European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 531–538

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Transport evaluation of salicylic acid and structurally related compounds across Caco-2 cell monolayers and artificial PAMPA membranes

Maija Koljonen [a,*], Katja Rousu [b], Jakub Cierny [a], Ann Marie Kaukonen [a,c], Jouni Hirvonen [a]

[a] Division of Pharmaceutical Technology, University of Helsinki, Helsinki, Finland
[b] Orion Corporation Orion Pharma, Research and Development, Espoo, Finland
[c] Drug Discovery and Development Technology Center (DDTC), University of Helsinki, Helsinki, Finland

ARTICLE INFO

Article history:
Received 10 October 2007
Accepted in revised form 19 May 2008
Available online 6 June 2008

Keywords:
Caco-2
PAMPA
Transport mechanisms
Anionic compounds
pH-dependency

ABSTRACT

The purpose of this study was to evaluate passive vs. proton-dependent active transport mechanisms of salicylic acid (SA) and four structurally related anions. Transport was studied across Caco-2 cell monolayers and artificial lipid membranes (PAMPA) under pH-gradient and iso-pH conditions. Kinetic permeability parameters were provided by bidirectional Caco-2 experiments and concentration-dependency measurements. The transport route and putative transporters involved in SA transport were studied using EDTA and several inhibitors. SA and lipophilic 5-chlorosalicylic acid and 2-hydroxy-1-naphthoic acid reached saturation with increasing compound concentration indicating active transport. Permeation of 5-hydroxysalicylic acid and 5-hydroxyisophthalic acid was not saturated indicating passive transport. PAMPA with pure passive diffusion underestimated the transport of SA compared to Caco-2. Opening up the paracellular tight junctions by EDTA did not increase the transport of SA under the pH-gradient conditions confirming the hypothesis of pure transcellular transport of SA. Active transport of SA remained concentration-dependent even without the pH-gradient, and was reduced by the known MCT1 and OATP-B inhibitors and structurally related anions. Overall, several permeability test protocols are needed to obtain a more complete picture of transport properties of salicylic acid and structurally related compounds.

1. Introduction

Many naturally occurring monocarboxylates, such as acetate, propionate and butyrate, which are fermentation products of carbohydrates in the colon, are reported to be transported by monocarboxylic acid transporters (MCT) [1–2]. In addition to these physiological molecules, many drugs and food-derived substances possess a carboxylic acid moiety in their structure and are ionised at the prevailing pH conditions in the intestine. According to the pH partition theory and the high degree of ionisation of carboxylic acids at physiological pH, it would be expected that these compounds are poorly permeable. Despite this, many of them are highly permeable compounds and, hence, well absorbed. It has been suggested that these molecules are actively transported, presumably by MCTs (monocarboxylic acid transporter family, SLC16), OATPs (organic anion transporting polypeptide family, SLC21) or other proton-dependent transport systems. Examples of such anionic compounds are salicylic acid [3–4], naproxen, ketoprofen, diflunisal, diclofenac [5], atorvastatin [6], pravastatin, fexofenadine [7–8], nateglinide [9–10] and telmisartan [11]. Additionally, merely passive, pH-dependent mechanism has been suggested for the acids, where the high gradient of the permeable protonated form between the acidic extracellular and intracellular (pH 7.5) compartments facilitates the rapid permeation and then dissociation of the protonated form until the intracellular pH decreases to the level of the outside [12].

The monocarboxylate cotransporter family comprises nowadays 14 members, of which MCT1 (SLC16A1) is the most investigated [13]. MCT1 is localised in the apical membranes of human intestine, and its expression increases along the length of the intestine [14]. OATP represents a family of transporters, which are expressed in several tissues and organisms. One of them, the OATP-B (SLCO2B1; SLC21A9), is localised at the apical membrane of intestinal epithelial cells in humans [15], and has been suggested to participate in the transport of anionic compounds such as estrone-3-sulfate [16], pravastatin, fexofenadine [7] and salicylic acid [4]. For both MCT1 and OATP-B, the driving force for compound transport has been linked to the presence of a proton gradient across the cell membrane. MCT1 functions as a proton-coupled transporter [17], but the importance of a proton gradient for OATP-B is still unclear, as it has been shown to be active even without the pH-gradient [7].

* Corresponding author. Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56 (Viikinkaari 5 E), 00014 Helsinki, Finland. Tel.: +358 9 191 59 674; fax: +358 9 191 59 144.
E-mail address: maija.koljonen@helsinki.fi (M. Koljonen).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.05.017

Recently published results revealed that the Caco-2 cells express similar transporters as the human jejunum, MCT1 and OATP-B among them [18], and are thus useful for screening purposes and, in particular, for mechanistic studies. In order to avoid the formation of subpopulations, the cells should be used at a well-defined passage range. Bidirectional studies across the Caco-2 cells under pH-gradient (acidic apical pH) and non-gradient conditions are valuable when evaluating the role of active transport [19]. However, in the case of acidic drugs, bidirectional studies under pH-gradient conditions can give misleading information of the transport mechanism, since the high influx ratio under pH-gradient conditions may be interpreted falsely as active transport [4]. Kinetic studies with increasing compound concentration under different pH conditions and the use of inhibitors/substrates of putative active transporters give more detailed information of the transport mechanism and of the pH-dependency.

Permeation experiments across an artificial lipid membrane (PAMPA, parallel artificial membrane permeability assay) are used as a rapid screening method for new drug candidates. Even though it is a fast and good predictor of passive permeation, PAMPA membrane lacks the paracellular spaces and active transporters and, thus, may underestimate the permeation of actively transported compounds. Therefore, it has been suggested that PAMPA and Caco-2 permeation methods should be used parallel for the evaluation of drug permeation mechanism(s) in drug discovery [20].

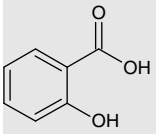
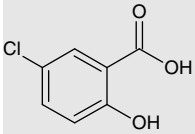
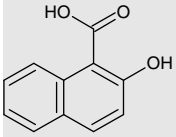
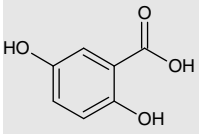
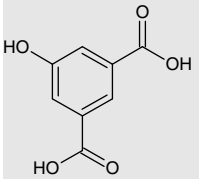
The aim of our study was to probe a range of permeation methods in the evaluation of passive vs. proton-dependent active transport mechanisms of salicylic acid (SA) and four structurally related anions. Firstly, the applicability of bidirectional and concentration dependency studies in the evaluation of permeation mechanisms (active and passive) was explored with Caco-2 cell monolayers. Passive permeation was evaluated also using the PAMPA method in order to utilize the information obtained from the Caco-2 and PAMPA experiments in a complementary fashion. Salicylic acid (SA) was used as a model compound, as present evidence suggests that it is a substrate of both MCT1 and OATP-B [4]. The other structurally related anions have similar ionisation behaviour, but differ from each other by their substituents, amount of ionisable/polar groups and lipophilicity (Table 1). These physico-chemical characteristics of the model anions were considered in relation to their potential active and passive transport. Second, the feasibility of paracellular disruption (by EDTA) in elucidating the contribution of paracellular and transcellular routes was explored using SA and known markers for transcellular (antipyrine) and paracellular (mannitol) permeation. Finally, transport inhibition studies of SA were performed using MCT1 and OATP-B inhibitors as well as other structurally related compounds, and proton-dependency of the active transport examined.

2. Materials and methods

2.1. Compounds

Salicylic acid (SA), 5-chlorosalicylic acid (5-Cl-SA), 2-hydroxy-1-naphthoic acid (2-OH-NA), 5-hydroxysalicylic acid (5-OH-SA), 5-hydroxyisophthalic acid (5-OH-IPA) (Table 1), as well as the inhibitors, pravastatin sodium and probenecid, were purchased from Sigma–Aldrich Chemie (Steinheim, Germany). Passive transcellular marker molecule antipyrine, the inhibitors α-cyano-4-hydroxycinnamic acid (CHC) and 2-hydroxy-3-isopropylbenzoic acid (2-OH-3-IPBA) were from Aldrich Chemical Company Inc. (Milwaukee, WI), and the paracellular membrane integrity marker

Table 1
Structures and physico-chemical parameters of the studied compounds

Compound		pK_a	Log P	Log $D_{5.5}$	log $D_{7.4}$
	Salicylic acid (SA)[a] (MW 138)	2.90	2.23	−0.37	−2.27
	5-Chlorosalicylic acid (5-Cl-SA)[a] (MW 173)	2.68	2.96	0.20	−0.62
	2-Hydroxy-1-naphthoic acid (2-OH-NA) (MW 188)	3.08	3.28	0.88	−0.29
	5-Hydroxysalicylic acid (5-OH-SA)[a] (MW 154)	2.72 (COOH) 10.07 (5-OH)	1.66	−1.12	−3.02
	5-Hydroxyisophthalic acid (5-OH-IPA) (MW 182)	3.44 (1-COOH) 4.16 (3-COOH) 9.35 (5-OH)	1.54	−1.69	−4.02

[a] pK_a and log P values from Ref. [21].