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Research paper

Biorelevant *in vitro* dissolution testing of products containing micronized or nanosized fenofibrate with a view to predicting plasma profiles

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ABSTRACT

The ability of *in vitro* biorelevant dissolution tests to predict the *in vivo* performance of nanosized fenofibrate (Lipidil 145 ONE®) and microsized fenofibrate (Lipidil – Ter®) was evaluated in this study. *In vitro* dissolution was carried out using USP apparatus 2 (paddle method) with updated biorelevant media to simulate the pre- and postprandial states. Membrane filters with different pore sizes were evaluated for their ability to hold back undissolved, nanosized drug particles. It was shown that filters with pore sizes of 0.1 μm and 0.02 μm were able to separate molecularly dissolved drug from colloidal and undissolved particles. *In vitro* results obtained with a suitable filter were used to generate simulated plasma profiles in combination with two different models using STELLA® software: (a) under the assumption of no permeability restrictions to absorption and (b) under the assumption of a permeability restriction. The simulated plasma profiles were compared to *in vivo* data for the nanosized and the microsized formulation in the fasted and fed states. The first model approach resulted in good correlation for the microsized fenofibrate formulation, but the plasma profile of the formulation containing nanosized fenofibrate was overpredicted in the fasted state. The second model successfully correlated with *in vivo* data for both formulations, regardless of prandial state. Comparison of simulations with the two models indicates that in the fasted state, absorption of fenofibrate from the nanosized formulation is at least partly permeability-limited, while for the microsized formulation the dissolution of fenofibrate appears to be rate-determining.

1. Introduction

Many new chemical entities possess physicochemical characteristics unfavorable for oral absorption. A major hurdle is their low aqueous solubility and subsequent slow dissolution, leading to insufficient bioavailability. One of the classical approaches to increase the rate of dissolution is through decreasing particle size. The underlying basis for the correlation between particle size and dissolution rate can be explained by the following modification of the Noyes–Whitney equation:

$$DR = \frac{A_{Drug} \cdot D_{Drug}}{\delta} \cdot (C_S - C_t) \quad (1)$$

where DR is the dissolution rate, A_{Drug} is the drug surface area, D_{Drug} is the diffusion coefficient of the drug, δ is the diffusion layer thickness, C_S is the saturation solubility of the drug and C_t is the concentration of the dissolved drug at time t.

Reducing the particle size, e.g. by micronization leads to an increase in the dissolution rate and frequently to a higher and more reliable bioavailability. The success of applying this approach has been well documented in many studies [1–4]. More recently, nanosizing technology has been introduced to further decrease the particle size of the active pharmaceutical ingredient (API) and hence improve its bioavailability. Potential advantages of nanonization over micronization include the very substantial additional increase in particle surface area and, potentially, a modest increase in saturation solubility.

The scientific backgrounds of these effects are well described in the literature [5–8]. In addition, the possibility of mucoadhesion and/or direct uptake of colloidal particles across the gut membrane has been raised [6,7,9,10].

A second advantage of reducing API particle size to the nanometer range is that the dependency of absorption on food intake can in some cases be attenuated [11,12].

Fenofibrate, a lipid lowering agent, is virtually insoluble in water and exhibits a positive food effect [13]. Its oral formulation has been improved over time. An early formulation consisted of a capsule containing coarse fenofibrate in a dose of 200 mg

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(Lipanthyl®). This formulation exhibited high interindividual variability in the plasma profiles, as well as a pronounced food effect and was recommended to be administered with meals. In 2000, "suprabioavailable" tablets (Lipidil – Ter®) were introduced. This formulation combines micronization of fenofibrate with a spray-coating process resulting in a particle size of 5–15 μm [13]. Micronization leads to a higher dissolution rate and this "suprabioavailable" tablet containing 160 mg fenofibrate was demonstrated to be bioequivalent to the 200 mg coarse powder capsule, with less variability and a reduced food effect. More recently, it has become possible to reduce the particle size of fenofibrate to a D_{50} less than 500 nm with a novel wet-milling technique (Tricor®, Lipidil 145 ONE®). These formulations exhibit even better bioavailability, so that the dose could be lowered to 145 mg fenofibrate and still maintain bioequivalence to the 200 mg conventional capsule. With the nanosized formulation, it is possible to administer fenofibrate independently of meal intake, since no food effect is observed [14]. Additionally, formulations with reduced particle size have been demonstrated to provide a more efficient and better tolerated treatment of hypercholesterolemia and hypertriglyceridemia.

The goal of this work was to investigate the dissolution of fenofibrate from microsized and nanosized formulations. In doing so, the study was designed to differentiate between colloidally suspended drug particles and molecularly dissolved drug, which has been an issue for years [15,16]. First, filters of various pore sizes were identified. Second, dissolution behavior in biorelevant media (FaSSIF, FeSSIF, FaSSGF, FaSSIF-V2 and FeSSIF-V2 [17–19]) was studied to determine whether food effects can be explained by dissolution behavior as a function of prandial state. Biorelevant media have been successfully applied over the past decade to obtain *in vitro–in vivo* correlations (IVIVC) [17,20–23]. Third, *in vitro–in silico–in vivo* correlations were established by combining dissolution tests with the STELLA® 9.1.1 software (isee systems, NH, USA).

2. Materials and methods

2.1. Chemicals and materials

Lipidil 145 ONE® (lot 85900, Solvay Arzneimittel, Germany) and Lipidil – Ter® (lot 87247, Solvay Arzneimittel, Germany) were purchased from pharmacies in Germany. Egg phosphatidylcholine (Lipoid E PC®, 97.9% pure, lot 108015-1-/042) was kindly donated from Lipoid GmbH, Ludwigshafen, Germany. Glycerylmonooleate (GMO, Rylo MG19 Pharma®, 99.5% monoglyceride, lot 173403-2202/107) was a gift from Danisco Specialities, Brabrand, Denmark. Hydrochloric acid (31–33%) was obtained from Hedinger, Stuttgart, Germany. Ortho-phosphoric acid (85%) and pepsin (Ph. Eur., 0.51 U/mg, lot 1241256) were purchased from Fluka Chemie AG, Buchs, Switzerland. Fenofibrate drug substance (lot 016K1644) and maleic acid (99% pure, lot 056K5473) were purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. Sodium oleate (82.7% pure, lot 51110) was obtained from Riedel-de Haën, Seelze, Germany. Sodium taurocholate (NaTC, >97% pure, lot 2007100274) was purchased from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy. All other chemicals were of analytical grade and obtained from Merck KGaA, Darmstadt, Germany. Long-life milk (3.5% fat) was obtained from Milfina, Germany.

The syringe filters used in this study are listed in Table 1.

2.2. Solubility measurements

The apparent solubilities of fenofibrate in FaSSIF and FeSSIF were taken from the literature [24]. Additional solubility measurements of fenofibrate were performed in FaSSGF, FaSSIF-V2 and FeSSIF-V2 using the shake-flask method. An excess of coarse fenofibrate in the medium was shaken on an orbital shaker at 37 °C ($n = 6$). After 24 h, the samples were filtered through a filter with a pore size of 0.45 μm (Rezist® 30, PTFE). The filtrate was analyzed by HPLC. Further, the measured solubility (24 h) of drug substance in FeSSGF was determined by HPLC using the shake-flask method for 24 h, filtering the medium through a filter of 2.7 μm and precipitating the proteins with acetonitrile [25].

2.3. Dissolution studies

The dissolution testing conditions for Lipidil 145 ONE® and Lipidil – Ter® consisted of USP apparatus 2 Erweka DT6 (Erweka, Heusenstamm, Germany), 500 ml medium volume, stirring at 75 rpm and a temperature of 37 °C. FaSSGF, FaSSIF and FaSSIF-V2 were used to simulate the preprandial state. FeSSIF and FeSSIF-V2 were used to represent the fed state in the small intestine.

The dissolution profiles were constructed from samples of 5 ml withdrawn after 5, 10, 15, 30 and 60 min, which were filtered immediately through filters with different pore sizes and diluted appropriately with methanol. The samples were replaced with fresh medium.

It was not possible to perform the dissolution test in FeSSGF, since this medium contains milk, which cannot be filtered using filters with a pore size in the range of 20–500 nm. As an approximation and despite the different colloidal structures in the two media, the dissolution rate in FeSSGF was assumed to be similar as in FeSSIF-V2, since the solubility in these media was not significantly different. To confirm that this assumption was not important to the outcome of the simulation, a sensitivity analysis with respect to dissolution rate was conducted (see Sections 2.7 and 3.4.2).

The initial dissolution rate of Lipidil – Ter® was calculated from the complete dissolution profiles. The initial dissolution rate of Lipidil 145 ONE® in FaSSGF, FaSSIF-V2 and FeSSIF-V2 was calculated after performing additional tests during which samples were withdrawn at 1, 2, 3, 4 and 5 min, filtered and immediately diluted appropriately with methanol. All experiments were carried out in triplicate.

2.4. Quantitative analysis of fenofibrate

Samples from the dissolution tests were analyzed by HPLC. The HPLC system consisted of a LaChrom® L-7110 pump, a LaChrom® L-7400 UV–Vis-Detector, a LaChrom® L-7200 autosampler (Merck Hitachi, Darmstadt, Germany) and the EZ-Chrom Elite Data System Software® (Biochrom Ltd., Cambridge, UK). The analysis was performed on a LiChroCART® RP-18 5 μm, 125 × 4 mm column (Merck, Darmstadt, Germany). The mobile phase consisted of 80% acetonitrile and 20% MilliQ-water. The pH value was adjusted with ortho-phosphoric acid to 2.5. The flow rate was set at 0.85 ml/min resulting in elution of fenofibrate at approximately 4.5 min. The concentration of drug was determined using a UV detector set at 254 nm.

2.5. Analysis of in vitro dissolution data

Differences in the *in vitro* dissolution profiles obtained using the different pore size filters were assessed using the model-independent similarity factor (f_2). The f_2-value can range from 0 to 100, values above 50 indicate a difference of less than 10% and therefore similarity between two dissolution profiles [26,27].

2.6. Analysis of in vivo pharmacokinetic data

An oral administration, two-compartment analysis (WinNonlin® model 12) was applied for evaluation of pharmacokinetic parameters using WinNonlin® Professional Edition version 4.1 software