton-X-100 and 0.5% sodium deoxycholate. Sample aliquots of 100 µl were mixed with 1 ml of scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany). The intracellular accumulation of the respective radio-labeled probe substrate was detected by a liquid scintillation beta counter (type 1409; LKB-Wallac, Turku, Finland). The determination of the unspecific protein content was performed using the bicinchoninic acid assay according to the manufacturer's instructions (Pierce, Rockford, USA).