European Journal of Pharmaceutical Sciences 88 (2016) 147–157

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Quantitative structure–retention relationships of flavonoids unraveled by immobilized artificial membrane chromatography

Adriana Leandra Santoro [a], Emanuel Carrilho [a,b], Fernando Mauro Lanças [a], Carlos Alberto Montanari [a,*]

[a] Instituto de Química de São Carlos, Universidade de São Paulo, 13566-590 São Carlos, SP, Brazil
[b] Instituto Nacional de Ciência e Tecnologia de Bioanalítica, 13083-970, Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 9 November 2015
Accepted 6 December 2015
Available online 23 February 2016

Keywords:
Flavonoids
Immobilized artificial membrane chromatography
Partition coefficient logarithm
Human colon epithelial cancer cell line
Partial least squares
Quantitative structure–property relationships

ABSTRACT

The pharmacokinetic properties of flavonoids with differing degrees of lipophilicity were investigated using immobilized artificial membranes (IAMs) as the stationary phase in high performance liquid chromatography (HPLC). For each flavonoid compound, we investigated whether the type of column used affected the correlation between the retention factors and the calculated octanol/water partition (log P_{oct}). Three-dimensional (3D) molecular descriptors were calculated from the molecular structure of each compound using i) VolSurf software, ii) the GRID method (computational procedure for determining energetically favorable binding sites in molecules of known structure using a probe for calculating the 3D molecular interaction fields, between the probe and the molecule), and iii) the relationship between partition and molecular structure, analyzed in terms of physicochemical descriptors. The VolSurf built-in Caco-2 model was used to estimate compound permeability. The extent to which the datasets obtained from different columns differ both from each other and from both the calculated log P_{oct} and the predicted permeability in Caco-2 cells was examined by principal component analysis (PCA). The immobilized membrane partition coefficients (k_{IAM}) were analyzed using molecular descriptors in partial least square regression (PLS) and a quantitative structure–retention relationship was generated for the chromatographic retention in the cholesterol column. The cholesterol column provided the best correlation with the permeability predicted by the Caco-2 cell model and a good fit model with great prediction power was obtained for its retention data ($R^2 = 0.96$ and $Q^2 = 0.85$ with four latent variables).

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The practiced paradigm of drug discovery, based only on the optimization of bioactive compounds in order to gain potency and selectivity (Hughes et al., 2011; Kwong, 2015), relegates drug development to a high failure rate (Kola and Landis, 2004). This is so because physicochemical properties, PK/PD and toxicity among other compound properties do not enter early in the drug discovery process (Prentis et al., 1988). However, due to increased efforts to add new tools into the drug design pipeline endeavor as early as possible (Cucurull-Sanchez et al., 2012), failure has dramatically changed in recent years (Arrowsmith and Miller, 2013; Kola and Landis, 2004; Waring et al., 2015). Partitioning in biological membranes is the chief step governing the passive transport of drugs through biological barriers and, as a result, the occurrence of several pharmacokinetic phenomena (*e.g.*, intestinal absorption and blood–brain barrier passage), takes place (Kaliszan et al., 1994; Sugano et al., 2010). It is usually related to lipophilicity and expressed as the logarithm of the octanol/water partition coefficient, log P_{oct} (Giaginis and Tsantili-Kakoulidou, 2008). A possible way to gauge ADME/Tox-related physicochemical properties is to rely on *in vitro* assays (Talevi et al., 2011). Caco-2 cells are a human colon epithelial cancer cell line used as a model of human intestinal absorption of drugs and other compounds (Artursson and Karlsson, 1991). Caco-2 cells are currently the favorite *in vitro* tool for prediction of the potential oral absorption of new drugs (Larregieu and Benet, 2013; Paixao et al., 2010). Yet, the validation of integrity and proper functionality of the Caco-2 cell monolayer is often tedious and never straightforward (Chan et al., 2005). For a recent 7-days analysis, see Cai et al. (2014).

A suitable alternative to model oral drug absorption has emerged, which measures drug retention on phospholipid stationary phases, the so-called immobilized artificial membrane (IAM), by high performance liquid chromatography (HPLC) (Abraham et al., 2013; Giaginis and Tsantili-Kakoulidou, 2013). IAMs are used to obtain lipophilic drug candidate properties (Van de Waterbeemd et al., 1996), since they can better mimic the interactions with membrane systems like buffer/octanol (Giaginis and Tsantili-Kakoulidou, 2008; Van de Waterbeemd et al.,

* Corresponding author.
E-mail address: Carlos.Montanari@usp.br (C.A. Montanari).

http://dx.doi.org/10.1016/j.ejps.2015.12.009
0928-0987/© 2015 Elsevier B.V. All rights reserved.

2001). IAM-HPLC is a simple, reproducible and fast method, which combines the robustness of HPLC with a partitioning model of phospholipids. For neutral test compounds, a good correlation generally is found between log P_{oct} and the chromatographic behavior in the IAM column, although the latter lies on a lower lipophilicity scale, owing to the reduced hydrophobic environment of the IAM stationary phase (Vrakas et al., 2005). In addition to the hydrophobic/solvophobic interactions, polar interactions, mainly involving hydrogen bond acceptor basicity, are the predominant factors in IAM retention (Abraham et al., 2013; Sprunger et al., 2007).

Beyond phospholipids, cholesterols also have been used in chromatographic systems (Bocian et al., 2012; Ogden and Coym, 2011; Soukup and Jandera, 2013). The cholesterol phase, which has properties of a liquid crystal, shows distinct selectivity and retention behavior due to its apparent ability to recognize specific structures in molecules, discriminate among derivatives, or subsequently recognize modifications of the structures (Lewis et al., 2015; Martinez-Mayorga et al., 2015). The cholesterol phase can also function as a chiral separation material for compounds with a variety of chemical structures. Hence, pharmaceutical analysis using cholesterol columns offers interesting possibilities for future applications.

We have previously reported a predictive model for potency against the *Trypanosoma cruzi* enzyme glyceraldehyde-3-phosphate dehydrogenase (*Tc*GAPDH). A set of flavonoids was studied for their *in silico* properties (Freitas et al., 2009b), and a principal component analysis (PCA) score plot roughly clustered them in the top-left corner of the plot. Considering, nonetheless, that such results did not display the partitioning of *Tc*GAPDH flavonoid inhibitors, we wanted to unveil the influence of lipophilicity on their partitioning behavior. Flavonoids are a large group of phenolic compounds that occur ubiquitously in food of plant origin and are treated as dietary supplements and functional ingredients of beverages, food grains, herbal remedies, and dairy products. The use of flavonoids has been the subject of extensive research (Hollman, 2004), including investigations of their permeability using Caco-2 cell monolayers. Caco-2 cell monolayers have been employed to investigate (i) the structure–permeability relationship of dietary/nutraceutical flavonoids and (ii) the transepithelial transport and cellular uptake of some flavonoids (Tian et al., 2009; Wang et al., 2011). An alternative to the Caco-2 cell model would be desirable in order for a high-throughput drug absorption screening method to become available for early candidate profiling in drug discovery. An encouraging possibility is to model Caco-2 permeability of drugs using immobilized artificial membrane (IAM) chromatography and physicochemical descriptors (Chan et al., 2005).

In this study, the elution behavior of a set of flavonoids was determined with two IAM columns of differing degrees of lipophilicity and with a cholesteryl ester column. The predictive relationship between the IAM partition coefficient values and the calculated log P_{oct} was investigated. The 3D physicochemical descriptors were calculated with VolSurf software for each compound based on their 3D molecular structures and then projected on the predictive model of permeability in Caco-2 cells, available in the VolSurf software, as described by Cruciani et al. (2000). The extent to which the data recorded on different columns differed amongst them, how they compared with the calculated log P_{oct} and the permeability predicted in Caco-2 cells were examined by PCA. The results from the three chromatographic systems were analyzed in relation to the molecular descriptors (Ghasemi and Saaidpour, 2007; Montanari et al., 2005) using partial least square regression (PLS) and a quantitative structure property relationship (QSPR) was obtained for the chromatographic retention on the cholesteryl ester column (Héberger, 2007; Montanari et al., 2000a). Herein, we show that IAM chromatographic retentions can be used to assess drug permeability and describe a robust QSPR model that should be of interest to further our knowledge on the phospholipophilicity of flavonoids. Moreover, it is also shown that retention on a cholesteryl ester column can be used for predicting the Caco-2 cell permeability of flavonoids.

2. Materials and methods

2.1. Materials

All chemicals obtained from commercial sources were of HPLC grade, and used without further purification. Acetonitrile and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared with a Milli-Q water purification system from Millipore (Milford, MA). Flavonoids where generously donated by the Laboratory of Natural Products of the Federal University of Sao Carlos — UFSCar, São Carlos/SP, Brazil (see Fig. 1).

2.2. Instrumentation

A liquid chromatographic apparatus (LC-10AD, Shimadzu, Japan) equipped with a Rheodyne 7725 injection valve (with a 20 μL loop) and SPD-10AV UV detector (Shimadzu), set at a wavelength of 330 nm, was used to carry out the separation of the analytes. Separations were carried out in stainless steel immobilized artificial membrane phosphatidylcholine drug discovery columns designated IAM.PC.DD2 and IAM.PC.DD. The first column (IAM.PC.DD2, dimensions = 100 mm × 4.6 mm I. D., 12 μm, 300 Å; Regis Technologies, Morton Grove, IL, USA) is used for the prediction of drug-membrane permeability and it is hydrophobic in nature, which gives longer retention times to compounds not well retained on the IAM.PC.DD column. This second column (IAM.PC.DD, dimensions = 100 mm × 4.6 mm I. D., 5 μm, 300 Å; Regis Technologies) lacks the glycerol moiety of natural phospholipids (Barbato et al., 2004). The structures of the IAM.PC.DD2 and IAM.PC.DD chromatography stationary phases are described elsewhere (Ong et al., 1995), but their 2D representations can be found in Appendix A, Fig. A.1 of the Supplementary material. The cholesteryl-10-undecenoate bonded phase column (Pesek et al., 1995; Pesek et al., 1998) whose archetypal synthesis strategy can be found in (Courtois et al., 2008) has the following dimensions: 100 mm × 4.6 mm I. D., 4 μm, 100 Å (Kromasil CHOL-HIC-H/T). See Appendix A, Fig. A.2 in the Supplementary material for its 2D representation. Distinctive from the other two, the cholesteryl phase characteristically presents three structural requirements that are fundamental to the molecular recognition processes: the hydrocarbon chain that ensures its mobility, the carbonyl group that can interact with various substituents and the cholesteric moiety that may involve π–π interactions as well as steric effects (Catabay et al., 1999).

The eluents were mixtures of 100 mM triethanolamine buffer at pH 7.4 (adjusted with hydrochloric acid) and various percentages of methanol, at a flow rate of 0.5 or 1.0 mL min^{-1}. EDTA (1 mM) and 2-mercaptoethanol (1 mM) were added to the buffer in order to reproduce as closely as possible the conditions used in the determination of the activity of these compounds against the enzyme *Tc*GAPDH (Freitas et al., 2009a; Malhotra et al., 2009). The aqueous solution of the mobile phase was filtered through a nylon membrane (pore size 0.20 μm, diameter 47 mm, MF-Millipore, HAWP04700 — USA) and degassed prior to use. All samples were filtered through a hydrophobic membrane (0.50 μm syringe filter, Advantec, California, USA).

The chromatographic experiments were carried out at room temperature. The compounds were dissolved in methanol at concentrations of 20 μg mL^{-1}. The volume of sample injected into the column was 20 μL. Chromatographic retention data are expressed as the logarithm base 10 of the retention factor, log k, defined as $\log k = \log [(t_r - t_0) / t_0]$, where t_r and t_0 are the retention times of the analyte and of a non-retained compound (citric acid for IAM columns, and uracil for cholesteryl ester columns), respectively. Separations were performed in isocratic mode with aqueous mobile phases containing methanol in percentages (ϕ) ranging from 20 to 80% (v/v). The values referring to 100% aqueous eluent are indicated as log k_w and were found by extrapolation. All reported values of log k are the average of at least four measurements. Partition coefficients in n-octanol, log P_{oct}, were calculated

Download English Version:

https://daneshyari.com/en/article/5809669

Download Persian Version:

https://daneshyari.com/article/5809669

Daneshyari.com