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Soluplus® as an effective absorption enhancer of poorly soluble drugs *in vitro* and *in vivo*

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ABSTRACT

As many new active pharmaceutical ingredients are poorly water soluble, solubility enhancers are one possibility to overcome the hurdles of drug dissolution and absorption in oral drug delivery. In the present work a novel solubility enhancing excipient (Soluplus®) was tested for its capability to improve intestinal drug absorption. BCS class II compounds danazol, fenofibrate and itraconazole were tested both *in vivo* in beagle dogs and *in vitro* in transport experiments across Caco-2 cell monolayers. Each drug was applied as pure crystalline substance, in a physical mixture with Soluplus®, and as solid solution of the drug in the excipient. In the animal studies a many fold increase in plasma AUC was observed for the solid solutions of drug in Soluplus® compared to the respective pure drug. An effect of Soluplus® in a physical mixture with the drug could be detected for fenofibrate. *In vitro* transport studies confirm the strong effect of Soluplus® on the absorption behavior of the three tested drugs. Furthermore, the increase of drug flux across Caco-2 monolayer is correlating to the increase in plasma AUC and C_{max} *in vivo*. For these poorly soluble substances Soluplus® has a strong potential to improve oral bioavailability. The applicability of Caco-2 monolayers as tool for predicting the *in vivo* transport behavior of the model drugs in combination with a solubility enhancing excipient was shown. Also the improvement of a solid dispersion compared to physical mixtures of the drugs and the excipient was correctly reflected by Caco-2 experiments. In the case of fenofibrate the possible improvement by a physical mixture was demonstrated, underscoring the value of the used tool as alternative to animal studies.

1. Introduction

An increasing number of new active pharmaceutical ingredients (APIs) show highly lipophilic properties, resulting in poor oral bioavailability (Lipinski et al., 1997). According to the biopharmaceutical classification system (BCS) the dissolution of poorly water-soluble drugs in gastrointestinal media is the limiting step for its permeation and absorption through the intestinal system (Amidon et al., 1995; Dressman and Reppas, 2000). Thus, the aim of many formulation attempts is to increase drug solubility in physiological fluids by means of surface-active excipients, which can act as wetting agents and solubilizers. Other methods for improving oral drug solubility include nanosizing or the use of cyclodextrin complexes. (Hong et al., 2006; Leuner and Dressman, 2000; Rabinow, 2004). In recent years, the emergence of the hot melt extrusion technique has enabled the formulation of solid solutions of poorly soluble drugs in polymer matrixes such as polyethyleneglycol, polyvinylpyrrolidone or cellulose derivatives (e.g. hydroxypropylmethylcellulose). In a solid solution the drug is molecularly dispersed in the solid matrix. After fast dissolution, a supersaturated solution is formed, allowing for high drug fluxes (Leuner and Dressman, 2000).

In the present study polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer (Soluplus®), a new polymer with amphiphilic properties was used (see Supplementary data for chemical structure). Soluplus® shows excellent solubilizing properties for BCS class II substances and offers the possibility of producing solid solutions by hot-melt extrusion. The dissolution of poorly soluble drugs in aqueous media can be highly improved by the use of solid solutions with Soluplus® (Hardung et al., 2010). The ability of Soluplus® to improve oral bioavailability of poorly soluble drugs was investigated using three different BCS

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class II drugs, danazol, fenofibrate and itraconazole, their physicochemical parameters are shown in Table 1 (see Supplementary data for the chemical structure of the used drugs).

Danazol, a testosterone derivative suppressing luteinizing and follicle stimulating hormone used for the treatment of endometriosis (Buckett et al., 1998), shows low relative bioavailability of 5.1 ± 1.9% as determined in beagle dogs, due to its poor aqueous solubility (10 μg/ml) (Liversidge and Cundy, 1995). Its oral bioavailability could be raised by different formulation techniques using nanoparticles or cyclodextrin complexes up to 82.3 ± 10.1% and 106.7 ± 12.3%, respectively (Liversidge and Cundy, 1995). Furthermore, danazol formulations with polyvinylpyrrolidone obtained by ultra-rapid freezing or spray freezing processes showed enhanced dissolution rates *in vitro* (Hu et al., 2004; Overhoff et al., 2007).

The lipid modifying drug fenofibrate with an aqueous solubility of 0.1 μg/ml is less soluble than danazol. Sant et al. discovered polymeric micelles of poly(ethylene glycol)-block-poly(alkyl acrylate-co-methacrylic acid) as an efficient solubility enhancer (Sant et al., 2005). Fenofibrate dissolution can also be improved by nanosizing, spray-drying or cogrinding of the drug (Vogt et al., 2008). Buch et al. showed the effectiveness of a melt-extruded solid solution, lipid microparticles, micronized and nanosized fenofibrate on oral drug absorption versus fenofibrate bulk ware *in vivo* as well as in a combined dissolution/permeation system *in vitro* (Buch et al., 2009).

The antifungal drug itraconazole is practically insoluble in water and has the highest log*P* of the drugs used in this study. It is a weak base with a pK_a of 3.7 and appears ionized in gastric fluids with a pH lower than 3 resulting in pH dependent oral absorption (Jaruratanasirikul and Sriwiriyajan, 1998). Itraconazole is commercialized as Sporanox® capsules, where it is applied as a drug/polymer layer with hydroxypropylmethylcellulose (HPMC) on neutral pellets. Six et al. developed a fast dissolving, but not homogenous solid solution of itraconazole and HPMC (Six et al., 2003). Other solid solutions of itraconazole were introduced by Miller et al. who generated a ternary solid solutions, obtained by hot-melt extrusion of itraconazole in a matrix formed by Carbopol® 974P and Eudragit® L100-55 (Miller et al., 2008). Buchanan et al. showed an increased oral bioavailability applying itraconazole as a formulation with hydroxypropyl-β-cyclodextrin (HPβCD) HPβCD is as well used as solubilizer for itraconazole in the commercialized Sporanox® for injection (Buchanan et al., 2007).

Solid solutions are usually characterized via their crystallinity and their dissolution behavior. However, as permeation across the intestinal epithelium is missing, merely performing *in vitro* solubility will not always afford an *in vivo* prediction. In particular the interplay between drug, excipient and epithelial cells cannot be investigated in a cell free setup.

Caco-2 monolayers are a widely used *in vitro* tool to predict permeation behavior of drugs over the intestine (Artursson, 1990; Artursson et al., 2001; Yamashita et al., 2000). In general, drugs are applied as solutions to the apical side of the *in vitro* model and drug permeation rate to the basolateral side is correlated to the *in vivo* absorbed fraction of the drug or directly to the AUC. The Caco-2 model has also been successfully applied to study the influence of pharmaceutical excipients on drug permeation not only via solubility enhancement but also via modulation of tight junction functionality and active transport systems (Collnot et al., 2006; Rege et al., 2002a; Thanou et al., 1999). Hence, the Caco-2 monolayer assay has been successfully used to evaluate complex formulations such as nanoparticulate formulations and self-emulsifying systems. Nevertheless, there is still need for further evaluation in particular with regards to experimental design and acceptor and donor media chosen. (Komura and Iwaki, 2007).

In the present study we used the Caco-2 *in vitro* model to predict the effect of Soluplus® on oral bioavailability of three BCS class II compounds. Drug permeation from the pure crystalline substance and from the physical mixtures of drug and excipient were compared to the respective aqueous dispersed solid solutions of drug and Soluplus®. Drug affinity to Soluplus® was studied by equilibrium dialysis to allow interpretation of possible differences in Soluplus® efficacy. Data obtained in the transport experiments was compared to data from *in vivo* studies in beagle dogs, validating the findings from the cell culture setup and demonstrating its strengths and weaknesses for the testing of complex formulations

2. Materials and methods

2.1. Chemicals

The solubility enhancer Soluplus® was provided by BASF SE (Ludwigshafen, Germany). Danazol and itraconazole were donated by Selectchemie AG (Zurich, Switzerland). Dulbecco's modified Eagle's medium (DMEM) and non-essential amino acids were obtained (NEAA) from PAA Laboratories GmbH (Pasching, Austria). Fetal bovine serum (FBS) was purchased from PAN-Biotech GmbH (Aidenbach, Germany). Vitamin E TPGS 1000 was supplied by Eastman Chemical Company (Kingsport, TN, USA). Transwell permeable membrane inserts (type 3460, 0.4 μm pore size, polycarbonate) were from Corning Incorporated Life Science (Lowell, MA, USA). Fenofibrate and all other chemicals were from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Solid solutions

Solid solutions were prepared by hot melt extrusion in a ThermoFisher Polylab twin-screw extruder (ThermoFisher Scientific

Table 1
Physico-chemical parameters of model drugs.

	Molecular weight (g/mol)	Melting point (°C)	log*P*	pK_a	Solubility (water) (μg/ml)	Solubility (10% Soluplus; phosphate buffer) (μg/ml)	Solubility in used physical mixture formulation (μg/ml)
Danazol	337.46	224.4–226.8	4.5[a]	–	~1[a]	730[b]	4.8
Fenofibrate	360.831	80.5	5.2[c]	–	0.1[d]	170[b]	5.2
Fenofibric acid	318.76	179–183	3.99[e]	3.09[e]	70[e]	n.d.	n.d.
Itraconazole	705.64	166.2	5.7[f]	3.7[f]	~0.001[g]	130[b]	2.7

[a] Dressman and Reppas (2000).
[b] BASF (2010).
[c] Vogt et al. (2008).
[d] Meng et al. (2009).
[e] Calculated using ACD/Labs.
[f] Yi et al. (2007).
[g] Peeters et al. (2002).

Table 2
Melt extrusion parameters for production of the used solid solutions.

	% API (w/w)	Temperature (°C)	Powder feed rate (kg/h)	rpm
Danazol	15	140	0.9	200
Fenofibrate	20	95	0.7	200
Itraconazole	15	150	1	200

Inc., Watham, MA, USA). Extrusion parameters were adjusted for each drug and are summarized in Table 2. The solid solutions employed in this study contained 15% API for danazol and itraconazole and 20% for fenofibrate, respectively.

2.3. Caco-2 cell culture

Caco-2 cells of clone C2BBe1 were obtained from American Type Culture Collection (Manassas, CA, USA). Cells were grown in an incubator at 37 °C with an atmosphere of 5% CO_2 and 80% relative humidity. Cell culture medium was DMEM, containing 10% FBS and 1% NEAA and was changed every 2–3 days. Every 7 days cells were passaged at a confluence of about 90%. Cell passages 65–75 were used for transport experiments. For transport experiments cells were seeded on Transwell permeable membrane inserts (0.4 μm pore size, 1.12 cm^2 membrane area) at a density of 60,000 cells per well. Fully differentiated cells were used for transport experiments after 21–23 days in culture. Only monolayers with a transepithelial electrical resistance (TEER) higher than 500 Ohm cm^2 were qualified for transport assay.

2.4. Caco-2 transport experiments

Transport medium was Krebs–Ringer buffer (KRB) containing 114.2 mM NaCl, 30 mM KCl, 4.0 mM D-glucose, 1.4 mM $CaCl_2$, 2.6 mM $MgCl_2$ and 10.0 mM Hepes. pH was adjusted by NaOH to a final value of 7.4. 0.2% Vitamin E TPGS 1000 was added to the buffer to maintain sufficient drug solubility in the receiver compartment, as described by Mellaerts et al. (2008). For itraconazole, a known substrate of the P-glycoprotein (P-gp) efflux system, transport studies were conducted using KRB + 1% HPβCD as acceptor medium, since Vitamin E TPGS is known to inhibit P-glycoprotein and thus might interfere with the transport experiment.

The following formulations of each drug were used for transport experiments: (I) the pure crystalline drug, (II) a physical mixture of the drug and Soluplus® in the same ratio as in the respective solid solution and (III) the solid solution of the drug in Soluplus®. All formulations were suspended in KRB and diluted to a final drug concentration of 0.5 mg/ml for danazol and itraconazole and of 1.0 mg/ml for fenofibrate. The suspensions were stirred for 1 h on a magnetic stirrer to simulate gastrointestinal dissolution. Throughout this timescale the solid solutions dissolve and enable a micellar-stabilized supersaturation of the respective drug. Cells were washed twice with buffer and pre-incubated with KRB for 1.0 h. Five hundred microliters of each drug suspension was applied on the apical side, while 1500 μl of the respective acceptor medium was placed on the basolateral side. Transport experiments were conducted in a cell culture incubator at 37 °C, 80% rH, 5% CO_2 and the system was agitated on an orbital shaker at 100 ± 20 rpm. Samples of 200 μl volume were taken at 30, 60, 90, 120 and 180 min from the basolateral side. The lost volume was replaced by fresh tempered buffer. TEER measurements as control for membrane integrity took place at the beginning and after the experiment.

Drug flux was calculated over the linear range of the permeation curve for each well, using the formula:

$$\text{Flux} = \frac{dc}{dt} \times \frac{1}{A}$$

whereas dc/dt indicates the slope of the permeation and A the surface of the monolayer.

2.5. Sample preparation and HPLC analysis

Samples, as drawn from the basolateral compartment, were diluted in a 1:1 ratio either with acetonitrile (fenofibrate, itraconazole) or with methanol (danazol). For danazol a methanol:water:phosphate buffer pH 6.8 [800:170:30 (v/v/v)] mixture was used as mobile phase. Fifty microliters of the samples was injected on a reversed phase column [LiChroCART® 125-4 LiChrospher® 100 RP18 (5 μm) by Merck KGaA (Darmstadt, Germany)] and detected by UV–vis absorption at 288 nm. Fenofibrate samples were injected at a volume of 50 μl on a reversed phase column [RP 18 (5 μm) as above] using a mixture of 30 volumes water acidified to pH 2.5 by phosphoric acid and 70 volumes acetonitrile. Due to enzymatic hydrolysis fenofibrate was detected as the mother compound and as its metabolite fenofibric acid by UV–vis absorption at 286 nm with retention times of 6.4 and 2.3 min, respectively.

The mobile phase for itraconazole consisted of a mixture of acetonitrile, water and pH 6.8 phosphate buffer (700:270:30 [v/v/v]). A volume of 80 μl was injected on a reversed phase column (RP 18 (5 μm) as above) and detected at a wavelength of 263 nm (see Supplementary data for further details).

2.6. Animal studies

The investigated formulations were administered as oral application via capsules to female fasted beagle stock dogs (n = 5/per test substance preparation). Purebred beagle dogs (age: 7–8 months for danazol; 20–22 months for itraconazole and fenofibrate) were selected, as this non-rodent species is recommended by the guidelines and there is extensive laboratory experience with this strain of dogs. Dogs were housed in animal rooms with forced ventilation system and additional heating in winter. The animals had free access to inner and outer kennels of at least 6 m^2. Dog diet was offered in the mornings for a period of 2 h. The animals had free access to water by an automatic watering device.

Food was withdrawn before drug administration for at least 22 h; free access to water was maintained. On days of experiment, dogs were fed about 2 h after administration of the test substances (following the blood sampling after 2 h). Appropriate amounts of drug preparations, adjusted on the basis of each individual animals weight, were weighed and placed in gelatin capsules, size 11 (Torpac Inc., Fairfield, NJ 07004, USA) and administered to the dogs in the morning: (I) Crystalline API, (II) a physical mixture of API and Soluplus® and, (III) a solid solution of API and excipient (as described in Section 2.2). The respective preparations with Soluplus® were administered orally at dose levels of 10 mg/kg bodyweight for itraconazole and fenofibrate and 30 mg/kg bodyweight for danazol, respectively. Dose levels were selected based on literature data: fenofibrate (Brodie, 1976; Brodie et al., 1976; Weil, 1988; Weil et al., 1988), itraconazole (Buchanan, 2007; Buchanan et al., 2007), danazol (Liversidge, 1995; Liversidge and Cundy, 1995). Experiments were performed according to German Animal Welfare legislation under the license number 1.577-07/873-50. The laboratory is AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) certified. Blood samples for kinetic investigations (approx. 3 ml each) were taken prior to administration and after 0.5, 1, 2, 4, 6, 8 and 24 h from the *vena cephalica antebrachii* and collected into lithium heparin blood collection tubes. After centrifugation at 1900 *g* for 10 min (22 °C), plasma was

divided into two equal parts and transferred into plastic tubes and placed on dry ice until storage at −20 ± 5 °C. One aliquot of each sample was subsequently transferred frozen to the analytical laboratory of BASF SE for analysis. The remaining aliquot was stored as contingency sample. Plasma samples were diluted by addition of acetonitrile [1:9 (v/v)]. After centrifugation at 3800g for 10 min, the received supernatant was analyzed by appropriate LC–MS-, LC–MS/MS-analysis (see Section 2.7) in the linear range of the quantification method. The linear range was assessed by standard solutions of the test compounds in the same matrix.

Area under the data (AUD_{0-24h}) levels were determined based on analyzed plasma concentrations by application of the linear trapezoid calculation method using a BASF-internal Microsoft Excel based kinetic assessment tool. For the performed experiments single animal plasma values at the different time points were transferred into the program and single animal AUD_{0-24h} values were calculated. Mean AUD_{0-24h} values and standard deviations were used for graphics.

2.7. LC–MS analysis of animal plasma samples

Danazol (molecular weight 337.5) was analyzed on a TSQ Quantum Ultra Surveyor (ThermoFisher Scientific Inc., Watham, MA, USA) by chromatography on a Waters Symmetry C18 150 × 2.1 mm 5 μm separation column (Waters Corp., Milford, MA, USA) with eluent A: highly deionized water; eluent B acetonitrile and a gradient, starting with 50% B, increased linearly to 100% B in 10 min, returning to initial conditions within 0.1 min. The flow was 0.3 ml/min. Injection volume was 25 μl. Detection by MS was performed by ESI-MS positive ionization in a single reaction mode (SRM; 160 °C; 4000 V) with mass transition m/z 338 → 310, 295, and 148. The collision energy was 20 V. Quantification was based on external calibration. The limit of quantification was 10 ng/ml.

Itraconazole (molecular weight 705.64) was analyzed on a WATERS 2695 Separations Module with a Quattro Micro mass detector (Waters Corp., Milford, MA, USA) by chromatography on a Luna C18 50 × 2mm, 2.5 μm separation column (Phenomenex, Torrance, CA, USA) with eluent A: highly deionized water acidified with formic acid (1 ml/l); eluent B: acetonitrile acidified with formic acid (1 ml/l) and a gradient method, starting with 50% B, increased linearly to 95% B in 2 min, kept until 5 min, and returning to initial conditions within 0.1 min. Re-equilibration was performed until 10 min. The flow rate was constantly 0.3vml/min. Injection volume was 10 μl for each sample. Detection by MS was performed by ESI-MS negative ionization in a single ion monitoring (ES−, mass 705; source temperature 120 °C; desolvation temperature 250 °C; 3200 V). Quantification was based on external calibration. The limit of quantification was 1.3 ng/ml.

2.8. Equilibrium dialysis

Dialysis experiments were performed to investigate binding behavior of the investigated drugs to Soluplus®. Studies were performed in a DIANORM® apparatus (identical to DIALYZER™ by Harvard Apparatus, Holliston, MA, USA) with a working volume of 2 ml per dialysis chamber. For our experiments suspensions of a physical mixture of 0.5% Soluplus® and 0.5 mg/ml (danazol and itraconazole) or 1 mg/ml (fenofibrate and fenofibric acid), respectively, were prepared in KRB by stirring on a magnetic stirrer for 24 h. The dispersions were centrifuged to eliminate non-dissolved drug, resulting in saturated drug–excipient solutions. The donor chambers of the dialysis cell were filled with 2 ml of these suspensions. The acceptor sides, separated by a cellulose membrane with a cut-off of 10 kDa (Harvard Apparatus, Holliston, MA, USA), were filled either with KRB + 1% BSA or KRB + 0.2% Vitamin E TPGS 1000 at the same volume. Dialysis was performed over 4 h at a rotation speed of 20 rpm at 37 °C. A control experiment using fluorescein sodium as model substance showed that equilibrium was reached throughout this time scale (data not shown). Samples from donor and acceptor were analyzed by HPLC as described above. Results are expressed as donor/acceptor ratio (D/A ratio) serving as an indicator for drug binding to Soluplus®.

3. Results

3.1. Danazol

Danazol transport across Caco-2 monolayers was demonstrated for all three formulations. As shown in Fig. 1a, the highest transport rate *in vitro* was reached from the solid solution of Soluplus® and the drug. The pure crystalline substance and the physical mixture showed no significant difference in transport rate. The solid solution enabled a drug flux of 20.61 ng min^{-1} cm^{-2} a 8-fold increase (Fig. 2) compared to the pure drug which afforded a flux of 2.67 ng min^{-1} cm^{-2}.

In vivo experiments in beagle dogs showed a 15-fold increase in plasma AUC from 329 ng h ml^{-1} for the pure drug up to 5081 ng h ml^{-1} for the solid solution after oral administration. For the physical mixture no significant effect of Soluplus® was determined compared to the pure drug (Fig. 1b). Besides increased AUC, an increased C_{max} was shown for the solid solution compared to the pure drug. C_{max} and T_{max} values are summarized in Table 3.

3.2. Fenofibrate

Since fenofibrate is mostly hydrolyzed to fenofibric acid either by a first pass effect in the animal or by hydrolytic enzymes present in Caco-2 cells, all results refer to fenofibric acid.

With a drug flux of 99.33 ng min^{-1} cm^{-2} the solid solution of fenofibrate showed the highest transport rate, followed by the physical mixture at 53.91 ng min^{-1} cm^{-2} and the pure drug at 34.46 ng min^{-1} cm^{-2} (Fig. 3a).

In animal experiments increased absorption of the drug was detected when fenofibrate was formulated with Soluplus® (Fig. 3b). Both formulations containing Soluplus® showed a strong increase in plasma AUC as well as in C_{max} values (Table 3). No significant difference was detected between the solid solution and the physical mixture of drug and excipient, both showing an AUC of about 60 μg h ml^{-1} an almost 5-fold increase compared to the unformulated crystalline drug.

Comparing *in vitro* and *in vivo* results (Fig. 4), drug flux was highest for the solid solution and the AUC of solid solution and physical mixture were on the same high level. The physical mixture therefore showed a stronger effect on drug absorption *in vivo* than *in vitro*.

3.3. Itraconazole

Itraconazole is characterized by a very poor aqueous solubility below 0.1 μg/ml, resulting in a low donor concentration. Thus, from the pure crystalline substance and from the physical mixture only very low transport rates limits were detected (Fig. 5a). For the pure drug the transported amount of itraconazole was below the limit of quantification, therefore no calculation of a flux value was possible. The physical mixture showed a flux of 0.458 ng min^{-1} cm^{-2}. In contrast, the solid solution of itraconazole in Soluplus® showed a high transport rate across Caco-2 cells, resulting in a drug flux of 14.51 ng min^{-1} cm^{-2}, about 30 times increased compared to the physical mixture.

In vivo the solid solution showed a plasma AUC of 2212 ng h ml^{-1}. For the physical mixture the AUC was 77 ng h ml^{-1},

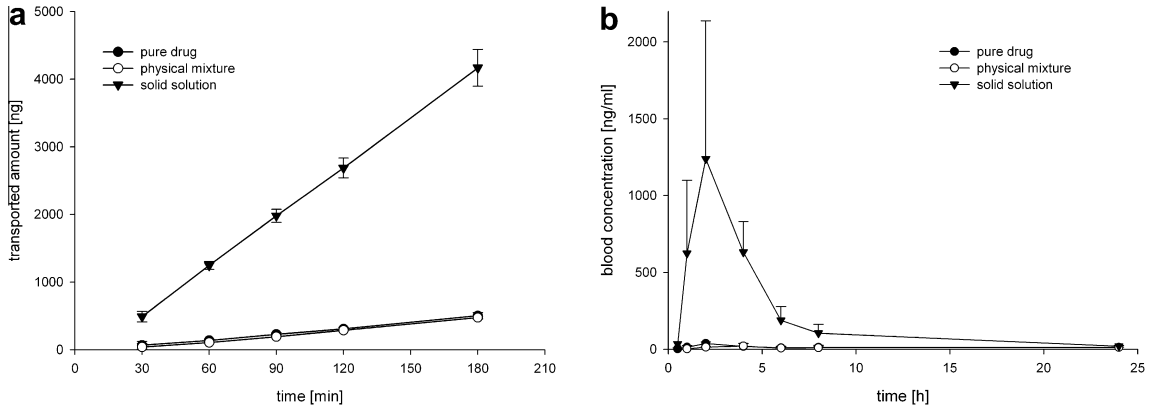

Fig. 1. (a) Danazol transport across Caco-2 monolayers ($n = 3–6$; mean ± SD). (b) *In vivo* blood levels of danazol applied to beagle dogs ($n = 5$; mean ± SD).

while no AUC could be determined for the crystalline substance, since no or only low drug amounts reached the blood (Figs. 5b and 6). The more than 30-fold increase in AUC is in line with strong *in vitro* effect of the solid solution of itraconazole in Soluplus®.

3.4. Dialysis

Equilibrium dialysis experiments were conducted to study drug interactions with Soluplus®. As indicated by donor/acceptor ratios >1 (Table 4) danazol, fenofibrate and itraconazole were retained on the donor side of the dialysis system, i.e. the side to which Soluplus® was applied, indicating complex formation or binding of drug to Soluplus®. In contrast, for fenofibric acid, a donor/acceptor ratio nearby 1 was reached for 0.2% TPGS as acceptor medium. This equal distribution to both sides of the system proves that no stable complex is formed with fenofibric acid. Mass balance of drug amounts on both sides of the dialysis system, afforded recovery rates of nearby 100% (data not shown). The donor/acceptor ratios were highest for itraconazole, followed by danazol and lowest for fenofibrate. An influence of the acceptor medium used was only detectable for fenofibrate and danazol, which significantly increased when Vitamin E TPGS (0.2% as used in the transport experiments) was replaced by 1% BSA to solubilize the drug in the acceptor compartment. In case of fenofibric acid, the equilibrium shifted to the acceptor side when BSA was added, probably by a bigger binding strength of fenofibric acid to the protein than to Soluplus®. However, the order of the strength of binding remained the same for both acceptor media (itraconazole > danazol > fenofibrate > fenofibric acid).

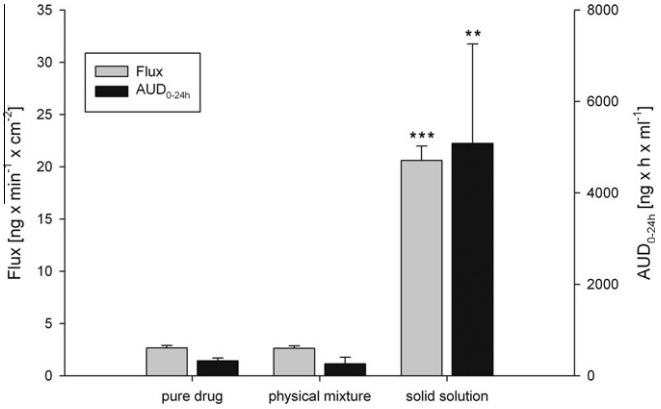

Fig. 2. Effect of Soluplus on danazol flux across Caco-2 monolayers and on AUD_{0-24h} in beagle dog animal study ($n = 3–6$ for *in vitro* data, $n = 5$ for *in vivo* data; mean ± SD). **Significantly very different from pure drug ($p \leq 0.01$). ***Significantly extremely different from pure drug ($p \leq 0.001$).

4. Discussion

As shown in many studies the Caco-2 monolayer model is an useful tool to predict drug permeation in the human intestine (Artursson, 1990; Artursson et al., 2001; Yamashita et al., 2000). As such the model has not only been used to investigate the permeability of pure drugs in solution but also of more complex systems studying the effect of solubility enhancers and other excipients on drug transport rates or even studying novel formulations such as nanoparticles or solid solutions (Takahashi et al., 2002; Yamashita et al., 2000). In the present study we looked at the effect of Soluplus® on oral absorption of poorly soluble, highly permeable BCS class II compounds itraconazole, fenofibrate and danazol. The novel excipient Soluplus® not only provides solubility enhancement by its amphiphilic structure but also offers the feasibility of forming solid solutions with poorly soluble drugs. Thus, both physical mixtures of drug and Soluplus® and solid solutions with respective drug/excipient ratios were investigated. As a validation of the *in vitro* experiments the different formulations were also orally applied to beagle dogs for *in vivo* monitoring of drug bioavailability via plasma levels.

For all three drugs the highest permeation over the Caco-2 monolayer was, for fenofibrate besides the physical mixture, realized by the solid solution. For danazol and itraconazole no or only limited effectiveness of a physical mixture of drug and excipient on drug permeation was detected. Although the drug solubility was increased in the presence of Soluplus® (Table 1) drug permeation remained on a low level, comparable to the pure drug. The superior bioavailability from the solid solution and the poor effect of the physical mixture predicted in the Caco-2 model was confirmed in the *in vivo* experiments. In particular, the magnitude of absorption change correlated in both setups when drug flux was compared with AUC. The failure of Soluplus® as a mere solubility enhancer in the physical mixture suggests that the amorphous state of the drug in the solid solution plays a crucial role for drug dissolution and permeation. Supersaturated drug solutions are only realized from the solid solutions allowing for steeper concentration gradient and higher drug flux across the epithelial barrier. Tight junction integrity and epithelial barrier properties are not affected by Soluplus® as TEER values stayed constant throughout the course of the experiments and no increase in transport of paracellulary transported marker dye fluorescein sodium could be observed in control experiments (data not shown). Furthermore, both danazol and itraconazole showed strong binding to Soluplus in equilibrium dialysis experiments, indicating that increased solubility in the presence of Soluplus is compensated by high affinity complex formation to the non-permeable excipient.

In contrast, for fenofibrate, Soluplus® in a physical mixture was as effective as the solid solution (in animal) and also significantly

Table 3
Enhanced bioavailability and drug flux of Soluplus/drug formulations (*in vivo* $n = 5 \pm$ SD; *in vitro* $n = 3 \pm$ SD).

Drug	Formulation	AUC (*in vivo*) (ng* h/ml)	C_{max} (*in vivo*) (ng/ml)	T_{max} (*in vivo*) (h)	Rel. AUC	Flux (*in vitro*) (ng/min/cm^2)	Rel. flux (*in vitro*)
Danazol	Crystalline	329 ± 58	37	2	1	2.67 ± 0.23	1
	Physical mixture	261 ± 143	20	4	0.8	2.63 ± 0.25	0.98
	Solid solution	5081 ± 2174	1238	2	15.4	20.61 ± 1.37	7.7
Fenofibrate	Crystalline	12201 ± 8080	1492	4	1	34.46 ± 2.76	1
	Physical mixture	64830 ± 9299	5509	4	5.3	53.91 ± 1.33	1.56
	Solid solution	54167 ± 14775	5262	4	4.4	99.33 ± 4.48	2.88
Itraconazole	Crystalline	–	–	–	–	–	–
	Physical mixture	77 ± 52	19	4	–	0.46 ± 0.01	–
	Solid solution	2212 ± 662	405	4	>29 (estimated)	14.51 ± 0.29	>30 (estimated)

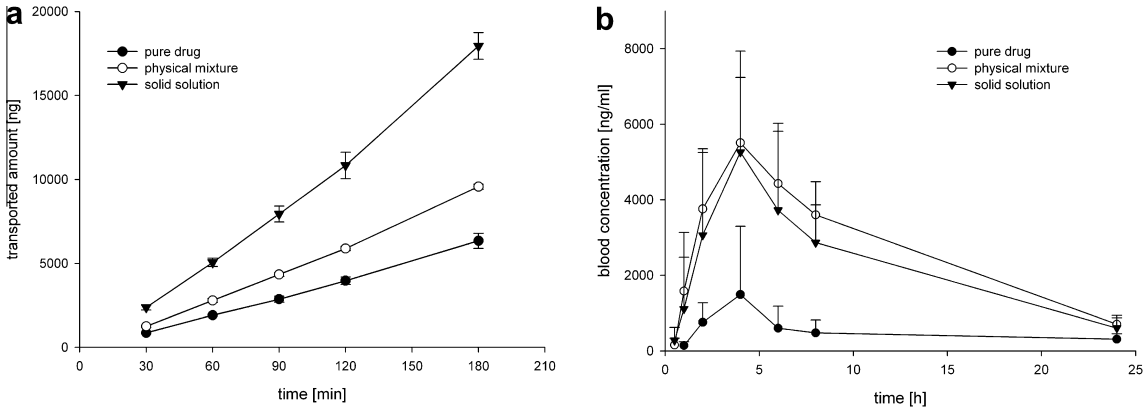

Fig. 3. (a) Fenofibrate transport across Caco-2 monolayers. transported amount expressed as fenofibric acid ($n = 3$–6; mean ± SD). (b) Fenofibric acid in blood samples of *in vivo* study in beagle dogs ($n = 5$; mean ± SD).

superior to the pure drug in increasing *in vitro* transport. Thus, for some drugs already a physical mixture could be an alternative for a solid solution, reducing production costs and avoiding stability issues as might be faced with heat-sensitive and polymorphous drugs. The mechanistic basis for the observed differences between danazol and itraconazole on one hand and fenofibrate on the other hand are not completely understood yet but might be connected to the low melting point of fenofibrate compared to the other tested drugs (Table 1, Chu and Yalkowsky, 2009). Chu and Yalkowsky introduced a melting point based absorption potential (MPbAP), which correlates the melting point to the absorbed fraction of BCS class II/IV drugs. Based on the MPbAP, a lower melting point leads to a higher absorption. Accordingly, fenofibrate shows higher *in vitro* and *in vivo* absorption than danazol and itraconazole, especially in case of the physical mixture.

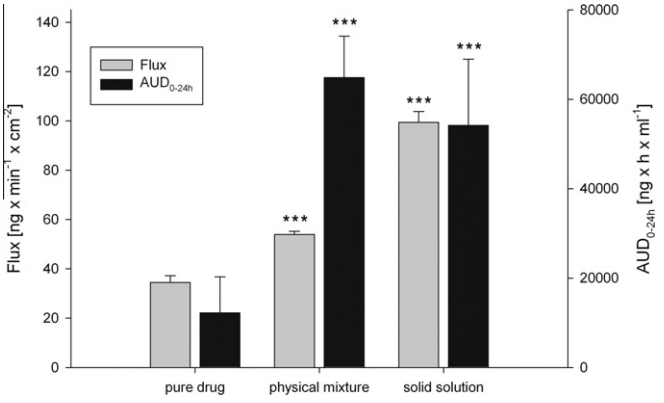

Fig. 4. Effect of Soluplus on fenofibrate flux across Caco-2 monolayer and on AUD_{0-24h} in animal study ($n = 3$–6 for *in vitro* data, $n = 5$ for *in vivo* data; mean ± SD). ***Significantly extremely different from pure drug ($p \leqslant 0.001$).

A discrepancy exists between fenofibrate *in vitro* and *in vivo* data with regards to the physical mixture: in animal studies the physical mixture performed equal to the solid solution, while in the *in vitro* experiments the observed drug flux was in between the solid solution and the pure drug, the observed increase in drug permeation rate being about 2-fold lower than the *in vivo* impact. This may be attributed to *in vivo* interaction of the drug and/or Soluplus® with the intestinal fluids, which contain bile salts and phospholipids leading to the formation of mixed micelles (Balfour et al., 1990; Sauron et al., 2006). The impact of dissolution and transport media composition for fenofibrate was previously confirmed by *in vitro* dissolution and permeation experiments using fasted or fed state simulated intestinal fluids (FaSSIF; FeSSIF). It was shown that dissolution and permeation rate is improved by these media (Buch et al., 2009). Therefore, it is probable that for the physical mixture of fenofibrate, Soluplus® and the micelles present in the intestine show a synergistic effect on drug absorption *in vivo*. Although food effects are also reported for danazol and itraconazole (Charman et al., 1993; De Beule and Van Gestel, 2001; Schmidt and Dalhoff, 2002), in our study no effect of the physical mixture was shown *in vitro* and *in vivo*. We suggest that danazol and itraconazole are already well solubilized by Soluplus® micelles and show strong binding to the excipient. Thus the mixed micelles present *in vivo*, do not have an additional effect. The differences in binding affinity between the three compounds are not yet mechanistically understood, although it seems that higher lipophilicity is associated with stronger binding to the excipient structures. Furthermore the pH may

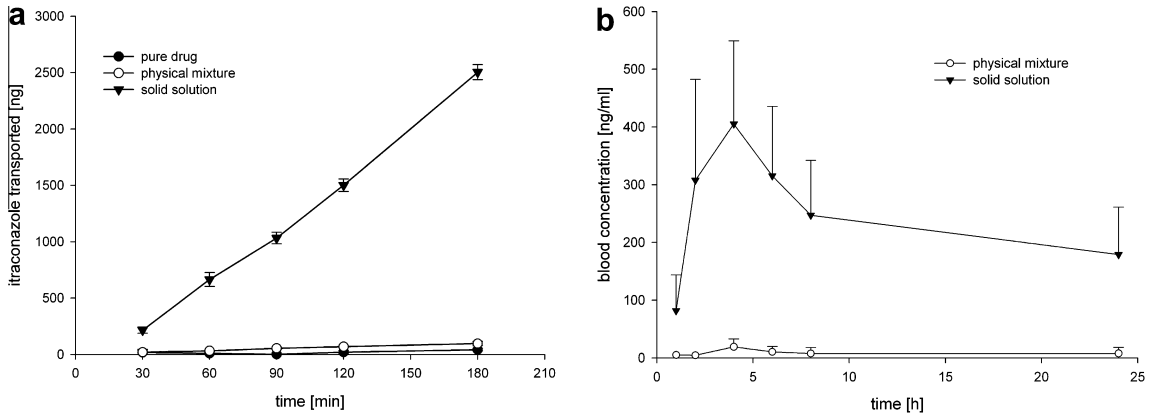

Fig. 5. (a) Itraconazole transport across Caco-2 monolayers ($n = 3$; mean ± SD). (b) Itraconazole bioavailability study in beagle dogs ($n = 5$; mean ± SD).

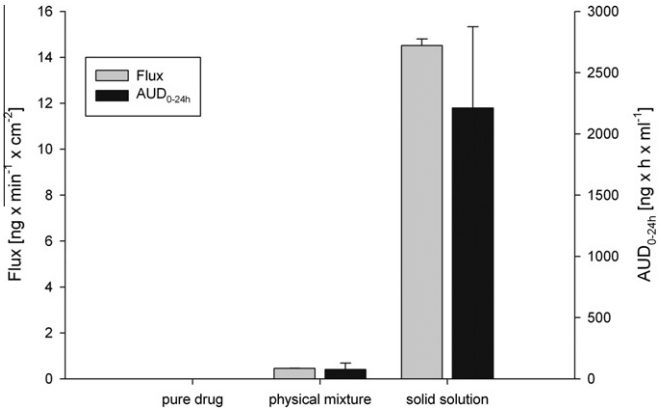

Fig. 6. Effect of Soluplus on itraconazole flux across Caco-2 monolayer and on AUD_{0-24h} in animal study ($n = 3$ for *in vitro* data, $n = 5$ for *in vivo* data; mean ± SD).

Table 4
Results from dialysis experiments of a physical mixture of 0.5% Soluplus and the respective drug. Data given as donor/acceptor ratio ($n = 5 \pm$ SD).

	Donor/acceptor (KRB + 1% BSA)	Donor/acceptor (KRB + 0.2% TPGS)
Danazol	9.9 ± 0.4	3.7 ± 0.3
Fenofibrate	4.9 ± 0.2	1.4 ± 0.2
Fenofibric acid	0.4 ± 0.1	1.0 ± 0.1
Itraconazole	16.4 ± 0.7	21.0 ± 5.9

play a role in the differences between *in vivo* and *in vitro* absorption. While cell culture experiments were performed at a constant pH of 7.4, *in vivo* the formulations occur a pH gradient which will especially affect the ionizable drugs itraconazole and fenofibric acid. However, additional experiments conducted in our laboratory did not show an effect on fenofibrate or itraconazole flux by a simulated gastric passage, using stepwise dissolution in pH 2 and pH 7 buffer.

Additionally, it was shown that fenofibrate mainly appears as fenofibric acid on basolateral side. Samples drawn from the apical side of the Transwell system also show a significant amount of fenofibric acid. Therefore it is probable, that solubilized fenofibrate is hydrolyzed by enzymes located on apical side of the Caco-2 cells and that mainly the metabolite fenofibric acid diffuses across the cell monolayer. As fenofibric acid is less lipophilic than fenofibrate and provides a much higher aqueous solubility, it provides a reduced binding to Soluplus® as proven by equilibrium dialysis (Table 4). Also the effectiveness of the physical mixture might be explained by that fact: an extensive supersaturation of the drug provided by a solid solution is not necessary, since the transport is controlled by the hydrolysis to fenofibric acid and its diffusion. Further experiments conducted at 4 °C (reduced enzyme activity) confirmed this hypothesis. The drug flux and also the amount of fenofibric acid on apical side were many fold lower under these conditions (data not shown).

The same mechanism is as well suitable for the *in vivo* situation as the intestinal membrane also provides a high variety of hydrolyzing enzymes and the bioavailable form of fenofibrate detected in blood is also fenofibric acid (Chapman, 1987).

For itraconazole HPβCD was added in a concentration of 1% (w/w) to the acceptor medium. Although Mellaerts et al. considered an addition of 0.2% Vitamin E TPGS (as used for danazol and fenofibrate) with Caco-2 monolayers as equivalent to rat plasma as acceptor medium for itraconazole transport experiments (Mellaerts et al., 2008), we changed the constitution of the acceptor medium as Vitamin E TPGS 1000 is known to inhibit the P-gp efflux system (Dintaman and Silverman, 1999; Rege et al., 2002a). Since itraconazole is a substrate of P-gp, efflux inhibition would likely have altered drug transport in our experiments (Balayssac et al., 2005). In contrast HPβCD has no effect on P-gp efflux, drug permeability and monolayer integrity in Caco-2 transport experiments (Oda et al., 2004; Shah et al., 2004). However, an intrinsic P-gp inhibition afforded by Soluplus® cannot be excluded neither *in vivo* nor *in vitro* and may account for some of the observed increase in itraconazole absorption. Besides Vitamin E TPGS, P-gp inhibition was already reported for a number of non-ionic surface-active excipients such as Tween 80, Cremophor EL and several Pluronic block copolymers (Johnson et al., 2002; Rege et al., 2002a). A possible P-gp modulating effect of Soluplus® is still under investigation.

This study clearly proves the potential of Soluplus® to improve oral bioavailability. For danazol the observed bioavailability enhancement (15-fold increased AUC) was slightly lower compared to a 16- or 20-fold increased AUC (compared to the pure drug) in beagle dogs, which was reached using nanoparticulate or cyclodextrin formulations, respectively (Liversidge and Cundy, 1995). However, these formulation approaches never reached the market. Also conventional hard capsules of crystalline danazol (Danacrine®) are mostly off-market as gonadotropin-releasing hormone agonists offer a better benefit-to-risk profile for the treatment of endometriosis. In the case of fenofibrate, the marketed nanoparticulate formulation TriCor®/Lipidil® ONE enabled a 2.8-fold increased AUC (compared to bulk drug) in Wistar rats (Buch et al., 2009), while Soluplus® formulations showed an about 5-fold increased AUC compared to the pure drug in beagle dogs. However, inter-species differences might play an important role hereby and prohibit a direct comparison. Therefore a side-by-side comparison may be included in further studies. For itraconazole, Yi et al. ob-

served an AUC of 9.4 ± 2.5 μg h ml^{-1} after oral application of the commercialized itraconazole–HPβCD solution (Sporanox® oral solution) in a dose of 7.5 mg/kg to fasted beagle dogs (Yi et al., 2007). Although this AUC is about 4-fold higher compared to the AUC of the Soluplus® solid solution, a future direct comparison under identical experimental conditions may further elucidate if the commercial product is really superior to the Soluplus® solid solution.

5. Conclusion

Soluplus® effectively enhanced the absorption of itraconazole, danazol and fenofibrate through the intestinal barrier if applied as solid solution. This could be shown in the Caco-2 *in vitro* system as well as in beagle dogs *in vivo*. For fenofibrate additionally the physical mixture of the test compound with Soluplus® was able to increase absorption and bioavailability *in vivo* and *in vitro*. The current work underlines the potential of the Caco-2 monolayer model as an excellent predictive tool for oral absorption behavior, not only for pure drugs but also for more complex drug–excipient formulations e.g. as solid solutions. As demonstrated for fenofibrate, we were able to distinguish *in vitro* between drugs for which the use of a solid solution is effective and drugs for which a physical mixture is sufficient to achieve a good oral bioavailability in accordance to *in vivo* tests in beagle dogs. However, for a better understanding of the absorption behavior *in vivo* and *in vitro*, further studies investigating the mechanism of drug liberation and transport in the presence of Soluplus® are needed.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2011.11.025.

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