Research Paper

Advances and challenges in PBPK modeling – Analysis of factors contributing to the oral absorption of atazanavir, a poorly soluble weak base

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ABSTRACT

Many active pharmaceutical ingredients (APIs) exhibit a highly variable pharmacokinetic (PK) profile. This behavior may be attributable to pre-absorptive, absorptive and/or post-absorptive factors. Pre-absorptive factors are those related to dosage form disintegration, drug dissolution, supersaturation, precipitation and gastric emptying. Absorptive factors are involved with drug absorption and efflux mechanisms, while drug distribution and clearance are post-absorptive factors. This study aimed to investigate the relative influence of the aforementioned parameters on the pharmacokinetic profile of atazanavir, a poorly soluble weakly basic compound with highly variable pharmacokinetics. The pre-absorptive behavior of the drug was examined by applying biorelevant *in vitro* tests to reflect upper gastrointestinal behavior in the fasted and fed states. The *in vitro* results were implemented, along with permeability and post-absorptive data obtained from the literature, into physiologically based pharmacokinetic (PBPK) models. Sensitivity analysis of the resulting plasma profiles revealed that the pharmacokinetic profile of atazanavir is affected by an array of factors rather than one standout factor. According to the *in silico* model, pre-absorptive and absorptive factors had less impact on atazanavir bioavailability compared to post-absorptive parameters, although active drug efflux and extraction appear to account for the sub-proportional pharmacokinetic response to lower atazanavir doses in the fasted state. From the PBPK models it was concluded that further enhancement of the formulation would bring little improvement in the pharmacokinetic response to atazanavir. This approach may prove useful in assessing the potential benefits of formulation enhancement of other existing drug products on the market.

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1. Introduction

Oral drug absorption of poorly soluble active pharmaceutical ingredients (APIs) can be influenced by various factors, such as drug dissolution, gastric emptying and drug permeability. For compounds which have a highly variable pharmacokinetic profile, the identification of all factors and their influence on the *in vivo* performance can be particularly complex. Several factors, such as influx and efflux transporters, metabolism in the gut wall and the liver, protein binding, distribution and elimination, many of which can exhibit saturation kinetics, become important during or after absorption and need to be considered for the evaluation of pharmacokinetics of such APIs. Additionally, food components may have an impact not only on drug dissolution but also on permeability of some APIs [1,2]. Grapefruit juice is a typical representative of nutritional inhibition of P-glycoprotein (P-gp) and CYP3A4 in the intestinal epithelia [3]. Moreover, co-administration of other APIs can change drug bioavailability through induction or inhibition of

carrier and metabolizing proteins in the intestinal epithelia and in the liver [4–6]. Some of the inhibitory mechanisms are nowadays exploited in advanced therapies, especially for drugs that are known to undergo extensive first-pass metabolism [7,8]. Interestingly, drug permeability can be correlated with drug metabolism. Benet et al. reported that highly permeable drugs often undergo extensive metabolism. Thus, bioavailability determinants, which are not generally assumed to be directly correlated with each other, may indirectly impact one another [9,10].

In this paper we examine the absorptive and post-absorptive determinants of oral bioavailability of atazanavir sulfate, the salt of a poorly soluble weak base, using biorelevant dissolution, transfer experiments, and physiologically based pharmacokinetic (PBPK) modeling, in an attempt to identify which of the various factors are the most important determinants of its pharmacokinetic profile. This example was chosen because studies have shown that its pharmacokinetics is complex and that the food effects do not follow the usual paradigm of showing the strongest effect with a high fat meal [11–14].

Atazanavir sulfate (Fig. 1) is an azapeptide HIV-1 protease inhibitor for combination antiretroviral therapy (ART) of HIV-1 infected patients [15–19]. The marketed product is Reyataz® from Bristol-Myers Squibb. Atazanavir (IUPAC name: methyl N-[(1S)-1-{[(2S,3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-N′-{[4-(pyridine-2-yl)phenyl]methyl}butanehydrazido]-1-phenylbutan-2-yl]carbamoyl}-2,2-dimethylpropyl]carbamate) is marketed as a hydrosulfate salt and has a molecular weight of 704.86 g/mol, referring to the free base. It has a basic pK_a value of 4.7, a calculated octanol–water distribution coefficient ($\log P$) of 5.92 and a calculated polar surface area of 186.2 Å [9,11]. As would be expected from its structure, atazanavir sulfate has a highly pH dependent aqueous solubility. In acidic environments it shows high solubility (5.2 mg/ml at pH 1.9) which decreases rapidly with increasing pH to 0.002 mg/ml at pH 4.3 [11].

The absolute bioavailability of atazanavir is not known; however, it exhibits non-linear pharmacokinetics over the dose range of 100 mg to 1200 mg. Since the compound is a substrate for CYP3A4 and CYP3A5 and an inhibitor of CYP3A4 and UGT1A1, it is reasonable to consider that its microsomal biotransformation is saturable [11,20,21]. A positive food effect for atazanavir was observed under light meal conditions, but under high fat meal conditions a slight positive or even a negative food effect has been reported [11–14]. Its solubility in fasted and fed state simulated fluids (FaSSIF and FeSSIF) is in good agreement with its solubility in human intestinal fluids, which are similar in FaHIF (Fasted human intestinal fluid, 15.1 μM) and in FeHIF (Fed human intestinal fluid, 14.9 μM) [22]. However, studies of atazanavir behavior upon emptying from the stomach into the small intestine are lacking in the literature. The permeability of atazanavir in Caco-2 cell cultures is concentration dependent (apical to basolateral:

$1\ \mu M \sim 1.5 \times 10^{-6}$ cm/s and $100\ \mu M \sim 8 \times 10^{-6}$ cm/s), indicating a possible involvement of P-gp mediated efflux in atazanavir absorption [23]. Interestingly, a Caco-2 study which conducted permeability of atazanavir using biorelevant media (FaSSIF and FeSSIF) in the apical compartment revealed significantly higher permeability values in FeSSIF (1.25×10^{-5} cm/s) than in FaSSIF (1.5×10^{-6} cm/s) [2].

In summary, atazanavir exhibits unusual *in vivo* behavior, which may be the result of a combination of various factors. This study utilizes a combination of solubility, dissolution and transfer model experiments along with known permeability and post-absorptive pharmacokinetic behavior, combined with *in silico* PBPK modeling, to determine the relative contributions of the factors influencing atazanavir pharmacokinetic profile.

2. Materials and methods

2.1. Chemicals and reagents

Atazanavir sulfate pure compound (lot 229975-97-7) and 200 mg atazanavir sulfate capsules (Reyataz®, lot 2H5117A) were obtained from Bristol-Myers Squibb, New York, USA. Long-life heat-treated and homogenized milk, containing 3.5% fat (UTH) was purchased from AF Deutschland GmbH, Düsseldorf, Germany. Lipofundin® (Emulsion with 20% middle chain triglyceride, lot 132918084) was obtained from B. Braun Meisungen AG. Glyceryl monooleate (GMO, Rylo M19 Pharma, 99.5% monoglyceride, lot 173403-401989490) was kindly donated by Danisco Specialities, Brabrand, Denmark. Egg phosphatidylcholine (Lipoid E PC, 99.1% pure, lot 108015-1/42) was kindly donated by Lipoid GmbH, Ludwigshafen, Germany. Sodium taurocholate (lot 2011040152) was obtained from Prodotti Chimici E Alimentari S.P.A., Basaluzzo, Italy. Sodium oleate powder (82.7% pure, lot 51110) was purchased from Riedel-de Haën, Seelze, Germany. Pepsin (0.51 U/mg, lot 1241256) was purchased from Fluka Chemie AG, Buchs, Switzerland. SIF Powder (lot PHA S 1003 012/16) and SIF Powder FaSSIF-V2 (lot 02-1302-01) were kindly donated by Biorelevant.com. Maleic acid (lot BCBC3155) was obtained from Sigma–Aldrich, Steinheim, Germany. Ammonium dihydrogen phosphate (lot 12A310046), sodium acetate trihydrate (lot A792767747) and monobasic sodium phosphate monohydrate (lot A02140949112) were of analytical grade and purchased from Merck KGaA, Darmstadt, Germany. Acetic acid (lot 12B220509), acetonitrile (lot I691330327), hydrochloric acid (lot 10L060526), methanol (lot I630607212), ortho-phosphoric acid 85% (12K210017), sodium chloride (lot 10J110040) and sodium hydroxide (lot 09G300017) were of analytical grade and acquired from VWR International, Leuven, Belgium.

2.2. Biorelevant media composition

The compositions of simulated fasted and fed state gastric and intestinal media used for this work are summarized in Table 1. Biorelevant media were used for solubility studies as well as for dissolution tests and transfer experiments. The medium characterizing the preprandial conditions of the stomach was Fasted State Simulated Gastric Fluid (FaSSGF). [24,25]. To simulate fed state gastric conditions corresponding to the light meal which was administered in PK studies, two media, FeSSGF pH 2.75 (Fed State Simulated Gastric Fluid) and FeSSGEm pH 2.75 (Fed State Simulated Gastric Emulsion) were introduced. The pH of both media was reduced from the usual FeSSGF value of 5.0 to pH 2.75 to reflect the decrease in gastric pH resulting from the orange juice which was ingested with the light meals. Orange juice, which has a pH value of approximately 3, was assumed to have an
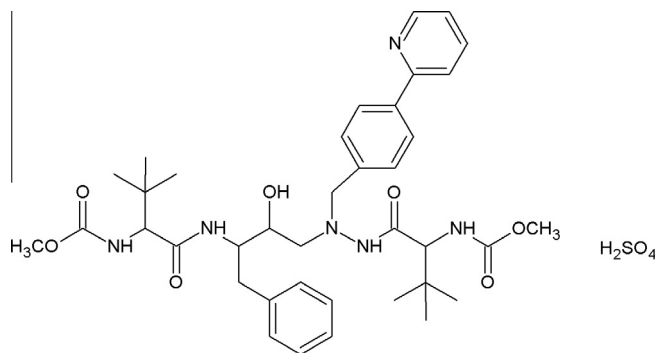

Fig. 1. Chemical structure of atazanavir sulfate.