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Research paper

Inulin solid dispersion technology to improve the absorption of the BCS Class IV drug TMC240

Marinella R. Visser [a], Lieven Baert [b,*], Gerben van 't Klooster [c], Laurent Schueller [b], Marian Geldof [e], Iris Vanwelkenhuysen [f], Herman de Kock [d], Sandra De Meyer [d], Henderik W. Frijlink [a], Jan Rosier [b], Wouter L.J. Hinrichs [a]

[a] Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, The Netherlands
[b] Chem-Pharm Development, Tibotec bvba, Mechelen, Belgium
[c] Preclinical Development, Tibotec bvba, Mechelen, Belgium
[d] Virology, Tibotec bvba, Mechelen, Belgium
[e] Drug Metabolism and Pharmacokinetics, Tibotec bvba, Mechelen, Belgium
[f] Bioanalysis, Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium

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ABSTRACT

TMC240 is a very poorly soluble and poorly permeating HIV protease inhibitor. In order to enhance its oral bioavailability, a fast dissolving inulin-based solid dispersion tablet was developed. During the dissolution test in water (0.5% or 1.0% SLS), this tablet released at least 80% of TMC240 within 30 min, while a tablet with the same composition, but manufactured as physical mixture, released only 6% after 2 h. In a subsequent single-dose study in dogs (200 mg of TMC240), plasma concentrations of TMC240 remained below the lower limit of quantification (<1.00 ng/mL) in all animals (n = 3 per tested formulation), except in one dog receiving the inulin solid dispersion tablet (C_{max} = 1.8 ng/mL, $AUC_{0\text{-}7\ h}$ = 3.0 ng h/mL). In the latter treatment group, ritonavir co-administration (10 mg/kg b.i.d.) increased TMC240 exposure more than 30-fold (mean $AUC_{0\text{-}7\ h}$ = 108 ng h/mL; F_{rel} = 3588%). Exposure was also 16-fold higher than after TMC240 administration as PEG400 suspension in the presence of ritonavir ($AUC_{0\text{-}7\ h}$ = 6.7 ng h/mL). The current data demonstrate that a solid dispersion of TMC240 in an inulin matrix allows considerable improvement in the release of poorly water-soluble TMC240, both *in vitro* in the presence of a surfactant and *in vivo* upon oral administration.

1. Introduction

Many new drugs can be categorized as Class II or IV drugs according to the Biopharmaceutics Classification System Guidance (BCSG) [1–3]. Class II drugs are poorly water soluble but once dissolved, they rapidly pass biological membranes like the gastro-intestinal wall. As a consequence, Class II drugs slowly dissolve in the aqueous environment of the gastro-intestinal tract after oral administration and result in a poor bioavailability, while increasing the dissolution rate will also improve bioavailability [4,5].

Application of solid dispersions is one of the strategies to increase the dissolution rate of Class II drugs [6,7]. Solid dispersions consist of two (or more) component systems in which the drug is dispersed monomolecularly or as small particles in a hydrophilic matrix. Increased dissolution rate can be attributed to a strongly enhanced surface area of the drug for dissolution [8], to an improved wetting of the drug [7] and to an enhanced solubility due to small size of the drug particles (Ostwald–Freundlich equation) and if applicable to the amorphous state of the drug [9,10].

Many studies on the application of solid dispersions for the improved dissolution behavior of lipophilic drugs have been published (reviewed in e.g. [6–8,11]). In most of these studies, either polyvinylpyrrolidone or polyethylene glycol was used as matrix material. Recently, we published on the application of sugar glass-based solid dispersions. In previous studies, we have investigated the dissolution behavior of tablets prepared from these solid dispersions [12]: it was found that in many cases, the release rate of the drug from tablets prepared from these solid dispersions was much higher than from tablets prepared from physical mixtures of the sugar and the drug. However, when small sugars like sucrose or trehalose were used and the drug load exceeded a certain threshold, the release of the drug was very slow [12]. This phenomenon was attributed to the extremely fast dissolution of the sugar which

* Corresponding author. Tibotec bvba, Chem-Pharm Development, Gen. De Wittelaan L11B 3, 2800 Mechelen, Belgium. Tel.: +32 15 46 16 00; fax: +32 15 46 19 36.
E-mail address: lbaert@its.jnj.com (L. Baert).

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resulted in a very high concentration of drug in the near vicinity of the dissolving tablet, resulting in the formation of large drug crystals which slowly dissolve. Due to its oligomeric nature, the oligofructose inulin 4 kDa dissolves slower than sucrose and trehalose. Therefore, it was subsequently investigated whether for a given drug load, the use of inulin instead of sucrose or trehalose as matrix material allowed to lower the drug concentration in the near vicinity of the dissolving tablet and, hence, lower the risk of crystallization. Whereas replacing the sucrose or trehalose in the solid dispersion tablets by inulin indeed allowed to increase the drug load threshold, above which drug release again decreases due to crystallization [12], increasing the molecular weight of inulin from 4 kDa to 7 kDa was not successful because in that case drug release became limited by slower dissolution of the matrix [13]. Therefore, inulin 4 kDa is currently used as matrix material for formulation studies. The surfactant sodium laurylsulphate (SLS) and superdisintregrant Primojel® (sodium starch glycolate) are added to overcome the limitations with regard to drug load [14,15]: the high surfactant concentration in the near vicinity of the dissolving tablet allows to increase the local drug solubility and limit the risks of its crystallization, this also due to the faster disintegration of the tablet itself. Fast *in vitro* release of active ingredients from inulin-4 kDa-based solid dispersion tablets, containing high drug loads and either SLS or Primojel®, has been confirmed for several poorly water soluble Class II active ingredients, including diazepam [12–14], nifedipine [13], cyclosporine A [16], fenofibrate [15] and Δ^9-tetrahydrocannabinol [17].

Class IV drugs pose an even bigger challenge as their absorption is not only limited by their slow dissolution in the aqueous environment of the gastro-intestinal tract, but also by their low permeation capacity, so that too low levels of dissolved drug in the intestine per definition will lead to poor bioavailability [1–3]. This is the case for TMC240 (Tibotec BVBA, Belgium), an experimental HIV-1 protease inhibitor (PI) with a broad-spectrum activity against a panel of highly PI cross-resistant viruses. Its structure is shown in Fig. 1. It is a structural analogue of a series of compounds that were designed to be active against multidrug-resistant viruses [18]. The compound is practically insoluble in water (2.07 mg/L) and has a low permeability (as also further confirmed in the results section of this paper). Permeation through the gastro-intestinal wall can be enhanced by the use of glycoprotein-P inhibitors, such as the protease inhibitor ritonavir, which can boost the plasma concentration levels of protease inhibitors in a number of ways: in the case of P-glycoprotein, it inhibits the protein efflux channels that take part in the active transport of protease inhibitors out of cells [19]. Ritonavir may also enhance drug exposure by hepatic and intestinal inhibition of CYP3A4 metabolism [19]. Ritonavir is commonly used in combination with currently marketed PIs in the treatment of HIV-infections.

In order to enhance absorption of TMC240, two strategies were studied: the use of the inulin solid dispersion technology to improve its dissolution behavior and the combined administration with ritonavir to improve its permeation over the intestinal wall. Therefore, inulin solid dispersion tablets were formulated and studied for their *in vitro* dissolution properties, as well as for their *in vivo* pharmacokinetics in dogs in the presence and absence of ritonavir, thereby comparing with physical mixture tablets of identical composition and with a liquid PEG400 suspension of TMC240.

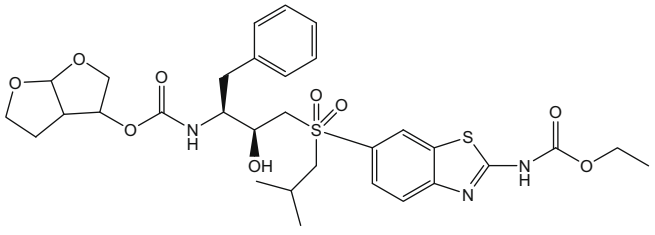

Fig. 1. Structure of TMC240.

2. Materials and methods

2.1. Materials

The HIV protease inhibitor TMC240 was supplied by Tibotec BVBA, Mechelen, Belgium and has been established to be stable under crystalline form (Tibotec, internal data on file). Inulin 4 kDa (further designated as inulin) was provided by Sensus, Roosendaal, The Netherlands. Primojel®, SLS and dimethyl sulfoxide (DMSO) were obtained from Avebe (Veendam), Bufa B.V. (Uitgeest) and Sigma–Aldrich Chemie B.V. (Zwijndrecht), respectively, in The Netherlands. Polyethylene glycol 400 (PEG400) was purchased at α-Pharma, Zwevegem, Belgium. Ritonavir oral solution (80 mg/mL) was purchased as commercially available solution (Norvir®) and diluted 1:10 (v/v) with PEG400 (stored at room temperature and protected from light).

2.1.1. Determination of solubility of TMC240

To determine its solubility in various dissolution media, an excess amount of TMC240 was added to pure water, 0.5% and 1.0% w/v SLS. These suspensions in closed vessels were stirred at 37 °C for two weeks and were then filtered with a 0.2-μm filter prior to analysis. The filtrate was analyzed by UV-spectrophotometry at wavelength of 280 nm. All measurements were performed in triplicate.

2.2. Production of TMC240 solid dispersion

Because of the extremely low aqueous solubility of TMC240, SLS and Primojel® were incorporated for formulation with the inulin solid dispersion technology. The TMC240-inulin solid dispersion was prepared as follows: a solution of 20 mg/mL TMC240, 180 mg/mL inulin, 20 mg/mL SLS and 9.2 mg/mL Primojel® in DMSO was prepared. Aliquots of 1 mL of this solution were pipetted in 20 mL glass vials and subsequently frozen in liquid nitrogen after which the frozen solutions were freeze dried with a Christ model Alpha 1-4 lyophilizer (Salm en Kipp, Breukelen, The Netherlands). Lyophilization was performed at a shelf temperature of −5 °C, a condenser temperature of −53 °C and a pressure of 0.05 mBar. The freeze-drying cycle took seven days during which the temperature was gradually raised to 25 °C. After freeze drying, the solid dispersion powder was equilibrated in a climate chamber conditioned at 20 °C and 45% relative humidity for at least one day.

2.3. Preparation of tablets

The TMC240-inulin solid dispersion was compressed with a hydraulic press (Hydro Mooi, Appingedam, The Netherlands) at a force of 5 kN and a compaction speed of 2 kN/s, to flat round tablets, containing 50 mg TMC240, having a diameter of 13 mm and weighing 573 mg.

In order to evaluate the impact of the inulin solid dispersion technology on the dissolution and pharmacokinetic behavior of TMC240, conventional formulated tablets were also prepared using the physical mixture of all ingredients in the same amounts as used for preparation of the TMC240-inulin solid dispersion tablets. Freeze-dried inulin was mixed with crystalline TMC240 (micronized at 1–3 μm) and the other excipients for 10 min in a Turbula mixer at 90 rpm (Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland). Subsequently, tablets were prepared as described

above. The tablets were equilibrated in a climate chamber conditioned at 20 °C and 45% relative humidity for at least one day.

2.4. Preparation of TMC240 suspension

A PEG400 suspension was prepared by suspending 960 mg of crystalline drug (micronized at 1–3 μm) in 120 mL of PEG 400 in order to obtain a suspension with a TMC240 concentration of 8 mg/mL.

2.5. Differential scanning calorimetry

The TMC240-inulin solid dispersion was characterized by differential scanning calorimetry (DSC), using a DSC Q2000 differential scanning calorimeter (TA instruments, Ghent, Belgium). A heating rate of 10 °C/min was used. During measurement, the sample cell was purged with nitrogen at a flow rate of 35 mL/min. Also, modulated DSC was carried out, thereby applying modulation amplitude of ±0.318 °C every 60 s and a heating rate of 2 °C/min. Measurements were done in duplicate.

2.6. In vitro dissolution test of tablets

The TMC240-inulin solid dispersion and physical mixture tablets were investigated for their dissolution behavior, in the absence and presence of 0.5% and 1.0% of SLS in the dissolution medium. A standardized paddle technique was applied, using a Prolabo USP dissolution apparatus II (Rowa Techniek B.V., Leiderdorp, The Netherlands). One tablet was stirred at 37 °C in 1000 mL of water, containing 0%, 0.5% or 1.0% SLS, at a paddle speed of 100 rpm. Each tablet was wedged in a paperclip weighing about 400 mg to prevent the tablets from floating. The dissolution vessel was connected to a Shimadzu UV 1601 spectrophotometer, using 1-cm cuvets for *in situ* assessment of the amount of TMC240 released from the tablet, by absorbance measurement at a wavelength of 280 nm. Under these conditions, a linear calibration curve of TMC240 was obtained in water containing 1.0% SLS, with the equation $y = 0.0334x + 0.0037$ ($R^2 = 1.0000$). All experiments were performed in duplicate.

2.7. In vivo study

The *in vivo* assessment of TMC240 release from the different formulations was performed in six male Marshall Beagle dogs (Marshall Farms, Green Hill 2001, Italy), aged 2 to 5 years and weighing between 8 and 14 kg at the start of the experimental phase. Animals were housed with free access to water all day and free access to dry food until the late afternoon. The study protocol and laboratory procedures were performed according to current ethical guidelines in animal research, as per Belgian laws and European convention (European Council Directives (1986) and European Commission's Protocol on the protection and welfare of animals used for experimental and other scientific purposes, 2007 online), as well as in accordance with the current GLP guidelines of the Organisation for Economic Cooperation and Development. The study was approved by the local ethics committee on animal experiments and performed in an AAALAC-accredited laboratory, complying with European and Belgian regulations for animal experiments.

Two parallel groups of three dogs were orally dosed, sequentially in a cross-over fashion. Each dog was given TMC240 as a 200 mg single dose and subsequent dosing in dogs was separated by a wash-out period of at least 10 days. The first group of three dogs received four inulin solid dispersion tablets each (50 mg TMC240/tablet), followed by four tablets of the identical physical mixture (50 mg TMC240/tablet) and finally by the solid dispersion tablet with ritonavir co-administration. The second group of three dogs first received TMC240 as a PEG400 suspension (8 mg/mL, 25 mL) and then the PEG400 suspension co-administered with ritonavir. The TMC240 suspensions and the solutions of ritonavir were administered by oral gavage. Ritonavir (10 mg/kg) was administered just prior to TMC240 administration and at 7 and 24 h post-TMC240 dosing.

2.8. Blood sampling

Blood samples [2 mL on ethylenediamine tetra-acetic acid (EDTA)] were collected from a jugular vein. Samples were taken at 30 min following TMC240 administration and then every hour thereafter up to 7 h, as well as at 24 and 31 h after administration. Blood samples were kept on melting ice and centrifuged within 1 h of sampling; plasma samples were stored in the freezer within 1 h after the start of centrifugation. At all times, the samples were protected from light.

2.9. Plasma preparation and determination of plasma concentrations

All study samples were analyzed using a qualified high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) research assay. The samples were first subjected to a selective sample cleanup using liquid–liquid extraction. In order to spike the plasma aliquots with internal standard, a structure analogue, was added to the calibration standard, quality control and unknown samples. The analytes were extracted once using 3 mL heptane/isoamylalcohol (90:10 v/v), after addition of 0.5 mL $Na_2B_4O_7$. $10H_2O$ (Borax). After equilibration and centrifugation, the organic layer was isolated and evaporated to dryness under a gentle stream of heated nitrogen. The extract residue was reconstituted in the mobile phase constituents and 5 μL was subjected to analysis.

After injection onto the HPLC-MS/MS system, chromatographic separation was achieved on a 3.5 μm C18 chromatographic column (4.6 ID × 50 mm) at a flow rate of 1.2 mL/min. An MS/MS system in the electrospray positive ionization, using multiple-reaction monitoring was used for detection. Transitions on an API-4000 instrument (Applied Biosystems) were monitored at m/z 677.2 to 521.0 for TMC240 and m/z 756.3 to 69.0 for the internal standard. The lower limit of quantification (LLOQ) of TMC240 was 1.00 ng/mL. The effective linear range of quantification was 1.00–5000 ng/mL.

Stability experiments, conducted in plasma from dogs, showed that TMC240 is stable in EDTA plasma after 2-h storage on ice, at room temperature and at 37 °C.

2.10. Pharmacokinetic analysis

Individual plasma concentration–time profiles were subjected to a pharmacokinetic analysis using validated PKAA R1.0a software. A non-compartmental analysis using the lin/log trapezoidal rule with lin/log interpolation was used for all data. Peak plasma concentrations (C_{max}), corresponding peak times (T_{max}), $AUC_{0\text{-}3\ h}$ and $AUC_{0\text{-}7\ h}$ and $AUC_{0\text{-}inf}$ values were calculated. Mean plasma concentrations and mean pharmacokinetic parameters were also calculated. The boosting effect of ritonavir was evaluated by comparing the AUC values of a given TMC240 formulation in the presence versus absence of ritonavir administration.

3. Results

3.1. Solubility of TMC240

Because the dissolution behavior of the tablets was determined in pure water and in 0.5% and 1.0% w/v SLS, the solubility of

TMC240 in these media was determined. As expected, the TMC240 solubility increased with increasing SLS concentration. The TMC240 solubility was 2.07 ± 0.08, 5.10 ± 0.52 and 11.75 ± 0.36 mg/L in pure water, 0.5% and 1.0% w/v SLS, respectively.

3.2. Production of TMC240 solid dispersion tablets

Application of the described technique allowed producing TMC240 as an inulin-based solid dispersion. The thermogram of pure TMC240 showed a melting endotherm at 226 °C. In the thermogram of the solid dispersion, this endotherm was no longer observed indicating that TMC240 was incorporated in the amorphous state. However, in the thermogram of the solid dispersion also no clear glass transitions could be observed. Therefore, the drug could be molecularly distributed over the solid dispersion or incorporated in the solid dispersion as amorphous clusters.

3.3. In vitro dissolution

Fig. 2 illustrates the dissolution behavior of solid dispersion tablets and physical mixture tablets at different concentrations of SLS in the dissolution medium.

Release of TMC240 from the inulin solid dispersion tablets was very fast, provided that the dissolution medium contained 0.5% or 1.0% of SLS (Fig. 2 top): in the presence of 0.5% of SLS, 80% of the drug was released within 30 min, and its maximum release (90%) was reached at 50 min. Thereafter, the amount of dissolved drug remained constant until ~100 min, after which its concentration slowly decreased again, indicating crystallization of the drug due to supersaturation. On increase in SLS concentration in the dissolution medium to 1%, an increased rate of drug release was observed, with 85% of the drug already dissolved within 30 min and remaining dissolved (drug) during the remaining 90 min.

However, in the absence of SLS in the dissolution medium, only ~10% of the drug was released after 2 h from this solid dispersion tablet.

In contrast, physical mixture tablets dissolved very slowly, even when the dissolution medium contained 1.0% SLS: only 6% of TMC240 was released after 2 h (Fig. 2 bottom).

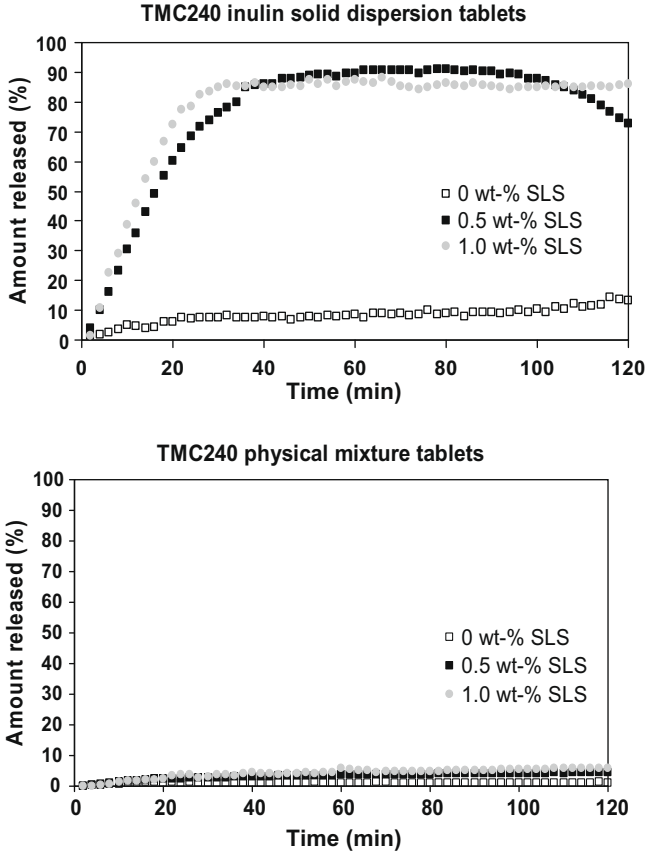

Fig. 2. Dissolution of TMC240 from inulin solid dispersion tablets (*top*) and physical mixture tablets composed of the same ingredients (*bottom*) and containing 50 mg TMC240, 450 mg inulin, 50 mg SLS and 23 mg Primojel®: effect of the SLS concentration on the dissolution method.

3.4. Pharmacokinetic study in dogs

The mean C_{max} and AUC values of TMC240 after a single oral administration to male beagle dogs are given in Table 1. In the absence of ritonavir, no pharmacokinetic release profiles could be generated except after a single oral administration of the TMC240-inulin solid dispersion tablets in one dog, in which values ranged between 1.09 and 1.78 ng/mL between 0.5 and 2 h after dosing (Fig. 3).

The plasma concentrations following administration of the TMC240 PEG400 suspension remained below the LLOQ, as did the levels after administration of the tablets prepared from a physical mixture of crystalline TMC240, inulin and the other ingredients, used in identical quantities as for the preparation of the TMC240-inulin solid dispersion tablets.

Ritonavir co-administration clearly increased the plasma levels of TMC240. After the single oral administration of the PEG400 suspension in the presence of ritonavir, plasma concentrations increased and remained above the LLOQ until 2–3 h after dosing. Plasma concentrations were between 1.07 and 3.73 ng/ml with C_{max} values reached at 0.5–1 h (T_{max}) and terminal half-lives ranging between 1.4 and 2.7 h.

The highest exposure of TMC240 was reached after single oral administration of the inulin solid dispersion tablets in the presence of ritonavir (C_{max} values 14.7–52.2 ng/ml). Plasma concentrations increased until 2–3 h after dosing (T_{max}) and remained above the LLOQ until 7 ($n = 2$) or 31 h ($n = 1$) after dosing. Terminal half-lives were 1.4 and 5.1 h. Exposure ($AUC_{0\text{-}7\ h}$, mean: 108 ng h/mL) increased more than 30-fold when compared with the inulin solid dispersion tablet alone. The F_{rel} values (based on the limited information on TMC240 plasma concentrations in the absence of ritonavir above the LLOQ) were 1748% and 3588% for the $AUC_{0\text{-}3\ h}$ and $AUC_{0\text{-}7\ h}$ values, respectively.

4. Discussion

The dissolution data in this study confirmed that the HIV protease inhibitor TMC240 is poorly water soluble. Furthermore, the solubility-enhancing effects of SLS were limited, i.e. the solubility of TMC240 is only increased about 2.5-fold by the addition of 0.5% SLS. This increase is usually much higher for lipophilic drugs, e.g. for fenofibrate, this increase is 2000-fold [20]. These data therefore show that TMC240 is not only poorly water soluble but will also have a low permeability. In conclusion, TMC240 behaves as a typical BCS Class IV drug.

The current study investigated two strategies to enhance the bioavailability of TMC240: one via changing the formulation, the other one via targeting pharmacokinetic enhancement of the gut wall permeation. It was shown that: (1) the inulin solid dispersion technology allows to produce a solid dispersion of TMC240 in an inulin matrix, (2) the resulting tablets lead to improved dissolution properties and enhanced bioavailability of TMC240 when compared to traditional formulations, and (3) the impact of ritonavir, a booster to enhance permeation through the gut wall, is larger

Table 1
Mean, standard deviation and range of values for the main pharmacokinetic parameters of various formulations of TMC240 after a single-dose administration of TMC240 in three male dogs.

Pharmacokinetic parameter[a]	Formulation administered as a single 200-mg dose of TMC240				
	Physical mixture tablets[b]	Solid dispersion tablets[b]	Solid dispersion tablets[b] + *ritonavir*[d]	PEG400 suspension[c]	PEG400 suspension + *ritonavir*[d,f]
C_{max} (ng/mL), mean	Below LLOQ	1.78[e]	31.5 ± 19.1 (14.7–27.5)	Below LLOQ	3.03 ± 0.76 (2.22–3.73)
$AUC_{0\text{-}3\ h}$ (ng h/mL), mean	Below LLOQ	3.01[e]	52.6 ± 28.5 (32.2–85.1)	Below LLOQ	5.83 ± 1.52 (4.88–7.59)
$AUC_{0\text{-}7\ h}$ (ng h/mL), mean	Below LLOQ	3.01[e]	108 ± 41.1 (73.4–153)	Below LLOQ	6.71 ± 2.17 (5.02–9.16)
Frel ($AUC_{0\text{-}3\ h}$), %[g]	–	–	1748	–	–
Frel ($AUC_{0\text{-}7\ h}$), %	–	–	3588	–	–

[a] The AUC_{inf} is not given because, even with the TMC240-inulin solid dispersion tablet in the presence of ritonavir, more than 30% of the AUC surface had to be extrapolated.
[b] Administered as four tablets containing 50 mg TMC240 per tablet.
[c] Two of three dogs slightly vomited within 5 min to 1 h after administration of the TMC240 PEG400 suspension, which may have in part influenced the results.
[d] Dose of ritonavir: 10 mg/kg, administered just before and 7 and 24 h after TMC240 dosing.
[e] Dataset from one dog, the plasma concentration values in the two other dogs being below the LLOQ.
[f] All developed diarrhoea 1 h after co-administration with ritonavir, which may have in part influenced the results.
[g] In presence versus absence of ritonavir.

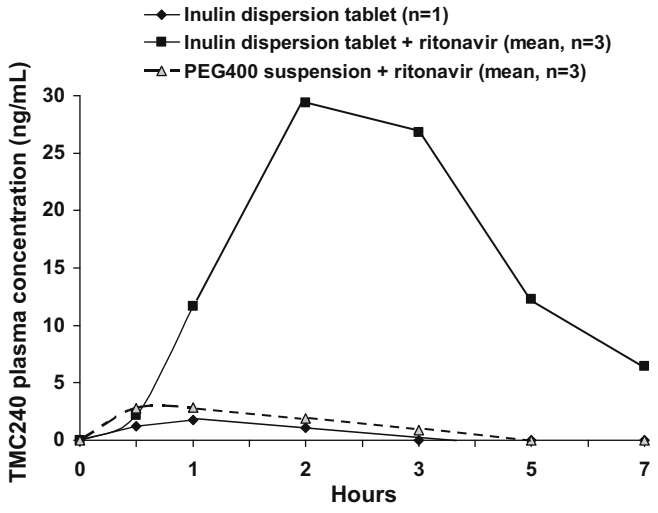

Fig. 3. Pharmacokinetic release profiles of TMC240 following a single 200-mg TMC240 dose, administered as four inulin solid dispersion tablets in the absence (◇–◇) or presence (□–□) of ritonavir, or as 8 mg/mL PEG400 suspension (▲–▲) in dogs (n = 3/treatment group, parallel design), with or without the co-administration of ritonavir (30 mg/kg, cross-over design). TMC240.[1]

than the formulation effect via solid dispersion, leading further to an additional 30-fold higher increase in exposure when combined with inulin solid dispersion technology.

With the inulin solid dispersion technology, tablets containing TMC240 could be prepared with an excellent *in vitro* dissolution behavior. The release of TMC240 in pure water was no more than ~10% which seems quite poor. However, as the tablets contained 50 mg TMC240 and the solubility of TMC240 in pure water is only 2.07 mg/L, about 2.5 times the saturation concentration was reached. The degree of supersaturation in 0.5% SLS was even higher. Since the maximal release was 90% and the solubility of TMC240 in 0.5% SLS is 5.10 mg/L, about 9 times the saturation concentration was reached. This high degree of supersaturation was maintained from 30 min until about 100 min after initiation of the dissolution test after which the concentration decreased due to crystallization of the drug. Also in 1% SLS, a high degree of supersaturation was reached. After 30 min, 85% of the drug was released while the solubility of the drug in this medium is 11.75 mg/L implying that about 3.6 times the saturation concentration was reached. This supersaturation was maintained without crystallization until the dissolution experiment was terminated at 120 min.

The lack of release of TMC240 from the solid dispersion tablets in the absence of SLS confirms the poor aqueous solubility of this drug. Yet, as SLS has strong surfactant properties, its addition to the dissolution medium led to rapid release of TMC240 from the TMC240-inulin solid dispersion tablet: in presence of 0.5% SLS in the dissolution medium, 90% of TMC240 dissolved within 50 min. However, after 100 min, TMC240 concentration decreased, indicating that supersaturation had been reached, followed by precipitation. When raising the SLS concentration to 1%, 90% of TMC240 dissolved within 30 min, and the decrease in TMC240 concentration at 100 min was not observed. It is unclear whether the saturation concentration was not reached in this dissolution medium or whether the dissolution medium was supersaturated without significant recrystallization within the duration of the experiment (2 h).

The improved dissolution properties of the TMC240-inulin solid dispersion tablet can be attributed to the solid dispersion technology used, as hardly any TMC240 was released from the physical mixture tablet containing crystalline TMC240 and the other ingredients in identical quantities, even when the dissolution medium contained 1% SLS. The results indicate that TMC240 was ultrafine dispersed in the inulin matrix. In addition, it is likely that also SLS and Primojel® are more evenly distributed in the solid dispersion tablet than in the physical mixture tablet which may also have played a role in the large difference in dissolution behavior [14,15].

In terms of formulation development, a novelty was the use of DMSO as solvent for both inulin and the drug. In previous studies, we prepared inulin-based solid dispersions using the following procedure [21]: two separate solutions were prepared; a solution of inulin in water and a solution of the lipophilic drug in tertiary butyl alcohol (TBA). The two solutions were mixed and subsequently freeze dried. A disadvantage of this procedure is that a solution of inulin and the active pharmaceutical ingredient in a mixture of water and TBA is thermodynamically unstable. Consequently, the drug can precipitate in the solution forming large particles in the ultimate solid dispersion resulting in poor dissolution behavior. Therefore, the solution had to be frozen fast and immediately after mixing to minimize the risks of the formation of these

[1] The TMC240 formulation was administered on Day 1 without ritonavir and on Day 72 in presence of ritonavir in the same dog. Ritonavir was administered as three 10 mg/kg doses, the first dose just prior to TMC240 administration and the second and third doses at 7 and 24 h post TMC240 dosing, respectively. Concentrations were below the LLOQ following administration as PEG400 suspension in absence of ritonavir, and conventional physical mixture tablets with an identical composition in absence and presence of ritonavir.

large particles. The advantage of using DMSO is that both the inulin and the TMC240 could be dissolved in this solvent to form a thermodynamically stable solution by which an ultrafine distribution of TMC240 in the inulin matrix could be obtained after freeze drying. A disadvantage of DMSO, however, is that it has an extremely low vapor pressure of 0.08 kPa (at 25 °C), while water and TBA have vapor pressures of 3.16 kPa and 5.49 kPa (at 25 °C), respectively. Because of the low vapor pressure, the freeze-drying process took 7 days, instead of the 2 days needed when a water/TBA mixture is used as a solvent. However, in this study, the freeze-drying process was not optimized. By adjustment of both shelf temperature and pressure, the process time might be shortened.

The pharmacokinetic study in dogs clearly showed that: (1) TMC240 – as Class IV drug – is not absorbed from a conventional formulation, such as a PEG400 suspension, (2) ritonavir (enhancing the permeation) allows to boost the exposure of TMC240 from the PEG400 solution to a limited extent, (3) inulin technology allowed to enhance absorption (via the enhanced dissolution of TMC240) and (4) the combined strategy of using the inulin solid dispersion technology (enhancing the dissolution) and co-administration of ritonavir (boosting via the permeation through the gut wall) considerably improved the bioavailability of TMC240. Yet, the observed plasma concentration levels remained still too low to support a clinical development of the formulation. The contribution of the solid dispersion state of TMC240 to the enhanced *in vivo* exposure was evidenced by the fact that: (1) the inulin solid dispersion tablet was the only formulation yielding measurable plasma levels of TMC240 in the absence of ritonavir, (2) the exposure ($AUC_{0\text{-}7\ h}$) in the presence of ritonavir was 16-fold higher with the TMC240-inulin solid dispersion tablet than with the PEG400 suspension. The impact of the booster ritonavir (enhancing the permeation through the gut wall via P-glycoprotein interaction [19]) on the exposure of TMC240 was at least as important as the contribution of the technology: simultaneous administration of ritonavir with the TMC240-inulin solid dispersion tablets or the TMC240 PEG suspension resulted in detectable plasma levels in all animals, while the $AUC_{0\text{-}7}$ of the TMC240 solid dispersion tablet in the presence of ritonavir increased more than 30-fold when compared to its administration without ritonavir.

In conclusion, this study shows that both the inulin solid dispersion technology and the pharmacokinetic enhancement of gut wall permeation with ritonavir each allow to increase the exposure of the very poorly water-soluble BCS Class IV drug TMC240 and that their combination may be a potentially useful strategy to improve its bioavailability.

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