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Research paper

In silico modelling of permeation enhancement potency in Caco-2 monolayers based on molecular descriptors and random forest

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ABSTRACT

Structural traits of permeation enhancers are important determinants of their capacity to promote enhanced drug absorption. Therefore, in order to obtain a better understanding of structure–activity relationships for permeation enhancers, a Quantitative Structural Activity Relationship (QSAR) model has been developed.

The random forest-QSAR model was based upon Caco-2 data for 41 surfactant-like permeation enhancers from Whitehead et al. (2008) and molecular descriptors calculated from their structure.

The QSAR model was validated by two test-sets: (i) an eleven compound experimental set with Caco-2 data and (ii) nine compounds with Caco-2 data from literature. Feature contributions, a recent developed diagnostic tool, was applied to elucidate the contribution of individual molecular descriptors to the predicted potency. Feature contributions provided easy interpretable suggestions of important structural properties for potent permeation enhancers such as segregation of hydrophilic and lipophilic domains. Focusing on surfactant-like properties, it is possible to model the potency of the complex pharmaceutical excipients, permeation enhancers. For the first time, a QSAR model has been developed for permeation enhancement. The model is a valuable *in silico* approach for both screening of new permeation enhancers and physicochemical optimisation of surfactant enhancer systems.

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1. Introduction

Development of oral delivery systems for proteins and peptides offers the promise of improved patient compliance compared to conventional parenteral administration. However, bioavailability is, in part, limited due to poor absorption of proteins across the intestinal epithelial barrier. To effectively deliver a protein systemically this barrier can be modulated by the presence of permeation enhancers [1].

Quantitative Structural Activity Relationship, QSAR methods have been applied extensively for exploration of structural properties of importance for oral absorption of new chemical entities, e.g., QSAR models have been developed for permeability [2] and solubility [3–5]. To our knowledge, no QSAR model for permeation enhancement has previously been published.

Some permeation enhancers have specific mechanisms of action, e.g., modulating the function of tight junctions in the plasma membrane such as zona-occludens-toxin [6], EDTA [7] or melittin [8]. However, the majority of permeation enhancers are primarily surfactants and will non-specifically disrupt the lipid bilayer packing of phospholipids in the epithelial membrane [1]. Surfactants are molecules having segregated lipophilic and hydrophilic domains. Water soluble surfactants tend to pool in the surfaces of water/air and water/lipid, lowering the surface tension. Lowering of surface tensions of water/air surfaces and the ability to enhance the permeability across lipid bilayers correlated

Abbreviations: C6, sodium hexanoate; C8, sodium octanoate; c8G, octylglucoside; C10, sodium decanoate/caprate; c12PC, dodecylphosphocholine; c12GPC, dodecanoylglycerophosphocholine; c14GP, myristoylglycerophosphate; CART, classification and regression tree; CDC, chenodeoxycholate; DDM, dodecylmaltoside; EDTA, ethylenediaminetetraacetic acid; GCC, glycochenocholate; GH, glycyrrhizinate; LCC, lauroylcarnitinechloride; LOO-CV, leave-one-out cross validation; MOE, molecular operating environment; PCC, palmitoyl carnitine chloride; QSAR, quantitative structural activity relationship; SM, simomenine; RMSE, root mean square error; r_p, Pearsons correlation coefficient; r_s, Spearman rank correlation coefficient; SD, standard deviation; TEER, transepithelial electrical resistance; TDM, tetradecylmaltoside; TDS, sodium tetradecyl sulphate; TDM, tetradodecyl maltoside; TC, taurocholate; T_{pot}, TEER potency; UC, Ursocholate.

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well for a selection of surfactant-like permeation enhancers [9]. General relations between molecular structures and physicochemical properties of surfactants are thoroughly described by Rosen [10]. Several properties of surfactants, including surface pressure, have previously been modelled with a QSAR approach applying both linear regression and non-linear machine learning models as artificial neural networks, support vector machine or random forest [5,11,12]. Combining the above mentioned concepts, it seems plausible that a QSAR-model of surfactant-like permeation enhancement could be constructed.

Our modelling is based on a Caco-2 data set for 41 surfactant permeation enhancers from Whitehead [13,14] tested in cell monolayers across three concentrations. Hereby, trade-offs between potency, pathway and safety amongst a selection of mainly surfactant-like permeation enhancers were investigated. For this article only the potency data was used. *In vitro* Caco-2 monolayers are cultures of functional, differentiated enterocytes and are widely employed to evaluate permeability rates of drug candidates or pre-formulations [15]. The Caco-2 data for permeation enhancers from Whitehead [13,14] together with molecular descriptors calculated from structure of these surfactants were the basis for the QSAR model.

Non-linear machine learning models can have superior predictive capabilities compared to classical statistical explanatory modelling. However, such machine learning models are often complex "black boxes" – difficult to interpret and discuss [16]. This article presents a promising method to elucidate the interplay of features comprising good permeation enhancers within the complex non-linear model of random forest. Therefore, based on the developed model, we here can recommend ranges of the selected molecular descriptors to obtain high permeation enhancement potency.

2. Materials and methods

2.1. Materials

Caco-2 cells (ATTC-HTB-37) were obtained from American Type Culture Collection (Manassas, VA). Cell culture media (Dulbecco's modified essential media (DMEM)) and penicillin/streptomycin were purchased from Lonza (Verviers, Belgium). All other supplements (i.e., foetal bovine serum, HEPES buffer and non-essential amino acids (NEAA)) as well as Hanks' balanced salt solution (HBSS) and trypsin were purchased from Gibco, Life Technologies (Carlsbad, CA). Corning Transwell® filter inserts (1.12 cm^2 surface area, 0.4 μm pore diameter) were purchased from Fisher Scientific (Waltham, MA). Bovine serum albumin (BSA) was purchased from Sigma Aldrich (St. Louis, MO). All other reagents were of the highest analytical grade.

2.2. Cell culture and TEER measurements

Caco-2 cells (passage numbers 41–49) were seeded at a density of 2.5×10^5 cells/flask and grown to 70–90% confluence in DMEM (supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin and 1% (v/v) NEAA). For transport studies, Caco-2 monolayers were cultured on permeable Transwell® 12 mm diameter inserts at a density of 10^5 cells/cm^2 and used after 14–17 days in culture. Cells were cultured at 37 °C and 5% CO_2 atmosphere and the medium was changed every other day. Monolayers were equilibrated in HBSS-based transport buffer 1 h prior to testing. Transepithelial electrical resistance (TEER) was measured with a chop-stick electrode (Millicell-ERS®, Millipore, Billerica, MA) prior to testing, and monolayers with TEER values <600 Ω cm^2 were discarded. TEER was measured after 1 h exposure to permeation enhancers.

3. Data processing

3.1. Training set

Whitehead et al., tested the ability of 51 permeation enhancers to lower the barrier integrity marker %TEER in Caco-2 cells at 1%, 0.1% and 0.01% (w/v) and published the data set as supplementary materials in two papers [13,14]. Of the 51 permeation enhancers reported, forty-two had computable molecular structures (non-mixtures) and were a wide selection of enhancers which were ascribed to 10 different categories of surfactants: Anionic surfactants, cationic surfactants, zwitterionic surfactants, non-ionic surfactants, bile salts, fatty acids, fatty esters, fatty amines, sodium salts of fatty acids, nitrogen-containing rings and others [13]. EDTA (a calcium chelator) was excluded from the training set because of a non-surfactant-like mechanism together with high potency. The remaining 41 permeation enhancers had surfactant-like structures or low potency e.g., urea could be described as an ineffective surfactant without permeation enhancement effect.

TEER-potency (T_{pot}) was defined to concatenate measurements of TEER%-decrease (EP) at the three different concentrations (0.01%, 0.1% and 1% w/v) into one target variable. T_{pot} was simply defined as the mean TEER%-decrease across the three concentrations as given in Eq. (1). $T_{pot} = 1$ corresponds to a permeation enhancer lowering TEER% completely at 0.01% (w/v) and $T_{pot} = 0$ translates to no effect of a permeation enhancer on TEER% even at 1% (w/v). The TEER%-decrease EP is defined as in Eq. (2) and depends of the TEER% before and after treatment with enhancer plus $TEER\%_+$ the background filter resistance.

$$T_{pot} = \frac{EP[0.01\%] + EP[0.1\%] + EP[1\%]}{3} \quad (1)$$

$$EP = 1 - \frac{TEER\%_{AE} - TEER\%_+}{TEER\%_{noAE} - TEER\%_+} \quad (2)$$

From a statistical point of view the loss of information is minimal, as the TEER%-values of the three concentrations were highly correlated. The loadings of the first principal component of a principal component analysis resembled the definition of T_{pot} and this principal component explained 71% of the variance. From a practical viewpoint T_{pot} could be seen as a linear approximation of pEC50 (−log effective concentration (w/v) of where 50% TEER-decrease is observed), see Eq. (3). pEC50 itself is dimensionless.

Thus, for a given permeation enhancer having a potency of pEC50 = 1 the corresponding value of $T_{pot} = 0.5$.

$$T_{pot} = \frac{pEC50 + 0.5}{3}, \quad \text{for } pEC50 \in [-0.5; 2.5] \quad (3)$$

3.2. Software packages, descriptors and model design

The open source R statistical software (v 3.02) was acquired freely from http://www.r-project.org and Rstudio integrated development environment (v 0.98.501) also acquired freely from http://www.rstudio.com. The R-package 'randomForest' (v.4.6) [17,18] was used in the random forest-QSAR model. CAS identification numbers of compounds in the training set were converted to mol-files through SciFinder [19]. Mol-files bundled in sdf-files were imported to the software application MOE [20] and sequentially pre-processed with the following functions: '*wash*' (simulating an ideal solubilised molecular form), '*partial charges MMFFA96x*' calculating the electron densities necessary for a number of descriptor algorithms, and finally '*energy minimize*' relaxing the molecule in the minimum state. All 2D molecular descriptors provided by MOE were computed. The subgroup of 3D descriptors '*vsurf*' [21] plus the single 3D descriptor '*dipole*' were calculated as they were relatively fast to compute and therefore suitable for

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