LB-001 A physiologically based pharmacokinetic (PBPK) modeling of amlodipine: High enterocyte binding, not enterohepatic circulation, is responsible for the long T_{max}

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Backgrounds

Amlodipine is a second generation calcium channel blocker that has been widely used in the therapy of hypertension and angina pectoris. As a BCS class I drug with high aqueous solubility and high passive permeability, amlodipine has an unusually long T_{max} of 4~9 hours after oral administration. Raušl et al. explained this long T_{max} with enterohepatic circulation [1]. This hypothesis has been adopted in a recently published PBPK model [2].

However, the possibility of enterohepatic circulation of amlodipine is low.

- There is no evidence of amlodipine biliary excretion in human.
- While delayed T_{max} (~ 4 h) of amlodipine was observed in rat after aqueous oral solution administration, studies in bile duct-cannulated rats (Ni et al. [3] and Walker et al. [4]) reported that no or less than 1% of the total dose was detected in the bile and that metabolism is the main clearance mechanism of amlodipine.

On the other hand, lysosomal trapping could be a more plausible explanation of the long T_{max} of amlodipine. It has been proposed that lysosomal trapping could cause delayed absorption after oral administration of dextromethorphan [5] and pulmonary administration of Olodaterol [6]. Given that amlodipine has the identified common properties of lysosomotropic agents, such as a LogP > 2 (LogP = 3.0) and a basic pKa between 6.5 and 11 (basic pKa = 9.1), it could be sequestered in the lysosomes during the absorption and resulting in delayed T_{max}. In addition, its lysosomal trapping potential has been confirmed by several *in vitro* cell based assays [7].

Thus, we proposed that the high lysosomal trapping in the enterocytes rather than enterohepatic circulation, is responsible for the long T_{max} of amlodipine.

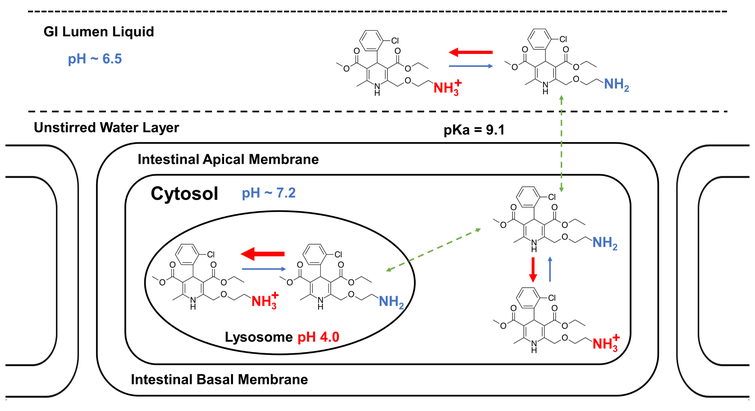

Figure 1: The mechanism by which amlodipine accumulates in the lysosomes.

METHODS

- All modeling and simulation was carried out using GastroPlus® version 9.5 (Simulations Plus, Inc., Lancaster, CA). Advanced Compartmental Absorption and Transit (ACAT™) model and PBPKPlus™ together with metabolism module were used to describe the amlodipine intestinal absorption, tissue distribution, gut and liver metabolism.
- Physicochemical and biopharmaceutical parameters of amlodipine were obtained either from literature or were predicted using ADMET Predictor™ 8.0 (Simulations Plus, Inc.).
- Human organ weights, volume, and blood perfusion rates were generated by the Population Estimates for Age-Related (PEAR™) physiology module in GastroPlus. The tissue distribution was modeled with perfusion-limited model in all tissues.
- The hypothesis that amlodipine would likely be influenced by lysosomal trapping was tested by conducting simulations of the *in vitro* Caco-2 permeability assay using MembanePlus™ (Simulations Plus, Inc.) with Kubinyi's model for passive membrane permeation. The simulated accumulation of amlodipine with lysosomal pH = 4.0 (physiological pH) was then compared to the simulated accumulation of amlodipine with lysosomal pH = 6.5.
- The tissue partition coefficients (Kps) of amlodipine were calculated using the default Lukacova method after adjusting the blood-to-plasma concentration ratio (R_{bp}) to 2.8 to account for lysosomal trapping in other tissues besides gut.
- The effect of lysosomal trapping in the enterocytes on amlodipine PK was simulated in the GastroPlus model by reducing the fraction of unbound drug in the enterocytes (Fu_{ent}) to 0.6%. The reduction of Fu_{ent} effectively reduces the rate of mass transfer from inside of the enterocytes across the basolateral membrane into the portal vein.

RESULTS AND DISCUSSIONS

A cellular simulation of the Caco-2 transwell permeability assay assuming the lysosomal pH = 4.0 can be seen in Figure 2A. At a lysosomal pH = 4.0, the simulated lysosome concentration is ~3 orders of magnitude higher than the cytoplasm concentration. As seen in Figure 2B, with the lysosomal pH = 6.5, the concentration in the lysosomal compartment is reduced to similar to that in the cytoplasm.

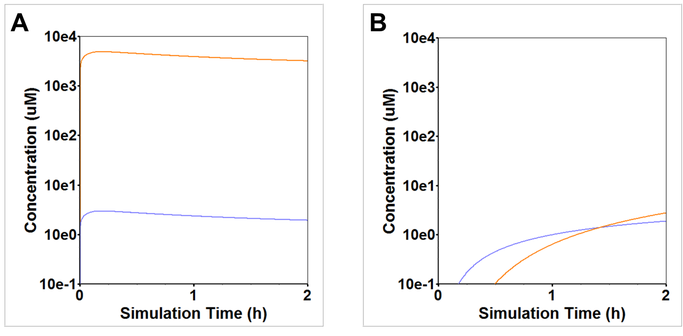

Figure 2: Simulated Caco-2 transwell permeability assay for amlodipine. (A) lysosomal pH = 4.0 and (B) lysosomal pH = 6.5. Concentration in lysosomes (orange lines), cytoplasm (purple lines).

As shown in Figure 3, the simulation with the adjusted R_{bp} of 2.8 accounting for lysosomal trapping matched with the observed plasma concentration time profile of amlodipine after intravenous administration better than the simulation with the experimental R_{bp} of 1.48.

The reason why we adjusted R_{bp} to scale Kps for taking into account of lysosomal trapping is: R_{bp} is commonly used as a substitute to account for the unknown interaction between drug molecules and acidic components of tissue cells when predicting Kps [8,9]. R_{bp} was changed back to the experimental value of 1.48 once Kps were scaled up since the experimental R_{bp} is good for the calculation of blood clearance.

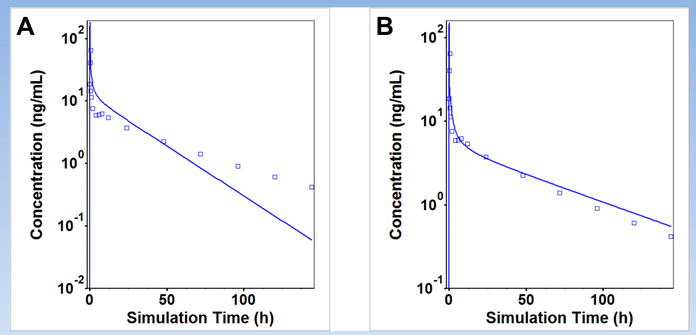

Figure 3: Observed (points) and simulated (lines) mean plasma concentration-time profiles of amlodipine after 10 mg intravenous infusion of amlodipine in 10 mins. (A) Kps predicted with experimental R_{bp} of 1.48 and (B) Kps predicted with fitted R_{bp} of 2.8. Experimental data were obtained from literature [10].

The developed PBPK model with high binding in enterocytes and increased Vd reflecting compound sequestration in tissues rich in lysosomes, but no biliary excretion, captured well amlodipine plasma profiles in Caucasian and Asian subjects after single and multiple oral administrations (Figure 4).

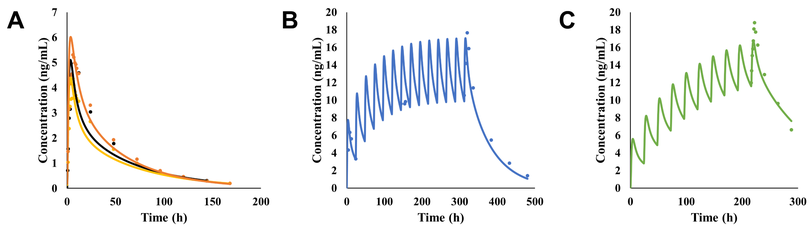

Figure 4: . Observed (points) and simulated (lines) mean plasma concentration-time profiles of amlodipine after 10 mg single dose PO administration of amlodipine in Caucasians (A), 15 mg QD PO administration in Caucasians (B), and 10 mg QD PO administration in Asians (C). Experimental data were obtained from literature [10-12].

CONCLUSIONS

- The long T_{max} of amlodipine, considering its physicochemical properties, is more likely to be caused by its high lysosomal trapping in enterocytes rather than enterohepatic circulation.
- Ignoring lysosomal trapping when predicting Kps causes underprediction of volume of distribution for lysosomotropic agents.

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