International Journal of Pharmaceutics 485 (2015) 229–234

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Inter-subject variability in intestinal drug solubility

Sarit Cohen Rabbie [a], Talia Flanagan [b], Paul D. Martin [b], Abdul W. Basit [a,*]

[a] UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London, UK
[b] AstraZeneca, Alderley Park, Cheshire, UK

ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 3 March 2015
Accepted 4 March 2015
Available online 7 March 2015

Keywords:
Oral absorption
Solubility
Inter-subject variability
Humans
Gastrointestinal fluids

ABSTRACT

Variability in oral drug absorption is a well-known phenomenon, but it is often overlooked for its potential effects in oral drug delivery. Understanding the mechanisms behind absorption variability is crucial to understanding and predicting drug pharmacokinetics. In this study, the solubility of furosemide and dipyridamole – drugs known to have highly variable oral bioavailabilities – was investigated in individual ileostomy fluids from 10 subjects with ulcerative colitis. For comparison, drug solubility was also determined in pooled upper gastrointestinal fluids from healthy human subjects and simulated intestinal fluids. Ileostomy fluid characterization revealed high variability in buffer capacity and to a lesser degree for pH. Drug solubility in ileostomy fluids showed high variability. Correlation analysis revealed that dipyridamole solubility in these fluids is pH-dependent, whereas furosemide solubility was highly correlated to buffer capacity and pH. The implications of these results might partly explain the high variability in bioavailability *in vivo*, assuming that most of the observed variability is due to the absorption, and not the elimination, process.

1. Introduction

Oral drug absorption is a complex process, and numerous factors in the gastrointestinal (GI) tract contribute to both low and erratic drug absorption. In a recent review, McConnell et al. (2008), stated that "there is no such thing as an average person. In every person physiology is variable, from gut contents to cellular mechanisms." Indeed, some factors that might cause variability in the gut on one occasion might not elicit such profound effects at other times (Varum et al., 2013). Furthermore, high inter- and intra-variability can lead to a lack of therapeutic response in some subjects, whereas adverse effects can be observed in others.

Hellriegel et al. (1996) examined the relationship between absolute oral bioavailability and inter-subject variability, and highlighted a significant positive correlation between the absolute oral drug bioavailability and its inter-subject coefficient of variation. Though the inter-subject variability of the fraction absorbed (f_a) has not been investigated, based on the published results from Hellriegel et al. (1996), it is reasonable to assume that increasing bioavailability by increasing f_a will lead to a corresponding decrease in variability. The factors which might contribute to variability in f_a are related to aspects of the formulation (disintegration and particle size), physicochemical attributes of the drug (dissolution/solubility and permeability) and variation in GI motility, pH, fluids volume and composition, and other parameters such as the disease state (Freire et al., 2011). It is becoming increasingly common to gain information on absorption variability during drug development by gathering individual plasma concentration and response data from large numbers of patients during phase III clinical trials. However, as conducting an *in vivo* experiment is costly both in terms of time and materials, early discovery efforts need to be focused on assessing *in vitro* or *in situ* the absorption potential of the drug compound. It is therefore beneficial during the early investigative stages of formulation development to understand the source of absorption variability. One major factor that is likely to have a role in this respect is the dissolution (and solubility) of the drug in the dynamic conditions of the luminal environment of the gut.

The composition of fluids in the upper GI tract is well-documented (Augustijns et al., 2013), however, limited studies have correlated the variability in the composition of upper GI fluids and variability in drug solubility (Annaert et al., 2010; Clarysse et al., 2009, 2011; Pedersen et al., 2000a,b). To this end, the solubility of two model drugs, dipyridamole and furosemide, were investigated in fluids from different individuals in the lower gut. Dipyridamole is a poorly soluble weak base with $pK_a = 6.4$, classified as BCS II (Fig. 1) (Williams et al., 1981). Its bioavailability is 40%, and varies significantly between subjects in the range of 20–70% (Rajah et al., 1977; Tyce et al., 1979). Furosemide, a weak acid with $pK_a = 3.8$

* Corresponding author at: UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK. Tel.: +44 20 7753 5865; fax: +44 20 7753 5942.
E-mail address: a.basit@ucl.ac.uk (A.W. Basit).

http://dx.doi.org/10.1016/j.ijpharm.2015.03.006
0378-5173/

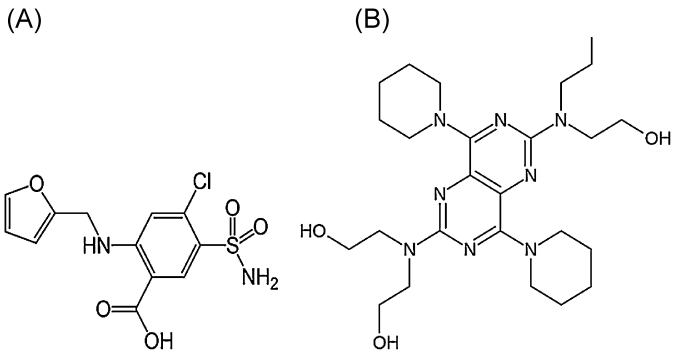

Fig. 1. (A) Furosemide (B) Dipyridamole.

(Fig. 1), is reported to be a poorly soluble and permeable drug classified as a BCS IV drug (Granero et al., 2010). Furosemide mean bioavailability is reported to be 60%, but is variable between 12% and 100% (Hammarlund et al., 1984; Kelly et al., 1974; Ponto and Schoenwald, 1990). Since neither drug is extensively metabolized in humans, it is reasonable to assume that variability in bioavailability might be attributed to the absorption rather than the elimination process. Despite the fact that dipyridamole and furosemide are commonly administered in their immediate release forms and their main site of absorption is the duodenum and jejunum, but due to their poor solubility, it is possible these molecules will reach the lower parts of the gut, namely the ileum. Therefore, in theory poorly soluble drugs, or modified release drug forms, will be exposed to the distal regions of the intestine and therefore solubility in these regions is of particular relevance and interest.

In this work, ileostomy fluids from 10 subjects with ulcerative colitis (UC) of the colon were characterized in terms of pH, buffer capacity, osmolality and surface tension, and solubility measurements were also made. For comparison, and to understand the regional differences in the solubility of dipyridamole and furosemide along the GI tract, solubility experiments were carried out in pooled gastric and intestinal fluids from healthy volunteers, and in simulated GI fluids

2. Materials

Dipyridamole (D9766) and furosemide (F4381) were obtained from Sigma–Aldrich Chemicals (Poole, UK). SIF powder was purchased from Biorelevant.com (UK). All salts to prepare the buffers were of analytical grade and purchased from VWR Chemicals Ltd. (Poole, UK). Solvents used in HPLC were: HPLC water, acetonitrile and phosphoric acid. All were of HPLC grade and purchased from Fisher Scientific (Loughborough, UK).

3. Methods

3.1. Human fluids

Healthy pooled gastric and intestinal fluids were supplied by AstraZeneca, Sweden. Gastric and intestinal fluids were aspirated from the stomach and proximal jejunum of fasted 10 healthy volunteers *via* an oral intubation tube (Loc-I-Gut synectics Medical, Sweden) (Bønløkke et al., 1997). The fluids were collected, pooled and stored in −80 °C until analysis. The sampling of the humans fluids was performed at the Clinical Research Department of the University Hospital in Uppsala, Sweden, and was approved by the Ethics Committee of the Medical Faculty at Uppsala University.

Ileostomy fluids were supplied from the Singleton Hospital in Swansea, UK. Fluids were collected from stoma bags in subjects who were undergoing a routine change of their stoma bag. Ileostomy fluids were collected from 10 different subjects. The samples were not pooled, and therefore each sample corresponds to one patient. Subjects were not fasting, and their diet was not controlled.

3.2. Sample preparation

The ileostomy fluids were centrifuged with Centrifuge 5415D (Eppendorf AG, 22331 Hamburg, Germany) at 16,110 × *g* rcf (relative centrifugal force, equivalent to 13,200 rpm) for 20 min. The supernatant was stored in a freezer (−80 °C) until analysis.

3.3. Osmolality measurement

Osmolality was determined with a Digital Micro-Osmometer (Type 5R), (Hermann Roebling Messtechnik, Berlin, Germany). The operating principle of this instrument is based on freezing point depression. Samples were thawed to room temperature before measurements were taken, and a volume of 100 μL was used for each measurement. All measurements were carried out in triplicates.

3.4. Surface tension measurement

Surface tension was measured with a Delta 8 Tensiometer (Kibron Inc.), controlled by Delta-8 manager software (version 3.8). The measurement was performed using DynePlates (96-well plate designed for tensiometer) with a 50 μL sample in each well. All measurements were carried out in triplicates.

3.5. Buffer capacity and pH measurement

Buffer capacity was measured by using a pH meter (HI99161) equipped with an FC202 electrode, designed for viscous and semi-solid materials (Hannah Instruments, Bedfordshire, UK). Buffer capacity was measured at a pH change of 1.0 unit. This was performed by the addition of accurate amounts of HCl to a 300 μL sample to achieve the desired pH change. Buffer capacity was then calculated using Eq. (1). Buffer capacity measurement was only performed in one direction due to the limited availability of gastrointestinal fluids.

$$\beta = \left[\frac{M_{acid/base} \times V_{acid/base(mL)}}{\Delta pH}\right] \times \frac{1000}{V_{sample\ (mL)}} \quad (1)$$

Eq. (1): modified equation for buffer capacity calculations.

In Eq. (1), $M_{acid/base}$ and $V_{acid/base}$ are the molarity and volume of the acid/base added to the V (mL) of the sample to produce a pH change of ΔpH in the sample. All measurements were carried out in triplicates.

3.6. Simulated intestinal fluid preparation

FaSSIF was prepared based on the recipe from Dressman et al. (1998). NaOH, NaH_2PO_4 and NaCl were dissolved in 0.9 L of purified water, pH was adujsted to 6.5 with either 1 N NaOH or 1 N HCl solution and the volume was completed to 1 L with purified water. SIF powder was added to 0.5 L of the buffer and was mixed until clear solution was achieved, the volume was then completed to 0.5 L with the buffer.

3.7. Solubility measurements

Solubility measurements were performed in healthy GI fluids, ileostomy human fluids and simulated intestinal fluids. An excess