

Research paper

# Absorption of poorly water soluble drugs subject to apical efflux using phospholipids as solubilizers in the Caco-2 cell model

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## Abstract

The purpose of this work was to determine the influence of liposomal solubilization of poorly water soluble drugs exhibiting apical efflux on permeation kinetics and cell toxicity in Caco-2 cells. The HIV-protease inhibitors indinavir and saquinavir were incorporated in phosphatidylcholine liposomes at maximal drug-to-lipid mass ratios and their absorption was determined in Caco-2 cell cultures grown on Transwell inserts using purely aqueous drug solutions as reference. A novel mathematical model was developed to quantitatively delineate the contribution of passive membrane permeation and carrier mediated efflux to transport across the cell monolayer and passive permeability coefficient and maximal efflux rate and affinity constant of the transporter system were determined. Cell toxicity of phospholipids was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and the lactate dehydrogenase (LDH) assay. Cell integrity was not significantly affected by phospholipid concentrations of up to 150 mg/ml with respect to the used standard tests. Maximum drug concentration was increased 10- and 750-fold for indinavir and saquinavir, respectively, by the use of liposomes. The passive membrane permeability coefficient differed between the two drugs in accordance with their lipophilicity and the affinity for apical efflux transporters was on average 4-fold greater for saquinavir than for indinavir. Liposomal solubilization diminished the passive permeability coefficient of both drugs but the passive apical-to-basal delivery rate was increased by the liposomes compared to the purely aqueous solutions at maximal donor concentrations for at least one of the two drugs. Efflux rate reached a maximum for the liposomal formulations reflecting transporter saturation. Hence, liposomal solubilization considerably increased drug concentration in the media and altered absorption behavior by affecting both the passive diffusion and the carrier mediated efflux components of cell monolayer permeation.  
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## 1. Introduction

As guide for formulation development work aimed at optimizing bioavailability, drugs are classified by the biopharmaceutic classification system (BCS), which considers four classes of drugs having either a low or high solubility in water and either a high or low permeability for the biological membrane [1,2]. The intestinal permeation kinetics

of candidate drugs is routinely measured using the Caco-2 cell model and the PAMPA method [3]. The characteristics of the Caco-2 cell monolayer model have been well described [4–6]. The Caco-2 cell monolayer can be easily used for testing water soluble drugs. The necessary concentration gradient can be generated by simply using high concentrations of the drug in the donor compartment. However, poorly water soluble compounds are much harder to test in the Caco-2 cell model because of the insufficiently steep concentration gradient over the cell monolayer requiring very sensitive analytical tools such as radio-labelling for monitoring drug permeation. For this reason, addition of solubilizing agents is necessary to allow

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