European Journal of Pharmaceutical Sciences 106 (2017) 274–286

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Impact of capillary flow hydrodynamics on carrier-mediated transport of opioid derivatives at the blood-brain barrier, based on *pH*-dependent Michaelis-Menten and Crone-Renkin analyses

Siti R. Yusof [a], N. Joan Abbott [b], Alex Avdeef [c,*]

[a] HICoE Centre for Drug Research, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[b] King's College London, Institute of Pharmaceutical Science, Franklin Wilkins Building, 150 Stamford St., London SE1 9NH, UK
[c] in-ADME Research, 1732 First Avenue, #102, New York, NY 10128, USA

ARTICLE INFO

Keywords:
Blood-brain barrier
Rodent *in situ* brain perfusion
Brain permeability-surface area
pH-Dependent Crone-Renkin equation
pH-Dependent Michaelis-Menten analysis

ABSTRACT

Most studies of blood-brain barrier (BBB) permeability and transport are conducted at a single *pH*, but more detailed information can be revealed by using multiple *pH* values. A *pH*-dependent biophysical model was applied to the mechanistic analysis of published *pH*-dependent BBB luminal uptake data from three opioid derivatives in rat: pentazocine (Suzuki et al., 2002a, 2002b), naloxone (Suzuki et al., 2010a), and oxycodone (Okura et al., 2008). Two types of data were processed: *in situ* brain perfusion (ISBP) and brain uptake index (BUI). The published perfusion data were converted to apparent luminal permeability values, P_{app}, and analyzed by the *p*CEL-X program (Yusof et al., 2014), using the *pH*-dependent Crone-Renkin equation (*pH*-CRE) to determine the impact of cerebrovascular flow on the Michaelis-Menten transport parameters (Avdeef and Sun, 2011). For oxycodone, the ISBP data had been measured at pH 7.4 and 8.4. The present analysis indicates a 7-fold lower value of the cerebrovascular flow velocity, F_{pf}, than that expected in the original study. From the pyrilamine-inhibited data, the flow-corrected passive intrinsic permeability value was determined to be $P_0 = 398 \times 10^{-6}$ cm·s^{-1}. The uptake data indicate that the *neutral* form of oxycodone is affected by a transporter at pH 8.4. The extent of the cation uptake was less certain from the available data. For pentazocine, the brain uptake by the BUI method had been measured at pH 5.5, 6.5, and 7.4, in a concentration range 0.1–40 mM. Under similar conditions, ISBP data were also available. The *pH*-CRE determined values of F_{pf} from both methods were nearly the same, and were smaller than the expected value in the original publication. The transport of the cationic pentazocine was not fully saturated at *pH* 5.5 at 40 mM. The transport of the neutral species at pH 7.4 appeared to reach saturation at 40 mM pentazocine concentration, but not at 12 mM. In the case of naloxone, a *pH*-dependent Michaelis-Menten equation (*pH*-MME) analysis of the data indicated a smooth sigmoidal transition from a higher capacity uptake process affecting cationic naloxone (pH 5.0–7.0) to a lower capacity uptake process affecting the neutral drug (pH 8.0–8.5), with cross-over point near pH 7.4. Evidently, measurements at multiple *pH* values can reveal important information about both cerebrovascular flow and BBB transport kinetics.

Abbreviations: ABL, aqueous boundary layer adjacent to the cell surface (including glycocalyx); CM/PD, carrier-mediated (saturable)/lipoidal passive diffusion (non-saturable); D_{aq}, aqueous diffusivity (cm^2·s^{-1}); F_{pf}, cerebrovascular flow velocity of perfusion/injection fluid (mL·s^{-1}·g^{-1}); $F(r/R)$, Renkin molecular sieving function, dimensionless fraction in the range of 0 to 1; h_{ABL}, ABL thickness (cm); ISBP/BUI, *in situ* brain perfusion/brain uptake index; J_{max}, maximum transport rate (μmol·min^{-1}·g^{-1}) of the saturable uptake process in the MM equation; k_d, the non-saturable uptake first-order rate constant (mL·min^{-1}·g^{-1}); K_{in}, unidirectional transfer constant (mL·s^{-1}·g^{-1}), *cf.*, Eq. (B.3); K_m, half-saturation concentration (mM) Michaelis constant in the MM equation; MM, Michaelis-Menten, *cf.*, Eq. (B.6); *pH*-CRE, *pH*-dependent Crone-Renkin equation flow correction method; *pH*-MME, *pH*-dependent Michaelis-Menten equation analysis; P_{app}, *in vitro/in vivo* (planar/capillary) apparent permeability (cm·s^{-1}), *cf.*, Eq. (A.1) or (B.8); P_C, corrected-for-flow (or -for-ABL) permeability coefficient (cm·s^{-1}), depends on *pH* for ionizable permeants (hyperbolic function), basis of the *pH*-partition hypothesis, *cf.*, Eq. (A.2); P_i, flow-corrected luminal permeability coefficient (cm·s^{-1}) of the *ionized* form of drug (carrier-mediated process), depends on *pH* for ionizable permeants (hyperbolic function), *cf.*, Eq. (B.9); P_+, maximum permeability in the P_i - *pH* curve (cm·s^{-1}); P_0, maximum permeability in the P_C - *pH* curve: *in vitro/in vivo* intrinsic permeability of uncharged permeant (cm·s^{-1}); P_{para}, paracellular permeability (cm·s^{-1}), *cf.*, Eq. (A.3); *PS*, permeability-surface area product (mL·s^{-1}·g^{-1}), traditionally determined from K_{in} using Crone-Renkin equation, *cf.*, Eq. (B.5); *r*, hydrodynamic molecular radius (Å); *R*, effective cell layer junctional pore radius (Å); *S*, endothelial surface area in a gram of brain tissue (100 cm^2·g^{-1} assumed in the calculations); ε/δ, porosity of paracellular junction pores divided by the rate-limiting paracellular pathlength (size-restricted, cation-selective); $(\varepsilon/\delta)_2$, secondary porosity-pathlength ratio (charge/size nonspecific "free diffusion" term); $\Delta\varphi$, electrical potential drop (mV) across the electric field created by negatively-charged residues lining the junctional pores

* Corresponding author.
E-mail address: alex@in-ADME.com (A. Avdeef).

http://dx.doi.org/10.1016/j.ejps.2017.06.016
Received 9 January 2017; Received in revised form 10 April 2017; Accepted 9 June 2017
Available online 11 June 2017

1. Introduction

The relationship between membrane permeability and *pH* can reveal the extent of the contribution from several effects relevant to drug absorption/distribution, such as aqueous boundary layer (ABL) resistance, paracellular leakage, and drug ionization (Ho et al., 2000), including those related to carrier-mediated/facilitated transport (Thomson and Dietschy, 1977; Tsuji et al., 1994; Takanaga et al., 1994).

The mammalian intestinal barrier (Wilson, 1967; Högerle and Winne, 1983; Kararli, 1995; Collett et al., 1997; Desesso and Williams, 2008) possesses variable *pH* gradients (acidic on the luminal side, neutral on the blood side). So, it is not so uncommon to find studies of drug transport as a function of *pH* – most obviously, to mimic the physiological *pH* conditions, but more subtly, to tease out contributions to transport from several biophysical factors (Ho et al., 2000). For example, there have been many published *in vitro* studies using cell lines (*e.g.*, Caco-2, MDCK), or primary cell cultures (*e.g.*, porcine brain capillary endothelium), where permeability was measured at two different *pH* values in the interval 5.4–7.4, under gradient-*pH* and/or iso-*pH* conditions (Adson et al., 1995; Pade and Stavchansky, 1985; Raeissi et al., 1999; Yamashita et al., 2000; Laitinen et al., 2003; Alsenz and Haenel, 2003; Lee et al., 2005; Bhardwaj et al., 2005; Berginc et al., 2007; Kanaan et al., 2008). A few studies have been published where 3–4 different *pH* values were considered (Takanaga et al., 1994; Yu and Zeng, 2007; Agarwal et al., 2007; Korjamo et al., 2008; Yusof et al., 2014). The most comprehensive *in vitro* investigations, with up to nine different *pH* values per molecule considered, were reported by Artursson and coworkers (Palm et al., 1999; Neuhoff et al., 2003, 2005; Avdeef et al., 2005). Principally, these *pH*-dependent studies focused on modeling the intestinal barrier, with one exception (Yusof et al., 2014). Controls were included in the assays to confirm that the biological barrier stayed intact as a result of *pH* changes.

In contrast to the intestinal environment, the *in vivo* blood-brain barrier (BBB) has no appreciable *pH* gradient between the luminal and the abluminal sides. It may not appear obvious that *in vivo* studies of BBB permeability as a function of *pH* have any practical relevance. Indeed, there could be concern that non-physiological *pH* might disrupt the tightly-regulated BBB. As it turns out, the BBB can withstand a range of *pH* values, *provided the exposure time is short*. For example, Greenwood et al. (1989) saw no significant mannitol permeability increase when a pH 5.5 lactic acid solution was perfused through rat brain for 450 s. In the 30-s *in situ* rat brain perfusion study of several highly lipophilic drugs (amitriptyline, atomoxetine, imipramine, maprotiline, sertraline, indomethacin) in pH 5.5–8.5 buffers, physical integrity of the BBB was confirmed using markers (moderately permeable antipyrine and low-permeable atenolol) co-perfused in the sample-containing buffers (Avdeef and Sun, 2011).

With the possible opportunities to deconvolve/quantify several factors affecting BBB transport (*e.g.*, impact of cerebral perfusion fluid flow on assessment of lipophilic drug uptake, native/disease-state paracellular leakage, carrier-mediated/facilitated effects), surprisingly only a hand-full of *pH*-dependent *in vivo* studies of transport at the BBB have been reported (Suzuki et al., 2002a, 2002b, 2010a; Okura et al., 2008; Avdeef and Sun, 2011; Suzuki et al., 2016). Efficient computational tools to comprehensively evaluate such *pH*-dependent data have not been widely applied (Yusof et al., 2014).

In this study, we exploit what has been learned from the *pH*-dependent *in vitro* cell-based biophysical models (sharpened by intestinal applications), and apply such models to the novel mechanistic analysis of the published *pH*-dependent *in vivo* data for pentazocine (Suzuki et al., 2002a, 2002b), naloxone (Suzuki et al., 2010a), and oxycodone (Okura et al., 2008), to illustrate the potential value of *pH* modulation for *in vivo* BBB assays. Preliminary treatment of oxycodone data has been described (Avdeef and Sun, 2011). In the case of naloxone, a *pH*-dependent Michaelis-Menten equation (*pH*-MME) analysis indicated a smooth sigmoidal transition from a cation CM uptake process (pH 5.5–7.0) to a neutral-species CM uptake process (pH 8.0–8.5), with near equal contribution from both types at pH 7.4. The *p*CEL-X program (Yusof et al., 2014) used in the study was further expanded to accommodate new computational features related to carrier-mediated (CM) transport.

2. Materials and methods

2.1. Data sources

The data used here to illustrate the impact of capillary flow hydrodynamics on carrier-mediated transport of the opioid derivatives were from Suzuki et al. (2002a, 2002b, 2010a) and Okura et al. (2008). Table 1 lists the physicochemical properties of the three drugs studied. Table 2 lists the data taken from the original sources, but converted to capillary-based apparent permeability values as a function of *pH*.

2.2. Biophysical model

In vitro cell monolayers grown to confluence on porous filters have been used to model drug absorption properties of the *in vivo* cell membrane barriers. Such models factor in a number of effects, including (i) the resistance of the aqueous boundary layer (ABL) at the interface between the aqueous solution and the cell surface, (ii) the paracellular leakiness of the cell junctions, and (iii) the pH of the solution. Appendix A describes the equations applied to *in vitro* cell monolayer data analyses. Appendix B extends the construct to *in vivo* models of uptake at the BBB from perfusate/injectate flow in brain capillaries.

By way of introduction to the computational approach, a simple electrical circuit may be considered. In the circuit analogy, resistance and permeability are related inversely. In a *series circuit*, the total resistance is the sum of the individual resistances. In a *parallel circuit*, the total permeability is the sum of the permeabilities of each parallel path. For example, consider that a molecular transport barrier consists of three lamellae: (a) luminal aqueous boundary layer (ABL1), (b) layer of cells bound together *very tightly* (CELL), and (c) abluminal aqueous

Table 1
Physical properties of the compounds studied[a].

Compound	MW	pK_{a1}[37] [c]	pK_{a2}[37] [c]	Type[b]	log *P* octanol-water	log P_o PAMPA-BBB[c]	log P_o Caco-2[d]	*r*(Å)[e]	D_{aq} (10^{-6} $cm^2.s^{-1}$)[f]
Naloxone	327.37	7.82	9.25	BA	1.81	− 3.49	− 3.63	4.48	7.19
Oxycodone	315.36	8.73		B	0.7	− 3.92	− 3.70	4.42	7.31
Pentazocine	285.42	9.16	9.96	BA	3.31	− 1.88	− 2.29	4.48	7.65

[a] Intrinsic permeability, hydrodynamic radius, and aqueous diffusivity were calculated using *p*CEL-X (*in-ADME* Research).
[b] Aqueous diffusivity.
[c] B = base, BA = ampholyte. pK_a values from Wiki-pK_a database (www.in-adme.com/tools.html).
[d] Intrinsic PAMPA-BBB permeability.
[e] Intrinsic Caco-2 permeability.
[f] Hydrodynamic radius of molecule.