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Exploring drug solubility in fasted human intestinal fluid aspirates: Impact of inter-individual variability, sampling site and dilution

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ABSTRACT

One of the main factors defining intestinal drug absorption is the solubility of the compound in the gastrointestinal environment. This study reports the solubility of a series of 27 commonly used acidic, neutral and basic drugs in human intestinal fluid samples collected from the duodenum or jejunum of healthy volunteers under fasted state conditions. The interindividual variability as well as the impact of factors such as pH, sampling site and bile salts on the solubility in human intestinal fluids was investigated. The solubility measurements were evaluated using a statistical experimental design. Variability in solubility across volunteers and sampling sites was highly compound-specific and appeared to be substantial for weak acids and bases and for lipophilic drugs. Both pH of the samples and the abundance of amphiphilic components were responsible for the variability observed in the solubility values obtained. The results confirm strong interindividual differences in intraluminal solubility, especially for compounds with high lipophilicity and/or compounds with a pKa value within the physiological pH range. It is important to recognize this variability in intestinal drug solubility as it may considerably influence the therapeutic outcome among patients.

1. Introduction

Solubility is one of the key properties influencing intestinal drug absorption after oral intake. Determining the solubility in human intestinal fluids (HIF) is the gold standard to estimate the concentrations that can be reached in the intraluminal environment. A more thorough understanding of the properties governing the solubility in HIF will promote prototyping and improving the media simulating the environment in the upper small intestine in order to better reflect the intraluminal conditions. As oral intake remains the most convenient, patient friendly and least costly route of drug administration, most pharmaceutical companies focus on drugs that are effective after oral intake. The solubility of drug candidates is therefore assessed during early drug discovery in order to support candidate selection or to estimate formulation efforts required in further development stages. Appropriate buffer systems and media simulating the environment in the upper small intestine are often used in current solubility screenings in early discovery, and decision trees supporting the selection of the

Abbreviations: ΔH, change in melting enthalpy; CV, coefficient of variation; CDC, chenodeoxycholic acid; DOC, deoxycholate; DSC, differential scanning calorimetry; ESI, electrospray ionization; FD, full factorial design; pKa, dissociation constant; FFD, fractional factorial design; FaSSIF, fasted state simulated intestinal fluid; GC, glycholate; GCD, glycodeoxycholate; HIF, human intestinal fluid; HPLC, high performance liquid chromatography; HPLC/UV, high performance liquid chromatography; HPLC/MS, high performance liquid chromatography mass spectrometry; logP, lipophilicity; LLOD, lower limit of detection; LLOQ, lower limit of quantification; SD, standard deviation; RMSE, root mean squared error; rpm, revolutions per minute; ND, not determined; TCDC, taurochenodeoxycholate; TDC, taurodeoxychloate; Tm, melting point; TUDC, tauroursodeoxycholate; TC, taurocholic acid; λ, wavelength.

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appropriate solvent system are desirable to avoid bias in the solubility estimates leading to flawed compound selection (Bogman et al., 2003; Dressman and Reppas, 2000; Pedersen et al., 2000a; Neuhoff et al., 2003).

The solubility of a drug in the intraluminal environment is the result of a complex interplay of many factors. Several studies describe how intestinal drug solubility and/or dissolution is affected by pH (Avdeef et al., 2000; Hendriksen et al., 2003; Jinno et al., 2000; Schwartz et al., 1977), bile salts (Cai et al., 1997; de Castro et al., 2001a; Jinno et al., 2000; Mithani et al., 1996; Wiedmann et al., 2002), electrolytes (Crison et al., 1997) and food compounds (Sunesen et al., 2005). The effects of the wetting properties (Bakatselou et al., 1991) and surface tension (Efentakis and Dressman, 1998; Finholt and Solvang, 1968; Luner and VanDer, 2001; Vertzoni et al., 2005) on solubility have also been addressed. Ideally, the effect of all these parameters should be considered when performing solubility and/or dissolution assessments.

The first solubility studies in human intestinal fluids assessed the solubility of hydrocortisone and danazol in intestinal media of the fasted state (Pedersen et al., 2000a, 2000b). The study of the solubility of drugs in HIF of the postprandial state was first assessed for ketoconazole and dipyridamole (Kalantzi et al., 2006) and cyclosporine, danazol, griseofulvin and felodipine (Persson et al., 2005). Other studies on the determination of solubility in HIF followed, some of which focused on the solubility determination of extensive series of compounds (Heikkilä et al., 2011; Söderlind et al., 2010).

Augustijns and co-workers have published several studies reporting on the postprandial effects on the solubilizing capacity of HIF (Clarysse et al., 2009), the correlation between solubility in HIF and fasted state simulated intestinal fluids (FaSSIF) (Clarysse et al., 2009), and on the age dependency of the solubilizing capacity of HIF (Annaert et al., 2010). Additionally, the use of HIF as solvent system in absorption models such as Caco-2 cells (Brouwers et al., 2007), Ussing chambers (Wuyts et al., 2015) and in situ intestinal perfusion in mice and rats (Holmstock et al., 2013; Stappaerts et al., 2014) has also been investigated by this research group.

In most of the studies, HIF are pooled, although in some studies interindividual variability has also been presented (Annaert et al., 2010; Clarysse et al., 2009, 2011; Pedersen et al., 2000a, 2000b or Rabbie et al., 2015). In a recent paper (Rabbie et al., 2015), intersubject variability in intestinal drug solubility was determined in individual ileostomy fluid samples from subjects with ulcerative colitis.

In order to contribute to the continuously progressing knowledge of intraluminal fluid composition (Riethorst et al., 2016) and drug behaviour, we explored the solubility of a series of chemically diverse drugs in duodenal and jejunal fluids collected from five healthy volunteers under fasted state conditions. The characterisation of the collected fluids has been described (Perez de la Cruz Moreno et al., 2006). The present study focuses on the interindividual variability in solubility and the impact of sampling site and dilution on drug solubility. The drugs included in this study were selected to cover a wide range of physicochemical properties with the purpose of linking these properties to drug solubility in HIF. To accommodate the volume of human intestinal fluids collected, different experimental designs were used including a full factorial design and a fractional factorial design.

2. Materials and methods

2.1. Materials

The solubility of acyclovir, azithromycin, captopril, bepridil hydrochloride, buspirone hydrochloride, carbamazepine, citalopram hydrobromide, clomipramine hydrochloride, felodipine, furosemide, glyburide, haloperidol, itraconazole, levothyroxine, meclizine hydrochloride, metoprolol tartrate, moclobemide, norfloxacin, perphenazine, phenytoin, propranolol hydrochloride, ranitidine hydrochloride, risperidone, sulfasalazine, terbinafine hydrochloride, venlafaxine hydrochloride and ziprasidone was studied in HIF. All the drugs, except for sulfasalazine (Sigma, St. Louis, MO), were synthesized in the chemical department of Eli Lilly and company (Indianapolis, IN, USA) and had purities higher than 95%.

The solvents used for drug analysis (acetonitrile, trifluoroacetic acid and formic acid) were HPLC grade. Purified water was used for the preparation of aqueous solutions and mobile phases. Stock solutions were prepared in dimethylsulfoxide purchased from Merck (Belgium).

2.2. Characterization of model drugs

A chemically diverse series of drugs was selected, providing representative samples of neutral, acidic and basic drugs (pKa ranges from 1.3 to 10.5). The drugs also span a wide range in lipophilicity, with log P values ranging from −1.4 to 6.2 (Table 1). The solubility of lipophilic drugs is expected to be affected by the presence of bile salts (Wiedmann and Kamel, 2002), thus a broad range of log P values allowed us to evaluate the link between log P of the compounds and their solubility in HIF.

2.2.1. Thermal analysis

Differential scanning calorimetry (DSC) was used to characterize the different drugs. Thermal analysis was carried out using a DSC 822^e model (Mettler-Toledo Instruments, Zaventem, Belgium). Samples of approximately 5 mg were placed in a flat crimped aluminium pan with a hole in the middle; they were heated at a rate of 5 °C/min between 30 and 350 °C. The onset and the

Table 1
Physicochemical properties of the drugs used in this work. Determined parameters include dissociation constant (pKa_1 and pKa_2), lipophilicity (logP), melting point (Tm), change in melting enthalpy (ΔH) indicating an endothermic peak (positive value) or an exothermic peak (negative value). For the dissociation constant the nature of the function acid (A) or base (B) is indicated.

Compound	pKa_1		pKa_2		logP	Tm (°C)	ΔH (J/g)
Acyclovir	3.82	B	9.34	A	−1.42	ND	ND
Azitromycin	8.62	B	9.34	B	3.78	242.4	−70.2
Bepridil HCl	9	B	–		5.24	77.6	−97.6
Buspirone HCl	2.46	B	7.65	B	2.91	189.9	−177.6
Captopril	3.8	A	9.73	A	0.41	107.0	−102.2
Carbamazepine	10.49	A	–		2.04	190.9	−97.6
Citalopram HBr	9.23	B	–		3.27	187.7	−90.6
Clomipramine HCl	9.47	B	–		5.19	195.1	−98.1
Felodipine	4.41	B	–		4.09	131	−28
Furosemide	3.35	A	9.84[a]	A	2.36	210.9	90
Glyburide	4.99[a]	A	7.06[a]	A	3.33	173.8	−86
Haloperidol	8.34	B	–		2.79	151.4	−120.4
Itraconazole	3.74	B	10.87	B	6.19	166.9	−73.9
Levothyroxine	1.36[a]	A	7.68[a]	A	5.3[a]	236.5	−20.9
Meclizine HCl	7.45[a]	B	–		6.21	ND	ND
Metoprolol tartrate	9.42	B	–		1.71	123.7	−93.3
Moclobemide	6.16	B	–		1.52	137.8	−111.3
Norfloxacin	6.42	B	8.48	A	−0.11	220.5	−93.5
Perphenazine	3.44	B	7.41	B	3.47	98.6	−77.7
Phenytoin	8.85	A	–		1.52	296.7	−127.5
Propranolol	9.53[a]	B	–		3.48[a]	165	−111.5
Ranitidine HCl	3.44	B	8.27	B	0.27[a]	137.1	−112.8
Risperidone	3.49	B	8.09	B	2.68	170.7	−96.8
Sulfasalazine	2.65[b]	A	7.95[b], 10.51[b]	A	3.23[a]	260.3	−145.2
Terbinafine HCl	7.19	B	–		5.02	208.0	−75.5
Venlafaxine HCl	9.39	B	–		2.79	211.8	−106.8
Ziprasidone	2.93	B	7.2	B	4.42[a]	222.3	−101.8

ND: Not determined.
[a] Pallas v3.1.1.2 CompuDrug, 1994, 1995.
[b] pION (Avdeef, 2003).