European Journal of Pharmaceutical Sciences 96 (2017) 20–27

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Dynamic dissolution-/permeation-testing of nano- and microparticle formulations of fenofibrate

Daniel Sironi [a], Jörg Rosenberg [b], Annette Bauer-Brandl [a], Martin Brandl [a,*]

[a] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, DK 5230 Odense M, Denmark
[b] AbbVie GmbH & Co. KG, Knollstraße 50, D-67061, Ludwigshafen, Germany

ARTICLE INFO

Article history:
Received 18 July 2016
Received in revised form 26 August 2016
Accepted 1 September 2016
Available online 03 September 2016

Chemical compounds studied in this article:
Fenofibrate (PubChem CID: 3339)

Keywords:
Dissolution/permeation
Supersaturation
Solubilization
Nanoparticle
Microparticle
Enabling formulation
Dynamic

ABSTRACT

The aim of the current study was to evaluate a dynamic dissolution-/permeation-system for prediction of gastrointestinal and absorption-behavior of two commercial fenofibrate formulations. To this end, both dissolution and barrier-flux were followed simultaneously for fenofibrate powder, a microparticle formulation (Lipidil® 200 mg) and a nanoparticle formulation (LIPIDIL 145 ONE®) using a pair of side-by side diffusion cells separated by a cellulose hydrate membrane. Under such dynamic conditions, transient supersaturation arising from the nanoparticle formulation could be demonstrated for the first time.

Furthermore, the dissolution-/permeation-system introduced here allowed for in-depth mechanistic insights: Biomimetic media, despite enhancing the apparent solubility of fenofibrate *via* micellar solubilization, did not increase permeation rate, irrespective whether the micro-/ or nanoparticle-formulation was tested. Nondissolved nano-/microparticles served as a reservoir helping to maintain high levels of molecularly dissolved drug, which in turn caused high and constant permeation rates. The micelle-bound drug may also serve as a drug-reservoir, yet of subordinate importance as long as there are nano-/microparticles present.

Despite the limitations of the current experimental set-up, combined dissolution-/permeation-testing appears a valuable new tool to promote mechanistic understanding during formulation development. Last but not least, the *in vitro* dissolution and permeation behavior revealed here was in good qualitative agreement with human duodenal and plasma values reported in literature for the same formulations.

1. Introduction

Dissolution and permeation are among the most relevant drug properties for formulation development and drug substances are classified accordingly (Biopharmaceutics Classification System, BCS), where class II drugs exhibit high permeability but low solubility (Amidon et al. 1995). Based on the principles of BCS, the Developability Classification System (DCS) was proposed for identifying critical quality attributes in early drug development (Butler and Dressman 2010). In the DCS, class boundaries are modified and class II is divided into two subclasses: compounds belonging to class IIa exhibit poor dissolution rate, whereas the limiting factor for class IIB is the thermodynamic solubility. In order to overcome poor solubility as the limiting factor for drug absorption, formulation scientists seek to increase the solubility by the introduction of enabling formulations.

Formulation-induced increase of solubility and dissolution rate is commonly determined *in vitro* as a matter of routine. While extrapolating from *in vitro* dissolution curves to the performance *in vivo* is relatively likely to be successful for extended release formulations of BCS class I drugs (good solubility and permeability), the necessity to employ enabling formulations and the potential precipitation of the drug renders establishing a meaningful *in vitro-in vivo* correlation (IVIVC) more challenging for poorly soluble drugs. There are different levels of IVIVC, and ideally a linear correlation between the fraction released *in vitro* and the fraction absorbed *in vivo* can be established (Zhou and Qiu 2011).

Compendial dissolution testing aims for sink conditions. For poorly soluble drugs sink conditions can be maintained by using high volumes of dissolution medium and/or adding surfactants, both of which are unlikely to reflect *in vivo* conditions. Dissolution testing in biomimetic media is commonly expected to yield more meaningful results (Kostewicz et al. 2014). Biomimetic media (also referred to as "biorelevant media") simulate, among others, drug solubilization in mixed phospholipid/taurocholate micelles similar to those in intestinal fluids (Buckley et al. 2012). Poorly soluble drugs may be present in the gastrointestinal tract in the following states: undissolved, molecularly dissolved, and solubilized by components of intestinal fluids (Buckley et al. 2013). However, there is evidence that dissolution testing using biomimetic media does not necessarily yield a relevant IVIVC either (Do et al. 2011). At the same time recent studies indicate that there is an important difference (Frank et al. 2014) between an increase in concentration of molecularly dissolved drug, induced by an enabling

* Corresponding author.
E-mail address: mmb@sdu.dk (M. Brandl).

http://dx.doi.org/10.1016/j.ejps.2016.09.001
0928-0987/

formulation (Frank et al. 2012a, 2012c) and of solubilized drug, induced by the formulation (Fischer et al. 2011; Kanzer et al. 2010) or the dissolution medium (Fong et al. 2016; Frank et al. 2012b).

One approach to better reflect the interplay between drug dissolution and drug permeation is the simultaneous testing of both parameters. The first combined dissolution/permeation experiments were based on Caco-2 cell monolayers as a permeation barrier (Ginski and Polli 1999). Cell-based models were subsequently implemented by several groups (Kataoka et al. 2003; Kobayashi et al. 2001; Motz et al. 2007). Later, *ex vivo* models based on rat intestine (Li et al. 2011; Zhou et al. 2014) and *in vitro* models based on artificial barriers (Gantzsch et al. 2014; Kataoka et al. 2014) were developed. With the combined dissolution/permeation testing it as, for instance, possible to establish a Level A IVIVC (*i.e.* a point-to-point correlation between the *in vitro* dissolution curve and the *in vivo* plasma curve) for three different generic products of poorly soluble indapamide (Yaro et al. 2014). However, neither the influence of enabling formulations nor that of biomimetic media on the interplay between drug dissolution and permeation of poorly soluble drugs has been studied systematically.

Fenofibrate was chosen for the current study as a neutral, lipophilic model compound (BCS class II / DCS class IIb drug) and because there were recently published both human duodenal and plasma concentration time curves for its marketed nano- and microparticle formulations (Hens et al. 2015). Fenofibrate was first introduced to the market as a capsule containing 100 mg fenofibrate powder with a mean particle size of approximately 150 μm (Sauron et al. 2006). The recommended administration of three capsules together with food resulted in a bioavailability of approximately 60% (Shepherd 1994). Later, a capsule preparation containing micronized fenofibrate (Lipidil® 200 mg) was marketed. Increased bioavailability was achieved by reducing particles to sizes between 5 μm and 15 μm (Sauron et al. 2006): administration of 200 mg micronized fenofibrate once daily yielded the same area under the curve as 300 mg of the conventional formulation, along with less inter-individual variance of plasma levels (Munoz et al. 1994; Shepherd 1994). Using wet-milling, a nanoparticle formulation was developed (LIPIDIL 145 ONE®) with a mean particle size of <400 nm (Sauron et al. 2006) by which the bioequivalent dose was further reduced from 200 mg to 145 mg. Moreover, plasma levels varied even less and the nanoparticulate formulation (presented in the form of a coated tablet) can be administered independent from food intake (Sauron et al. 2006). Since the particle size of the nanoparticle formulation is smaller than 2 μm, (true) supersaturation is likely to occur (Mosharraf and Nyström 1995) which to some extent may account for the higher bioavailability as compared to the microparticle formulation. To the best of our knowledge, however, occurrence of supersaturation has not been experimentally proven for fenofibrate nanoparticles.

The objective of the present study was to investigate the contribution of both solubilization and (true) supersaturation on fenofibrate permeability in a dynamic setting.

2. Material and methods

2.1. Chemicals

Fenofibrate (≥99%, powder) was purchased from Sigma-Aldrich ApS (Brøndby, Denmark). Lipidil® 200 mg capsules (Mylan Healthcare GmbH, Hannover, Germany; lot 23102) and LIPIDIL 145 ONE® coated tablets (Abbott, Hannover, Germany; lot 22672) were purchased in a German pharmacy. Caprylocaproyl macrogol-8 glycerides (Labrasol®) were kindly donated by Gattefossé (Saint-Priest Cedex, France) and will be referred to by its tradename for reasons of readability. SIF powder for preparation of simulated intestinal fluids was purchased from biorelevant.com (London, UK). Highly purified water was prepared in-house using a Milli-Q® water purification system (Merck Millipore, Darmstadt, Germany). Acetonitrile, formic acid and buffer salts were purchased from Sigma-Aldrich ApS (Brøndby, Denmark).

2.2. Media

Phosphate-buffered saline (PBS) contained 1.73 g l^{-1} of sodium dihydrogen phosphate dihydrate and 4.92 g l^{-1} disodium hydrogen phosphate dodecahydrate in highly purified water. The pH was adjusted with sodium hydroxide to a value between 7.35 and 7.45; the osmolality was adjusted with sodium chloride to a value between 280 and 290 mOsmol kg^{-1}.

Fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) were prepared according to the protocol of the supplier.

2.3. Dissolution-/permeation-set-up

A pair of jacketed side-by-side diffusion cells of 5 ml volume each (PermeGear Inc., Hellertown, PA, USA) were separated by a hydrophilic cellulose hydrate membrane with an effective permeation area of 1.77 cm^2 (Pütz GmbH, Taunusstein, Germany), unless stated otherwise. High permeability and low non-specific adsorption of fenofibrate to the membrane were observed during preliminary studies. An appropriate amount of powdered sample was placed in the rear part of the donor cell; subsequently, the cells were assembled and filled with medium. Both, the donor and acceptor chamber were stirred with the provided cross-shaped stirbars at a fixed speed of 500 rpm (H-3 stirrer, PermeGear Inc., Hellertown, PA, USA) and the temperature was set to 37 °C.

Based on the assumption that a volume of 250 ml is the volume available in the intestinal lumen for dissolution of a single oral dose, an amount of formulation equivalent to 1/50 of the single oral dose was used in the dissolution- and in the combined dissolution-/permeation-experiments. For this purpose, LIPIDIL ONE 145® tablets (immediate-release coating) were thoroughly ground in a mortar; for Lipidil® 200 mg, conventional gelatin capsules were opened and an aliquot of the capsule content was used.

2.4. Sampling and sample handling

In order to minimize non-specific adsorptive loss from aqueous fenofibrate solutions, 1 ml and respectively 5 ml glass syringes (FORTUNA® Optima, Poulten & Graf GmbH, Wertheim, Germany) were used for sampling. All samples were immediately diluted with a twofold volume of acetonitrile (ACN) using Gastight® Syringes (Hamilton Company, Reno, Nevada, USA).

2.5. Ultra-high performance liquid chromatography (UHPLC)

Fenofibrate was quantified using a Dionex Ultimate 3000 Binary Rapid Separation LC system (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) in combination with a Hypersil GOLD column (C18, 1.9 μm, 2.1 mm × 100 mm) (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and a pre-column. Measurements were performed in isocratic flow mode with a mobile phase consisting of ACN and 0.1% formic acid in water in the ratio of 80:20 (v/v). The flow rate was 0.3 ml min^{-1} and the injection volume was 10 μl. The column temperature was 38 °C and the total run time was 3 min. Fenofibrate typically eluted after 2.1 min and was detected at a wavelength of 286 nm.

Standard series were prepared in ACN/water 2:1 (v/v). Calibration was carried out over the range of 10 ng ml^{-1} to 100 μg ml^{-1}.

For samples containing simulated intestinal fluids, the total run time was increased to 12 min. Under the chosen chromatographic conditions, fenofibrate appeared as rider peak. It was therefore verified that the peak area was determined correctly (data not shown).