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The effect of formulation additives on *in vitro* dissolution-absorption profile and *in vivo* bioavailability of telmisartan from brand and generic formulations

Enikő Borbás[a], Zsombor K. Nagy[a,*], Brigitta Nagy[a], Attila Balogh[a], Balázs Farkas[a], Oksana Tsinman[b], Konstantin Tsinman[b], Bálint Sinkó[b]

[a] Budapest University of Technology and Economics, 3 Műegyetem rkp, Budapest, Hungary
[b] Pion Inc., 10 Cook Street, Billerica, MA 01821, USA

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ABSTRACT

In this study, brand and four generic formulations of telmisartan, an antihypertensive drug, were used in *in vitro* simultaneous dissolution-absorption, investigating the effect of different formulation additives on dissolution and on absorption through an artificial membrane. The *in vitro* test was found to be sensitive enough to show even small differences between brand and generic formulations caused by the use of different excipients. By only changing the type of filler from sorbitol to mannitol in the formulation, the flux through the membrane was reduced by approximately 10%. Changing the salt forming agent as well resulted in approximately 20% of flux reduction compared to the brand formulation. This significant difference was clearly shown in the published *in vivo* results as well. The use of additional lactose monohydrate in the formulation also leads to approximately 10% reduction in flux. The results show that by changing excipients, the dissolution of telmisartan was not altered significantly, but the flux through the membrane was found to be significantly changed.

These results pointed out the limitations of traditional USP dissolution tests and emphasized the importance of simultaneously measuring dissolution and absorption, which allows the complex effect of formulation excipients on both processes to be measured. Moreover, the *in vivo* predictive power of the simultaneous dissolution-absorption test was demonstrated by comparing the *in vitro* fluxes to *in vivo* bioequivalence study results.

1. Introduction

Traditional USP dissolution tests have been used in the pharmaceutical industry to perform quality control (QC) of manufacturing process for drug products and to carry out stability tests after production. Dissolution tests have also been used in research and development (R&D) to compare performance of different drug product formulations during the late stage of development process as well as to compare brand and generic products in biowaiver studies (Food and Drug Administration, 2015). Although USP dissolution tests provide a simple, reproducible, and cost-effective way of analyzing final dosage forms and, therefore, are well suited for QC purposes, the *in vitro* results often show poor correlation with the *in vivo* data (Buckley et al., 2012).

A common reason for this phenomenon is that dissolution and absorption are consecutive processes *in vivo* while they are separated artificially when carrying out *in vitro* tests, which can result in misleading information. The solubility-permeability interplay must be taken into consideration when formulating poorly water-soluble active pharmaceutical ingredient (API) because solubilizing agents, such as surfactants, polymers, and cyclodextrins, influence not only dissolution but also the permeation of the drug molecules through biological membranes (Dahan et al., 2010; Dahan and Miller, 2012; Fenyvesi et al., 2011; Loftsson and Brewster, 2011). For example, when the API has low aqueous solubility, the use of solubility enhancer is a possible way to improve the dissolution behavior (Frank et al., 2012; Qi et al., 2012). However, when using formulation techniques, it is important to keep in mind that while enhancing dissolution, the modification of the API's solubility can hinder absorption (Borbás et al., 2016; Miller et al., 2012; Raina et al., 2015). For that reason, it seems advantageous to improve dissolution without altering the thermodynamic solubility of the API. Creating supersaturated solutions by dissolving amorphous solid dispersions (ASD) is an approach that has already shown superior fluxes in many cases compared to results from saturated or subsaturated solutions (Beig et al., 2015; Frank et al., 2012, 2014; Iervolino et al., 2000;

* Corresponding author.
E-mail address: zsknagy@oct.bme.hu (Z.K. Nagy).

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Kumprakob et al., 2005; Pellett et al., 1994; Raghavan et al., 2001; Raina et al., 2015; Santos et al., 2011). In the case of *in vitro* dissolution tests, the concentration of the API is not lowered by transports like in biological systems, meaning that the probability of precipitation from a supersaturated solution is higher *in vitro* than *in vivo*. Therefore, dissolution-absorption tests have the possibility to be more predictive to *in vivo* results if precipitation occurs (Borbás et al., 2015; Stewart et al., 2017).

There have been many attempts to improve the *in vitro in vivo* correlation (IVIVC) by the simultaneous testing of dissolution and absorption, *e.g.* by connecting dissolution cells with Caco-2 tests (Ginski et al., 1999). Due to the fact that Caco-2 permeability assay is a living cell-based method, its time and cost effectiveness is low compared to non-cell-based tests like Parallel Artificial Membrane Permeability Assay (PAMPA). PAMPA has been shown to be predictive of passive transcellular permeability (Avdeef and Tsinman, 2006) while having the advantage of being more robust, reproducible, and applicable for formulations like creams and semi solid dosage forms (Sinkó et al., 2012). Therefore, it is proposed that a scaled-up version of a PAMPA-like setup could become a way of combining dissolution and absorption testing. The MacroFLUX™ apparatus is a novel device that incorporates an absorption compartment into USP 1 or 2 dissolution apparatus. Thus, by enabling simultaneous measurement of dissolution and absorption rates, it has the potential to improve IVIVC, which may help generic drug developers improve the outcomes of bioequivalence studies. In the case of formulation screening, it could be a more reproducible and cost-effective way of testing formulations compared to cell-based assays or animal tests.

Telmisartan, a poorly water-soluble antihypertensive drug, was chosen for this study as a model API. Because of telmisartan's amphoteric character, its solubility strongly depends on the pH. In the range of pH 3–8, its solubility is less than 1 μg/mL, while this value increases both towards more acidic or basic conditions (Chowhnan, 1978; Kramer and Flynn, 1972; Wienen et al., 2000). For that reason, the addition of a microenvironmental pH-modifying agent is a possible solution for improving the dissolution of the API (Tran et al., 2008, 2010; Yan et al., 2014).

In this study, brand and generic formulations of telmisartan were used in *in vitro* simultaneous dissolution-absorption, investigating the effect of different formulation additives on dissolution and on absorption through an artificial membrane. These *in vitro* results were compared to *in vivo* bioequivalence study results published by the developer of the generic products in public assessment reports.

2. Materials

Telmisartan (TEL) (514 g/mol, structure shown in Fig. 1), buffer components (NaH_2PO_4, NaOH, NaCl, KCl, HCl), D-mannitol, lactose monohydrate, and n-dodecane were purchased from Sigma-Aldrich Co. Llc. (St. Louis, MO, USA). Prisma™ HT buffer were obtained from Pion Inc. (Billerica, MA, USA). SIF powder was purchased from Biorelevant.com (London, UK). Telmisartan brand and generic tablets (40 mg API/tablet) were purchased from the listed companies: Micardis from Boehringer Ingelheim International Gmbh (Ingelheim am Rhein, Germany), Telmisartan Ratiopharm from Ratiopharm (Ulm, Germany), Telmisartan Tolura from Krka (Novo mesto, Slovenia), Telmisartan Actavis from Actavis (Parsippany-Troy Hills, NJ, USA), Telmisartan Mylan from Mylan (Canonsburg, PA, USA). Table 1 shows the qualitative composition of the tablets.

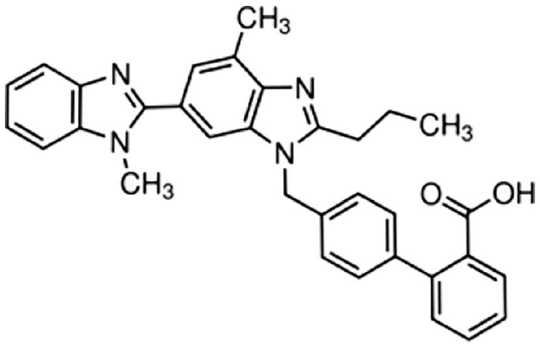

Fig. 1. Structure of telmisartan.

3. Methods

3.1. Thermodynamic solubility measurements

For measuring the thermodynamic solubility, crystalline TEL (10 mg) was added to 10 mL of pH 1.6 (SGF) and pH 6.5 FaSSIF full buffer Version 1 (n = 3). The resulting mixtures were stirred at 37 °C for 6 h (to the solution equilibrium). The concentration of the API in the buffers was determined without filtering the solutions by the Rainbow Dynamic Dissolution Monitor instrument (Pion Inc., Billerica, MA) using UV calibration.

3.2. pK_a determination of telmisartan

An automated potentiometric-optical system (PULSE™, Pion Inc.) was used to collect the UV spectra in the course of a titration experiment. The optical system consisted of a pulsed Xe light source, a photodiode array detector, and a fiber optic dip probe that was coupled to a mini-titrator composed of a temperature-controlled titrating vial, pH electrode, overhead stirrer, and syringe dispensers to dispense acid (0.5 M HCl), base (0.5 M KOH), solvent (0.15 M aqueous KCl solution), and/or cosolvent (*e.g.*, methanol). All experiments were carried out at an ionic strength of 0.15 M at 25 ± 2.0 °C. Phosphate buffer of 0.05 mM or the linear universal Prisma™ HT (Pion Inc.) were used to avoid any subtle change of the titration curve in the neutral pH region. The pH range of the titration experiment was set from 2 to 10.

3.3. Flux experiments

Brand and generic formulations of Telmisartan, were tested using MacroFLUX™ (Pion Inc.). Receiver chamber integrated with permeation membrane, overhead stirrer and fiber optic UV probe was inserted in the standard 900 mL vessel of USP 2 apparatus (Erweka DT 126 Dissolution Tester, Heusenstamm Germany) (Fig. 2). A filter-supported artificial membrane (hydrophobic PVDF, polyvinylidenfluoride, 0.45 μm pore size, 3.8 cm^2) impregnated with 50 μL of n-dodecane was separating the dissolution (donor) compartment from the receiver compartment containing 13 mL of pH 7.4 (Prisma™ HT, Pion Inc.). The experiment began in 850 mL of pH 1.6 buffer simulating gastric conditions (SGF) on the donor side and then after 30 min media in the dissolution vessel was converted to fasted state simulated intestinal fluid (FaSSIF V1) (pH 6.5) by adding 212.5 mL of specially formulated concentrate containing SIF powder. Donor stirring was set to 100 rpm, while the receiver stirring was set to 250 rpm to keep the thickness of unstirred water layer on minimum. The integrated fiber-optic UV probes were positioned in the donor and receiver compartments allowing real time concentration monitoring in both chambers. Concentration monitoring was enabled through fiber optic UV probes connected to the Rainbow Dynamic Dissolution Monitor instrument (Pion Inc., Billerica, MA, USA). The flux across the membrane was calculated using the following equation:

$$J(t) = dm/A*dt \quad (1)$$

where the flux (J) of a drug through the membrane is defined as the amount (m) of drug crossing a unit area (A) perpendicular to its flow per unit time (t).