Utilizing *In Vitro* and PBPK Tools to Link ADME Characteristics to Plasma Profiles: Case Example Nifedipine Immediate Release Formulation

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ABSTRACT: One of the most prominent food–drug interactions is the inhibition of intestinal cytochrome P450 (CYP) 3A enzymes by grapefruit juice ingredients, and, as many drugs are metabolized via CYP 3A, this interaction can be of clinical importance. Calcium channel-blocking agents of the dihydropyridine type, such as felodipine and nifedipine, are subject to extensive intestinal first pass metabolism via CYP 3A, thus resulting in significantly enhanced *in vivo* exposure of the drug when administered together with grapefruit juice. Physiologically based pharmacokinetic (PBPK) modeling was used to simulate pharmacokinetics of a nifedipine immediate release formulation following concomitant grapefruit juice ingestion, that is, after inhibition of small intestinal CYP 3A enzymes. For this purpose, detailed data about CYP 3A levels were collected from the literature and implemented into commercial PBPK software. As literature reports show that grapefruit juice (i) leads to a marked delay in gastric emptying, and (ii) rapidly lowers the levels of intestinal CYP 3A enzymes, inhibition of intestinal first pass metabolism following ingestion of grapefruit juice was simulated by altering the intestinal CYP 3A enzyme levels and simultaneously decelerating the gastric emptying rate. To estimate the *in vivo* dispersion and dissolution behavior of the formulation, dissolution tests in several media simulating both the fasted and fed state stomach and small intestine were conducted, and the results from the *in vitro* dissolution tests were used as input function to describe the *in vivo* dissolution of the drug. Plasma concentration–time profiles of the nifedipine immediate release formulation both with and without simultaneous CYP 3A inhibition were simulated, and the results were compared with data gathered from the literature. Using this approach, nifedipine plasma profiles could be simulated well both with and without enzyme inhibition. A reduction in small intestinal CYP 3A levels by 60% was found to yield the best results, with simulated nifedipine concentration–time profiles within 20% of the *in vivo* observed results. By additionally varying the dissolution input of the PBPK model, a link between the dissolution characteristics of the formulation and its *in vivo* performance could be established.
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Keywords: absorption; bioavailability; computational ADME; cytochrome P450; dissolution; drug metabolizing enzymes; first-pass metabolism; pharmacokinetics; quality by design (QbD)

INTRODUCTION

It is well known that the main factors that influence the bioavailability of a drug after oral administration are the solubility and dissolution of the drug at the site of absorption, degradation and complexation of the drug in the gut lumen, the ability of the drug to permeate the gut wall and enter into systemic circulation [including the possibility of the drug being a substrate for efflux transporters, e.g. P-glycoprotein (P-gp)], and metabolic degradation, that is first pass metabolism in the gut wall and liver.[1]

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Besides solubility and permeability issues, first pass metabolism, including in the gut wall, can be an important limitation to the oral bioavailability of certain drugs. With a contribution of approximately 80%, the most prominent enzymes responsible for intestinal first pass metabolism belong to the cytochrome P450 (CYP) 3A subfamily.[2] In proximal regions of the small intestine, CYP 3A4 is the most prominent enzyme of the CYP 3A subfamily. Other enzyme subfamilies present in the small intestine belong, for example, to CYP 2C and 2D. Interestingly, the individual contributions of the various CYP subfamilies to intestinal metabolism are different compared with hepatic metabolism.[2,3]

As the intestinal CYP enzymes are located in enterocytes, which constitute the surface cell monolayer of the intestinal mucosa,[4] drug molecules and food components can come in contact with these enzymes during the absorption process and thus be metabolized. *Vice versa*, intestinal CYP enzymes can be influenced by drug and food components, resulting in either increased (e.g., enzyme induction by increased enzyme expression) or decreased (e.g., enzyme inhibition) CYP activity. The interaction between grapefruit juice and dihydropyridine calcium channel blockers, such as felodipine and nifedipine, is one of the most well-known examples of enzyme inhibition caused by food components.[5–15] However, not only calcium channel blockers, but also other CYP 3A substrates show clinical interactions with grapefruit juice, for example terfenadine,[16,17] midazolam,[18] triazolam,[19] cyclosporine,[20,21] atorvastatin,[22] and saquinavir.[23]

Grapefruit juice contains flavonoids such as naringin, naringenin, quercetin, and kaempferol, and furanocoumarins, for example bergamottin and dihydroxybergamottin, which are known to be potent and selective inhibitors of CYP 3A enzymes. By contrast, other intestinal CYP enzymes, for example CYP 2D or CYP 1A, are not inhibited by grapefruit juice components.[9,10,13,14,24,25]

Ingestion of grapefruit juice leads to a pronounced and long-lasting inhibition of small intestinal CYP 3A enzymes, which, in turn, may lead to clinical implications for drugs that are metabolized via this enzyme subfamily. After ingestion of grapefruit juice, small intestinal CYP 3A protein activity decreases rapidly,[5,6,14] and the explanation for this decrease in CYP activity is an irreversible, concentration-dependent inhibition of the CYP enzymes because of protein degradation.[9,13] By contrast, short-term ingestion of grapefruit juice does not affect hepatic CYP 3A enzymes significantly, and inhibition of hepatic CYP 3A enzymes occurs only after long-term ingestion of grapefruit juice.[9–13]

After administration of grapefruit juice, the time to reach the maximum nifedipine plasma concentration, t_{max}, was found to be increased. After concomitant ingestion of grapefruit juice and nifedipine, Odou et al.[11] reported a delay in gastric emptying of approximately 30 min because of the low *p*H and the caloric content of grapefruit juice, and this delay in gastric emptying is a reasonable explanation for the increase in t_{max}. Similar observations have also been reported for felodipine pharmacokinetics.[7]

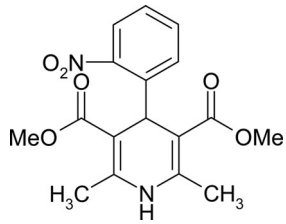

Figure 1. Structural formula of the calcium channel-blocking agent nifedipine.

Physiologically based pharmacokinetic (PBPK) modeling can be regarded as a mathematical, *in silico* (computer-assisted) approach to simulate and predict the absorption, distribution, metabolism, and excretion (ADME) properties of compounds, and this technique has gained increased importance in recent years. In terms of improving predictability of the simulations, biorelevant dissolution testing can be coupled with PBPK software to better reflect the *in vivo* dissolution, especially of poorly soluble drug compounds. In addition, the PBPK modeling approach allows the integration of information related to organ systems that may be influenced due to disease states and/or reflect values unique to special patient populations and various drug delivery profiles.[26–39]

The purpose of this work was to implement a detailed description of small intestinal CYP 3A first pass metabolism into the generic PBPK software tool PK-Sim® (Bayer Technology Services, Leverkusen, Germany) to simulate the pharmacokinetics after intravenous and oral administration of the model compound nifedipine. In a subsequent step, CYP 3A enzyme inhibition caused by grapefruit juice ingestion and its impact on the intravenous and oral pharmacokinetics of the model compound was simulated.

Nifedipine (Fig. 1) was chosen as model compound as the drug's pharmacokinetic properties are well described in literature and as it is subject to intestinal and hepatic first pass metabolism via CYP 3A enzymes. The drug is a calcium channel-blocking agent used in the therapy of angina pectoris, Raynaud's phenomenon, and hypertension. It inhibits the transmembrane calcium influx into smooth muscle cells and hence leads to a prompt and marked hypotensive effect. Immediate release (IR) nifedipine formulations may show adverse effects, such as headache, flushing, and palpitation, caused by a rapid and profound vasodilatation. For this reason, together with the short half-life of the drug, modified release nifedipine