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IVIVR in oral absorption for fenofibrate immediate release tablets using dissolution and dissolution permeation methods

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In a previous study it has been demonstrated that a dissolution/permeation (D/P) system can discriminate between different immediate release fenofibrate formulations. The fractions permeated were correlated with fenofibrate's *in vivo* exposure in rats following p.o. administration. In the present study more detailed investigations are presented using data from six fenofibrate tablets tested *in vivo* in humans. In these pharmacokinetic studies no significant differences between formulations in AUC but in C_{max} were found. Differences between the C_{max} values were not explained by the dissolution characteristics of the tablets but were rationalized on the basis of micellar entrapment and diminished mobility of the active ingredient by surfactants in the formulations. This was demonstrated by a permeation system using dialysis membranes. Thus a permeation step in addition to dissolution measurement may significantly improve the establishment of an IVIV relationship.

1. Introduction

Hydrophobicity and poor water solubility of new chemical entities represent an increasing problem in pharmaceutical development. As *in vivo* studies are time- and cost-intensive, a number of approaches simulating the *in vivo* situation for dosage forms for poorly soluble drugs have been introduced. For example, biorelevant media including FaSSIF and FeSSIF, representing the fasted and the fed state in the upper jejunum, respectively, were proposed by Galia et al. (1998). Dissolution studies conducted with these media better predicted the *in vivo* performance of formulations containing poorly soluble drugs compared to the use of compendial media (Lobenberg et al. 2000).

Dissolution is not the only determining parameter for absorption of a compound. Following its dissolution the dissolved drug has to permeate biological membranes to become absorbed. To simulate this second step *in vitro* permeation methods were established.

The prevailed *in vitro* permeability assay in pharmaceutical research is based on cellular models, such as Caco-2 (Pinto et al. 1983; Artursson and Karlsson 1991) and MDCK (Irvine et al. 1999). These permeation screening tools are used in early stages of drug discovery, but have limited applicability for testing of pharmaceutical formulations and evaluation of the effects of various excipients on permeability.

A combined dissolution/permeation step was first introduced by Ginski et al. (1999) who evolved a continuous dissolution/Caco-2 permeation system which allowed the simultaneous determination of a drug's permeability and solubility.

Kataoka et al. (2003) introduced a dissolution/permeation (D/P) system to determine solubility and permeation under physiological conditions for prediction of drug absorption capacity *in vivo*. They used biorelevant media as dissolution medium to evaluate the effect of food intake on the oral absorption of poorly water-soluble drugs (Kataoka et al. 2006). The original FaSSIF and FeSSIF could not be used as dissolution media because their components would destroy the integrity of the cell culture monolayer. Therefore, they applied sodium taurocholate (NaTC) and egg-phosphatidylcholine (lecithin) as physiological solubilizing substances, using the same concentrations as in FaSSIF and FeSSIF. Furthermore Hank's balanced salt solution (HBSS) was used as cell culture medium base for their biorelevant media, $FaSSIF_{mod}$ and $FeSSIF_{mod6.5}$. With this D/P system the fraction of fenofibrate permeated was correlated with its *in vivo* exposure in rats (Buch et al. 2009).

In the present study six immediate release fenofibrate tablet formulations were tested *in vivo* in humans. Fenofibrate belongs to the class of poorly soluble but highly permeable drugs (Granero et al. 2005). Bioequivalence with a reference product (formulation B) in fasted-state conditions should be demonstrated in these randomized crossover studies. We tried to correlate these *in vivo* data with data from several *in vitro* methods, in order to predict their *in vivo* performance. As *in vivo* studies were conducted in the fasted state, attempts were made to correlate these data with results obtained from a D/P system using $FaSSIF_{mod}$ as the apical medium. Besides the D/P system, dissolution studies of the fenofibrate tablets were evaluated and a permeation system was introduced to predict the *in vivo* performance in humans.

2. Investigations and results

The plasma concentration *versus* time profiles of fenofibric acid after single oral doses of 145 mg fenofibrate are shown in Fig. 1.

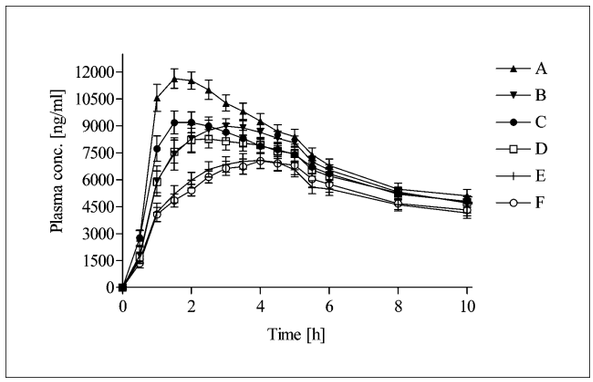

Fig. 1: Average (±SD) fenofibric acid plasma concentrations in humans ($n \geq 12$) after oral administration of different fenofibrate formulations. The data for formulations E and F were standardized to the values for formulation B obtained in clinical study 2. For a better differentiation between the formulations only the first 10 hours of the plasma profiles are shown

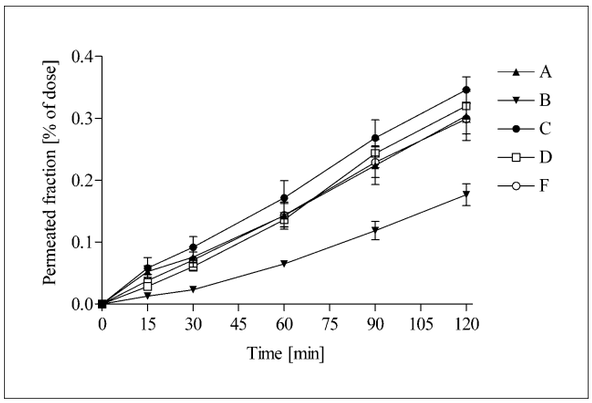

Fig. 2: Effect of different formulations on permeation of fenofibrate in the D/P system with $FaSSIF_{mod}$ as apical medium. Each data point is the mean ± SEM of three to six independent experiments

Table 1: Mean pharmacokinetic parameters (relative to formulation B, $n \geq 12$) following oral administration of formulations containing 145 mg fenofibrate

	In vivo data		*In vitro* data (D/P system)		
Formulation	C_{max} (%)	$AUC_{0\text{-inf}}$ (%)	Dissolved fraction (%)	Permeated fraction (%)	Predicted absorption (%)
A	123.4	107.53	7.49	0.304	132.07
B	100	100	7.67	0.177	100
C	95.7	103.93	5.71	0.346	139.94
D	86.33	102.08	6.35	0.32	135.18
E	73.93	99.91	n/a	n/a	n/a
F	72.43	100.11	6.37	0.299	131.05

Fraction of fenofibrate dose dissolved after 2 h (mean, $n \geq 3$) in the apical chamber of the D/P system. Fraction of fenofibrate dose permeated after 2 h (mean, $n \geq 3$) into the receiver chamber of the D/P system and the predicted absorptions, all *in vitro* data based on the results obtained from the D/P system using $FaSSIF_{mod}$

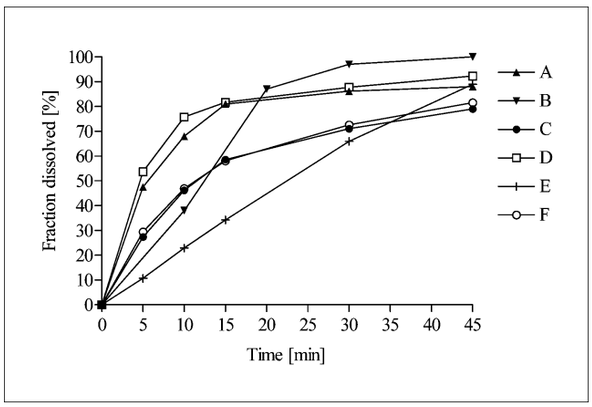

Fig. 3: Mean dissolution profiles ($n = 3$) of different formulations containing 145 mg fenofibrate per tablet using the USP paddle apparatus (1000 ml water, 1.5% SLS, 50 rpm, 37 °C)

The pharmacokinetic parameters following noncompartmental analysis are depicted in Table 1. The results based on $AUC_{0\text{-inf}}$ values in humans showed no significant differences between the six fenofibrate formulations, since all formulations were within the 80–125% confidence interval (log transformed). Concerning the C_{max} values the rank order of the formulations was as follows: A > B > C > D > E > F. Formulations B, D, E and F contained 10.15, 19.32, 41.9 and 40.22 mg sodium lauryl sulfate (SLS) per tablet, respectively. Formulations A and C contained different surfactants.

The fraction of fenofibrate dissolved in the donor medium of the D/P system is given in Table 1. By taking the solubility of fenofibrate into consideration (14.3 ± 1.05 µg/ml in $FaSSIF_{mod}$) it becomes obvious that all experiments were run under nonsink conditions. This explains the low fractions of drug which were dissolved over the 2 h time period. The fenofibrate dissolved from the tested formulations differed only marginally and was not useful to classify the formulations.

The results of permeation of fenofibrate from the tablet formulations A, B, C, D and F from the D/P system using $FaSSIF_{mod}$ as apical medium are depicted in Fig. 2 and, the *in vivo* absorption relative to formulation B was calculated (Table 1). Formulations A, C, D and F performed much better than B and their predicted absorptions were therefore approximately 25% higher than the calculated absorption from formulation B.

The permeation profiles of fenofibrate from formulations A, C, D and F showed almost the same characteristics which resulted in similar predicted absorptions. Formulation B's permeation profile of fenofibrate showed a flatter gradient which had a mandatory impact on its calculated absorption.

Dissolution testing in a USP paddle apparatus was carried out under sink conditions (Fig. 3). In 1000 ml water, containing 1.5% SLS, more than 65% of the fenofibrate content of the formulations were dissolved within 30 min. Formulation E showed the slowest dissolution in the first 30 min of all tested formulations, probably since it did not contain any disintegrants. Formulation B showed a slow initial dissolution rate as well, but after 20 min almost 90% of its fenofibrate dose were dissolved. Regarding the initial 15 min, formulations D and A showed the highest dissolution rate of fenofibrate.

As an additional *in vitro* tool, a modified permeation method was introduced to perform a closer analysis of the permeation step of fenofibrate in the presence of estimated intestinal surfactant concentrations. Whereas in the previously described D/P system 1% of the clinical applied dose was dissolved in 8 ml of apical medium corresponding to an intestinal volume of 500–1000 ml (Dressman et al. 1998), a more recent study by Schiller et al. (2005) suggested much smaller intestinal volumes available for drug dissolution. The reported minimum and median volumes under fasted conditions in the stomach (13/47 ml) and the small intestine (45/83 ml) provoked a review of the tablet mass to dissolution volume proportion to be used in the D/P system under fasted conditions. Thus higher surfactant concentrations needed to be employed. In this case the use of the original D/P system was not feasible because some surfactants (e.g., SLS) are

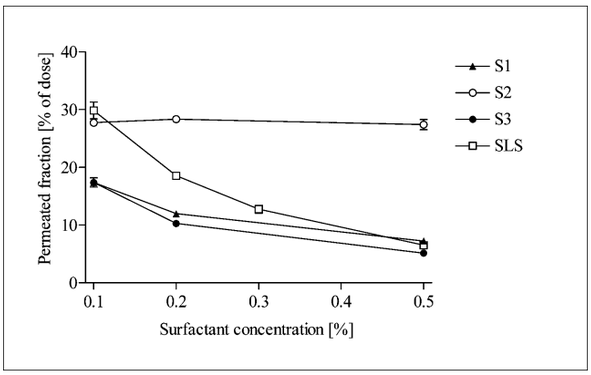

Fig. 4: Fraction of fenofibrate permeated after 4 h (mean ± SD, n = 3) across the dialysis membrane (neutral cellulose, molecular weight cut-off 10 000 Dalton) to the receiver side. The four tested surfactants were dissolved in $FaSSIF_{mod}$

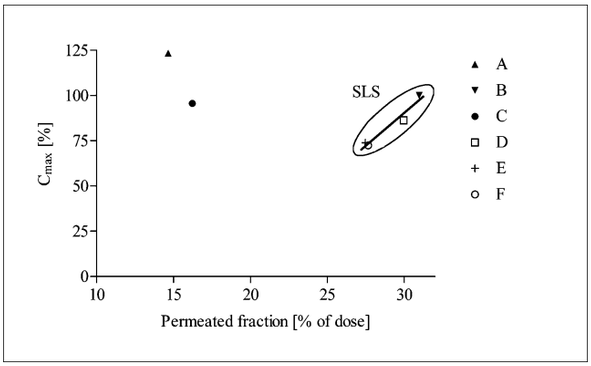

Fig. 5: Plot of predicted permeated fractions of fenofibrate from the formulations calculated from permeation data obtained with the permeation method using dialysis membranes (50 ml *in vivo* dissolution volume were assumed) versus C_{max} values for the fenofibrate formulations. Linear regression analysis was done on the formulations containing SLS

toxic to the epithelial monolayer at the concentrations needed in the modified D/P assay. That is why a permeation system using dialysis membranes was introduced in this manuscript.

The permeation of fenofibrate in $FaSSIF_{mod}$ containing different amounts of the surfactants which are used in the tablet formulations, i.e. SLS, two surfactants from the class of poloxamers (S1 and S2) and one surfactant from the class of polyethoxylated castor oils (S3), is depicted in Fig. 4. It becomes obvious that the permeated fractions of fenofibrate decreased with rising concentrations of S1, S3 and SLS. Low concentrations of SLS seemed to have the least negative impact on fenofibrate permeation. But with rising surfactant concentrations the permeated proportion of fenofibrate obtained with SLS approximated the values gathered with S1 and S3. Only S2 seemed not to affect the permeability of fenofibrate, as the fraction permeated remained at the same high level even with rising surfactant concentration. Based on the results obtained with this modified permeation method, the permeated fractions of fenofibrate from the formulations were calculated at their respective surfactant composition. A dissolution volume of 50 ml *in vivo* was assumed. From all tested surfactants S2 was excluded from calculations as its rising concentrations did not affect the permeated fraction of fenofibrate. The calculated permeated fractions of fenofibrate correlated well with C_{max} values for formulations containing SLS, indicating a negative influence of SLS on the permeation of the dissolved fenofibrate (Fig. 5).

3. Discussion

This study describes the elucidation of an *in vitro/in vivo* relationship for fenofibrate immediate release tablets using several *in vitro* methods. The outcome of the human studies was an obvious variation in C_{max} for the fenofibrate formulations, whereas the results based on $AUC_{0\text{-inf}}$ values showed no significant difference. Although the D/P system was developed to predict the *in vivo* systemic exposure in humans rather than absorption rates, it was tried to correlate the permeation profiles with the C_{max} values in humans. This was not successful. Using the D/P system, the predicted fenofibrate absorptions from formulations A, C, D and F showed almost the same performance which is consistent with the human *in vivo* studies. Only the calculated absorption from formulation B missed the result of the *in vivo* studies by being around 25% smaller compared to the predicted absorptions from the other formulations.

Similarly to the fraction permeated after 2 h, permeation results at other time points did not result in significantly improved IVIV relationships. Also traditional dissolution testing under sink conditions did not result in any useful IVIV-relationship between the dissolution profiles and C_{max} values in humans, demonstrating the inadequacy of the dissolution method to predict *in vivo* performance. For example, the good dissolution performance of formulation D was not reflected in humans. Likewise, the second highest fenofibric acid C_{max} value in humans was measured after the administration of formulation B, but its dissolution result after 10 min was only the second to last.

No improvement of prediction was seen when the solubilizing surfactant SLS in the dissolution medium was replaced by a bile salt based medium as, e.g., $FaSSIF_{mod}$. Again, no improvement of differentiation and interpretability of the dissolution data from the formulations was obtained (data not shown) with generally fractions dissolved of less than 1% of the fenofibrate dose from the tablets over a period of 45 min in 50 ml $FaSSIF_{mod}$. Up to 10% of the fenofibrate dose was dissolved from the tablets after 45 min in 900 ml $FaSSIF_{mod}$, but no rank order of the formulations based on their dissolution profiles was established.

By introduction of a permeation method using cellulose-based membranes, higher surfactant concentrations as compared to the original D/P system were tested. It was discovered that the permeated fractions of dissolved fenofibrate decreased with rising concentrations of S1, S3 and SLS.

This lower permeability may be due to surfactant forming mixed micelles with the sodium taurocholate and lecithin contained in $FaSSIF_{mod}$, which prevent micellar entrapped fenofibrate from permeation across the dialysis membrane. The rising number of micelles due to increasing surfactant concentrations can explain the concurrently decreasing fractions of fenofibrate dissolved in the donor chamber which permeated across the membrane. Only with S2 the fractions of drug which were detected in the receiver chamber remained at the same level, irrespective of the surfactant concentration. This confirms the hypothesis of micellar entrapment of fenofibrate regarding SLS, S1 and S3 because all tested S2 concentrations were below their critical micellar concentration (CMC = 6.3 mM at 37 °C) (Serbest et al. 2005). The CMCs for SLS, S1 and S3 are 1.6 mM, 0.0004 mM and 0.012 mM, respectively (Bennion et al. 1969; Wanka et al. 1994; Busoi et al. 2006).

The uptake of dissolved fenofibrate into micelles which reduced the free concentrations of drug in the intestinal lumen might serve as an explanation for the performance of these solid dosage forms in the human studies. The three RD formulations containing SLS in conjunction with S2 showed a poor performance in terms of C_{max} compared to the two formulations containing different surfactants instead of SLS and formulation B having the overall lowest surfactant concentrations. The micelles

containing SLS thus restrain dissolved fenofibrate from permeation across membranes by incorporation, as has been shown previously for tetracycline in combination with dioctyl sodium sulfosuccinate (Shah et al. 1986).

This theory is further supported by comparing the predicted permeated fractions of fenofibrate from the formulations with the C_{max} values for the fenofibrate formulations, assuming a 50 ml *in vivo* dissolution volume. The dissolution of tablet formulations containing low amounts of SLS will result in small numbers of SLS micelles or no micelles at all which could incorporate and therefore restrain dissolved fenofibrate from permeation across membranes.

In conclusion, even for highly permeable substances dissolution testing may not always be a sufficiently predictive *in vitro* method for *in vivo* performance. An additional permeation step may need to be incorporated into the *in vitro* prediction models. The D/P system which combines dissolution and permeation seems to be a useful tool to predict the *in vivo* performance in humans in terms of exposure. To enable the prediction of the performance of absorption rate, more concentrated surfactant solutions in a permeation model using artificial membranes need to be introduced.

Incorporation of dissolved fenofibrate in micelles containing SLS and subsequently reduced permeability of the fenofibrate/micellar complex was made responsible for the reduction in C_{max} of the three fenofibrate formulations which goes along with a reduced absorption velocity (permeability) of fenofibrate. In tablet formulations containing a poorly soluble drug like fenofibrate, SLS is a frequently used excipient to improve the drug's solubility which is a prerequisite for absorption. But formulation scientists should keep in mind that the dissolved drug has to permeate biological membranes to become systematically available. As shown in this study an increase in surfactant concentration in a tablet formulation cannot guarantee improved absorption.

4. Experimental

4.1. Materials

The Caco-2 cell line was purchased from American Type Culture Collection (Rockville, MD) at passage 17. Dulbecco's modified Eagle medium (D-MEM) was purchased from Sigma-Aldrich (St. Louis, MO). Nonessential amino acids (NEAA), fetal bovine serum (FBS), L-glutamate, trypsin (0.25%)-EDTA (1 mM) and antibiotic-antimycotic mixture (10000 U/ml penicillin G, 10000 μg/ml streptomycin sulfate and 25 μg/ml amphotericin B in 0.85% saline) were purchased from Gibco Laboratories (Lenexa, KS). Fenofibrate was purchased from Labochim (Milan, Italy). TriCor® (formulation B) was obtained from the manufacturer (Abbott Laboratories, Chicago, IL). RD 24, RD 25, RD 23, RD 22 and RD 18 were kindly provided by LifeCycle Pharma (Hørsholm, Denmark). Lecithin was purchased from NOF Corp. (Tokyo, Japan). NaTC was kindly donated by Prodotti Chimici e Alimentari S.p.A. (Basaluzzo, Italy) via their German agent Alfred E. Tiefenbacher GmbH & Co. KG (Hamburg, Germany). Bovine serum albumin (BSA) was purchased from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of analytical reagent grade.

4.2. Formulations employed

The RD formulations were based on the solid dispersion principle (MeltDose®-technique) and were used as delivered by the manufacturer (Holm and Norling 2007). RD 22 (formulation E) contains fenofibrate dispersed in a hydrophilic polymer and compressed to tablets following mixing with magnesium stearate. RD 24 (formulation A), RD 25 (formulation C), RD 23 (formulation D) and RD 18 (formulation F) additionally contain Avicel PH 200 and Polyplasdone XL as binder and disintegrant, respectively. Formulation A contains a surfactant from the class of poloxamers (S1). Formulations D, E and F contain besides SLS a poloxamer (S2) which is different to the one employed in formulation A. S2 and a surfactant from the class of polyethoxylated castor oils (S3) are used as emulsifying agents in formulation C.

Formulation B: This formulation comprises fenofibrate particles in the nanometer range (< 200 nm) downsized by wet-milling (Kesisoglou et al. 2007; Ryde et al. 2007). The tablet formulation additionally contains hypromellose 2910, docusate sodium, sucrose, SLS, lactose monohydrate, silicified microcrystalline cellulose, crospovidone, magnesium stearate, polyvinyl alcohol, titanium dioxide, talc, soybean lecithin and xanthan gum. It was used as received.

4.3. In vitro studies

4.3.1. Preparation of Caco-2 monolayer

Caco-2 cells were cultured in humidified air with 5% CO_2 at 37 °C in culture flasks (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) using D-MEM supplemented with 10% FBS, 1% L-glutamate, 1% NEAA and 5% antibiotic-antimycotic solution. Cells were harvested with trypsin-EDTA and seeded at a density of 3×10^5 cells/filter onto polycarbonate filters (0.3 μm pores, 4.20 cm^2 growth area) inside a cell culture insert (Nippon Becton Dickinson Co., Ltd.). Culture medium (1.5 ml in the insert and 2.6 ml in the well) was changed every 48 h for the first 6 days and every 24 h thereafter. Cells were used for the experiments between days 18 and 21 postseeding.

4.3.2. D/P system

For these studies the previously described D/P system was used (Kataoka et al. 2003). In short, the Caco-2 monolayer is fixed between two chambers, the donor and the receiver chamber. In the donor chamber the tested formulation dissolves, the dissolved drug permeates across the Caco-2 monolayer, and from the receiver chamber samples are taken for determining the concentration change over time. As master solution HBSS including 19.45 mM glucose and 10 mM HEPES was used (transport medium, TM).

$FaSSIF_{mod}$ was based on TM supplemented with NaTC 3 mM and lecithin 0.75 mM (Kataoka et al. 2006; Patel et al. 2006). The donor chambers contained 8 ml $FaSSIF_{mod}$, adjusted to pH 6.5. 5.5 ml of TM containing 4.5% w/v BSA (pH 7.4) was used as receiver medium in each receiver chamber (Yamashita et al. 2000). All the solutions and chambers were preheated to 37 °C and maintained at that temperature. The solutions in both chambers were stirred at 200 rpm with magnetic stirrers (8 mm × 2 mm in size).

4.3.3. Permeability studies in the D/P system

Before the Caco-2 monolayer was fixed between the chambers of the D/P system, it was incubated for 20 min in the culture well with TM (pH 6.5) for the apical and TM containing 4.5% w/v BSA (pH 7.4) for the receiver side. After positioning the Caco-2 monolayer with support filter in the D/P system $FaSSIF_{mod}$ was filled in the apical chamber and receiver medium was filled in the receiver chamber. Formulations A, B, C, D and F were ground in a mortar and added to the apical side as powder. The fenofibrate dose tested *in vitro* amounted to 1.45 mg, which corresponds to 1/100 of the clinically taken dose of 145 mg. Samples (200 μl) from the receiver solution were taken over 2 h and replaced by fresh medium. For HPLC analysis only the deproteinized supernatants (1.3 ml) of the samples containing BSA were used which were obtained by adding 1.4 ml of methanol followed by centrifugation (15 000 rpm, 20 min, 20 °C). After all samples from the receiver compartment were gathered, apical solution was taken and filtered through cellulose acetate filter (pore size, 0.2 μm) for determining the final concentration of the dissolved drug. Fenofibrate and fenofibric acid concentrations were both determined in all samples as Caco-2 cells metabolized only a fraction of fenofibrate.

The transepithelial electric resistance (TEER) values of the cell monolayers were monitored before and after the experiments by Millicell-ERS (MILLIPORE, Billerica, MA). The experimental conditions had no effect on the integrity of the cell layers, as TEER-values did not decrease significantly and were higher than 300 Ω*cm^2 (Kobayashi et al. 2001).

To estimate the *in vivo* absorption the *in vitro* permeated fraction of both fenofibrate and fenofibric acid (% of dose/2 h) in the D/P system was used in the following equation:

$$\text{Predicted absorption } (\%) = \frac{Abs_{max} \times PA^{\gamma}}{PA_{50}^{\gamma} + PA^{\gamma}} \quad (1)$$

Abs_{max} is the maximum absorption (defined as 100%), PA is the cumulative *in vitro* permeated fraction in the D/P system (% of dose/2 h), PA_{50} is the permeated fraction, which corresponds to 50% of the absorption *in vivo*, and γ is a Hill coefficient. PA_{50} and γ were obtained by a fitting procedure from the permeated fraction (PA) of drugs in the D/P system and their oral absorption in human (Kataoka et al. 2006) by using MULTI program developed by Yamaoka et al. (1981). The values for PA_{50} and γ were 0.334 ± 0.096 (SD) and 0.883 ± 0.178.

Table 2: Surfactant composition of the tablet formulations

Formulation	Surfactant (1)	Surfactant (2)	Surfactant (2) amount (mg/tablet)
A	S1	–	–
B	Docusate-Na	SLS	10.15
C	S2	S3	n/a
D	S2	SLS	19.32
E	S2	SLS	41.9
F	S2	SLS	40.22

S1 and S2 are surfactants from the class of poloxamers. S3 is a surfactant from the class of polyethoxylated castor oils

4.3.4. Analysis of D/P system samples by HPLC

Samples were analyzed using a reversed-phase HPLC system (LC-10A, Shimadzu Co., Kyoto, Japan) with a binary pump, a vacuum degasser, an autosampler, a column oven and a variable wavelength ultraviolet detector (SPD-10A, Shimadzu Co.). The mobile phase was composed of 50 mM phosphate buffer (pH 2.5) (A) and acetonitrile (B) and the flow rate was 1.0 ml/min. All drugs were trapped and eluted from the analytical column (J'sphere ODS-H80 75 × 4.6 mm ID, YMC, Kyoto, Japan) held at 40 °C using the following gradient conditions: 0–2.0 min, 50% A; 2.0–3.0 min, 50%–30% A; 3.0–10.0 min, 30% A; and 10.0–13.0 min, 50% A. Fenofibrate and fenofibric acid were detected at a wavelength of 288 nm.

4.3.5. Dissolution studies

Dissolution studies were performed on a fully calibrated dissolution apparatus using the paddle method at 50 rpm (apparatus: Sotax AT7, Sotax AG, Basel, Switzerland). The degassed dissolution medium consisting of 1.5% sodium lauryl sulfate (SLS) in 1000 ml water was maintained at 37 ± 0.5 °C. All dissolution studies were performed in triplicate.

4.3.6. Permeation studies

The permeation method using dialysis membranes was carried out using a DIANORM equilibrium dialysis equipment (Bachofer Laboratoriumsgeräte, Reutlingen, Germany). From all surfactants deployed in the tablet formulations (Table 2) four were chosen for these permeation studies. Fenofibrate was added in excess to $FaSSIF_{mod}$ containing different amounts of the four surfactants. The resulting suspensions were shaken over night in an orbital shaker (37 °C, 200 rpm). After a centrifugation step (5000 rpm, 10 min, 20 °C) the dissolved amount of fenofibrate in the supernatant was analyzed and 4 ml of each solution were placed in the donor chamber of the dialysis cell. The dissolved fenofibrate permeates through the dialysis membrane (neutral cellulose, molecular weight cut-off 10000 Dalton) to the receiver side, which contained the same volume and the same concentration of the deployed surfactant as the donor solution. After 4 h the permeated fraction of fenofibrate was analyzed in the receiver chamber.

4.3.7. Analysis of permeation samples by HPLC

Quantitative analysis of fenofibrate was carried out using an HPLC pump, a vacuum degasser, automatic sampler, and UV detector from Series 1050, Hewlett Packard (Wilmington, DE). Data processing was carried out with the software package HPLC Chemstation Rev. A. 03.01 (Hewlett Packard). Samples were separated using a 125 × 4.6 mm LiChrospher 100 RP-18 (end-capped) column (5 μm) (MZ Analysentechnik, Mainz, Germany). The mobile phase consisted of 0.1% diethylamine in a water/acetonitrile-mixture (20%/80%) which was adjusted with phosphoric acid to a pH of 4. Fenofibrate was detected at a wavelength of 289 nm.

4.4. In vivo studies

Two human studies were conducted with six solid dosage forms, four containing SLS. Bioequivalence with formulation B in fasted conditions should be demonstrated in these randomized crossover studies. The bioavailability studies were performed at Biovail Research Center, Toronto, ON, Canada and were approved by the Ontario Institutional Review Board.

4.4.1. Clinical study 1: formulation B, formulation E and formulation F

Healthy non-smoking human volunteers (6 male and 6 female), aged 20 to 73 years (mean 43.4 years), were selected. Each subject received one tablet in a randomized crossover manner. There was a washout period of 10 days between dosing. Tablets were administered at 7:00 AM following an overnight fast (min. 10 h). No water was permitted from 1.0 h predose to 1.0 h postdose, with exception of 240 ml dosing water. Food was withheld for at least 4 h postdose. At 4.5, 9.5 and 13.5 h postdose, standardized meals with beverages were provided to the subjects. All meals and beverages were free of alcohol, grapefruit products, xanthine and caffeine and were identical for the three study periods. 21 blood samples (4 ml) were drawn at 0.00 (predose), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 8.0, 10.0, 14.0, 24.0, 36.0, 48.0, 72.0, 96.0 h postdose.

4.4.2. Clinical study 2: formulation B, formulation A, formulation C and formulation D

Healthy non-smoking human volunteers (12 male and 8 female), aged 26 to 77 years (mean 47.5 years), were selected. Each subject received one tablet in a randomized crossover manner. The experimental protocol used in this study was the same as described in study 1.

4.4.3. Analysis of plasma samples

Fenofibric acid and its internal standard, naproxen, were extracted from human plasma (0.2 ml), using dipotassium EDTA as an anticoagulant, by solid phase extraction into an organic medium, evaporated under nitrogen and reconstituted in 200.0 μl of mobile phase. An aliquot of this extract was injected into a HPLC system and detected using a UV detector. The analytes were separated by reverse phase chromatography. Evaluation of the assay, using defined acceptance criteria, was carried out by the construction of an eight point calibration curve (excluding zero concentration) covering the range 49.996 ng/ml to 12798.8 ng/ml for fenofibric acid in human plasma. The slope and intercept of the calibration curves were determined through weighted linear regression analysis ($1/conc^2$). Two calibration curves and duplicate quality control samples (at three concentration levels) were analyzed along with each batch of the study samples. Peak height ratios were used to determine the concentration of the standards, quality control samples, and the unknown study samples from the calibration curves.

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