ABSTRACT SUMMARY:
We describe the development of a mechanistic mathematical model for the analysis of in vitro permeability assays that accounts for all major mechanisms contributing to observed apparent permeability. We used the model to study the effect of CYP3A4 metabolism of midazolam in Caco-2 monolayers. The effect of adding human serum albumin to the receiver compartment on the apparent permeability of midazolam was also predicted and compared with in vitro measurements.

INTRODUCTION:
In vitro permeability assays utilizing cultured cell monolayers (e.g. Caco2, MDCK) are routinely used to estimate a drug’s potential for absorption after oral administration; however, the simple models used to analyze measured in vitro profiles frequently do not account for various processes affecting the drug in a given experiment (e.g., metabolism, paracellular permeability, carrier-mediated transport, and accumulation in membrane or lysosomes), nor do they account for various experimental setup variables (e.g., shaking rate, solvent pH, filter support, sampling effects, and addition of protein to the media).

We describe the model including the above-mentioned mechanisms that we incorporated in MembranePlus™ (SimulationsPlus, Inc.), as well as its use in the simulation of midazolam in vitro concentration-time profiles after apical and basolateral administration.

Midazolam, a benzodiazepine, shows significant binding to serum proteins, unbound fraction in plasma around 3-4% [1,4]. It is metabolized mainly by CYP3A4, with 1'-hydroxymidazolam as 75% of the total metabolites. We validated the model with reported concentration-time profiles from in vitro experiments in Caco2 monolayers. We also explored the effects of some of the experimental conditions such as shaking rate, solvent pH, filter support, sampling effects and protein binding.

EXPERIMENTAL METHODS:
The transport model included in MembranePlus depends on the molecule’s physicochemical properties such as pKa, log P, water diffusivity, solubility, and metabolic properties. Physicochemical properties of midazolam used in the simulations were either obtained from literature (e.g. measured log P value of 2.7 from [3] and CYP3A4 metabolism from [5]) or from in silico predictions (e.g. predicted pKa values and water diffusivity from ADMET Predictor™ 6.0 (Simulations Plus, Inc.). Experimental parameters such as shaking rate, volume and pH of donor and receiver compartments, and cell growth area were used as described in the literature [4]. Paracellular diffusion was estimated from drug properties and experimental setup. CYP3A4 metabolism was modeled using Michaelis-Menten kinetics with the measured Km= 3.7 µM from microsomal data [5]. Four parameters accounting for passive transcellular diffusion and membrane accumulation, along with the Vmax for CYP3A4, were fitted to observed concentration-time profiles in the apical, basolateral and cellular compartments after both apical and basolateral administrations of 3 µM midazolam. The fitted model was then used to predict the concentration profiles after adding human serum albumin to the basolateral compartment. We employed linear binding kinetics with a fixed unbound fraction of 3.3%, as reported in [4].

RESULTS AND DISCUSSION:
Fitted and observed concentration-time profiles after apical and basolateral administrations of 3 µM of Midazolam during a 2-hour incubation are shown in Figure 1. The overall R² for A->B (Figure 1A) and B->A directions (Figure 1B) is 99%. The same transport model resulted in reasonably good prediction of concentration-time profiles after addition of human serum albumin (4 g/dL) in the basolateral compartment. Predicted concentrations in apical and basolateral compartments, as well as in cells, are shown together with experimental data in Figure 2.

Concentration-time profiles were well-predicted. The model can be also used to obtain pure passive transcellular permeability of the compound rather than an apparent permeability which is heavily affected by experimental conditions (e.g., chamber pH, shaking rate, and protein binding). Estimated passive transcellular in vitro permeability for midazolam was 2×10⁻⁵ cm/s.

CONCLUSION:
The utility of a new model that simulates in vitro permeability experiments for analyzing measured in vitro profiles as well as in predicting the effect of changing experimental conditions has been
demonstrated. It is a promising tool for *in vitro-in vivo* extrapolation and predictions of absorption and metabolism. It can also be used to explore effects such as transporters, cytotoxicity, lysosomal trapping and other relevant cellular processes; however, these were not explored in the work reported here.

**Figure 1.** Simulated (lines) and *in vitro* (squares) concentration-time profiles after apical (A) and basolateral (B) administration of 3 µM midazolam in Caco-2 cells. Predicted cellular (light blue) and cytosol (yellow) concentrations are also shown.

**Figure 2.** Predicted (lines) and *in vitro* (squares) concentration-time profiles after apical administration of 3 µM midazolam under protein binding effects.

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**REFERENCES:**