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Prediction of blood–brain barrier permeation of α-adrenergic and imidazoline receptor ligands using PAMPA technique and quantitative-structure permeability relationship analysis

Jelica Vucicevic *, Katarina Nikolic, Vladimir Dobričić, Danica Agbaba

Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, PO Box 146, 11000 Belgrade, Serbia

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ABSTRACT

Imidazoline receptor ligands are a numerous family of biologically active compounds known to produce central hypotensive effect by interaction with both α_2-adrenoreceptors (α_2-AR) and imidazoline receptors (IRs). Recent hypotheses connect those ligands with several neurological disorders. Therefore some IRs ligands are examined as novel centrally acting antihypertensives and drug candidates for treatment of various neurological diseases. Effective Blood–Brain Barrier (BBB) permeability (P_e) of 18 IRs/α-ARs ligands and 22 Central Nervous System (CNS) drugs was experimentally determined using Parallel Artificial Membrane Permeability Assay (PAMPA) and studied by the Quantitative-Structure-Permeability Relationship (QSPR) methodology. The dominant molecules/cations species of compounds have been calculated at pH = 7.4. The analyzed ligands were optimized using Density Functional Theory (B3LYP/6-31G(d,p)) included in ChemBio3D Ultra 13.0 program and molecule descriptors for optimized compounds were calculated using ChemBio3D Ultra 13.0, Dragon 6.0 and ADMET predictor 6.5 software. Effective permeability of compounds was used as dependent variable (*Y*), while calculated molecular parametres were used as independent variables (*X*) in the QSPR study. SIMCA P+ 12.0 was used for Partial Least Square (PLS) analysis, while the stepwise Multiple Linear Regression (MLR) and Artificial Neural Networks (ANN) modeling were performed using STASTICA Neural Networks 4.0. Predictive potential of the formed models was confirmed by Leave-One-Out Cross- and external-validation and the most reliable models were selected. The descriptors that are important for model building are identified as well as their influence on BBB permeability. Results of the QSPR studies could be used as time and cost efficient screening tools for evaluation of BBB permeation of novel α-adrenergic/imidazoline receptor ligands, as promising drug candidates for treatment of hypertension or neurological diseases.

1. Introduction

Predicting the Absorption, Distribution, Metabolism and Excretion (ADME) processes is a critical step during development of new compounds. Inappropriate pharmacokinetic properties are one of the main reasons for the failure of drug candidates in the preclinical and clinical trials.

Blood–Brain Barrier (BBB) plays an essential role in protecting Central Nervous System (CNS) from harmful agents present in the bloodstream. It is made of brain endothelium, astrocytes and neurons (Abbott et al., 2006). The brain capillary endothelial cells act as a physical barrier for the most compounds due to high-resistance tight junctions between them. Compounds can enter CNS by passive diffusion, or they can be carry by various transport system (Clark, 2003).

The pharmacological activity of CNS drugs does not depend only on receptor affinity but their ability to cross the BBB. On the other hand, blood brain penetration of peripherally acting drugs has to be minimal to avoid undesirable CNS side effects (Ecker and Noe, 2004).

Even though direct measurement of brain permeability is the most reliable method, it represents costly and labor intensive process. Moreover, a large number of factors, such as metabolism of compounds and their binding to tissues or proteins, affect the brain distribution, so this method does not necessarily give a clear picture of BBB permeability. As a consequence in order to estimate the central nervous delivery of compounds a lot of useful *in vitro* models have been developed (Abbott et al., 2006; Abbott, 2004; Gumbleton and Audus, 2001).

* Corresponding author. Tel.: +381 11 3951 259; fax: +381 11 3974 349.
E-mail address: jelicav@pharmacy.bg.ac.rs (J. Vucicevic).

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Parallel Artificial Membrane Permeability Assay (PAMPA) (Avdeef, 2005), Immobilized Artificial Membrane (IAM) chromatography (Reichel and Begley, 1998; Yoon et al., 2006) and cell based assays (Caco-2, Madin-Darby Canine Kidney (MDCK) and primary Bovine Brain Microvessel Endothelial Cells (BBMEC)) are the most used methods in pharmaceutical industry for predicting brain penetration of new compounds in early drug discovery phase (Terasaki et al., 2003; Garberg et al., 2005). Compared to primary cell lines PAMPA is time efficient and low cost method applicable for high-throughput in drug discovery process. These *in vitro* measures are usually necessary due to laborious, expensive and low-throughput *in vivo* techniques (Hitchcock and Pennington, 2006).

The PAMPA involves non-biological artificial membranes for the estimation passive transcellular permeability of small molecules (Kansy et al., 1998). It is used to mimic BBB and to predict passive permeability of compounds. One of its main disadvantages is inability to predict efflux by P-glycoprotein (Pgp), because the membrane is made of polar brain lipids in dodecane (Di et al., 2003).

The compounds used in this study were imidazoline receptor (IRs) ligands. Because the I-IR ligands exhibit additional CNS effects, we have decided to include several structurally related CNS drugs in this study. Using ADMET predictor 6.5 (Simulation Plus, Inc., 2013) software it was shown that those compounds are not substrates for efflux transporters, such as Pgp, so BBB-permeability could be accurately predicted by PAMPA method.

Imidazolines are a numerous family of biologically active compounds known from comprehensive therapeutic application. Recent hypothesis connect activity of those ligands with three types of imidazoline receptors (I_1-IR, I_2-IR, and I_3-IR) (Eglen et al., 1988; Head and Mayorov, 2006) and α_2-adrenoreceptors (α_2-AR) (Bousquet et al., 1984; Tibirica et al., 1988). The central hypotensive effect of imidazoline derivatives such as clonidine, rilmenidine and moxonidine is results of activation both I_1-IR and α_2-AR (Ernsberger et al., 1990; Bousquet et al., 1992; Head and Mayorov, 2006; Chan et al., 2007). I_2-IRs have been shown to be modulatory site for monoamine oxidase-B (MAO-B) (Tesson et al., 1995), enzyme responsible for several neurological disorders (Kimura et al., 2009). This type of imidazoline ligand receptors have high affinity for idazoxan and its analogues. I_3-IRs are involved in induction of insulin secretion from β-cells and maintaining glucose homeostasis (Eglen et al., 1988; Morgan and Chan, 2001). Therefore some IRs ligands are examined as novel centrally acting antihypertensives and drug candidates for treatment of various neurological diseases.

The main aims of this study were to evaluate BBB permeability of 40 compounds (18 IRs/α-ARs ligands and 22 CNS drugs) using PAMPA method based on porcine brain lipid extract and than to develop Quantitative Structure-Permeability Relationship (QSPR) models useful for prediction BBB permeability of related IRs/α-ARs ligands.

2. Materials and methods

2.1. Chemical and reagens

All used reagents were of analytical grade of purity. Methanol, Sigma–Aldrich (St. Louis, MO, USA), acetonitrile, Sigma–Aldrich (St. Louis, MO, USA), water (HPLC grade), triethylamine, Merck (Darmstadt, Germany), ortho-phosphoric acid 85%, Merck (Darmstadt, Germany), acetic acid ⩾ 99.8%, Sigma–Aldrich (St. Louis, MO, USA) and ammonia solution 25%, Carlo Erba (Milano, Italy) were used for the preparation of the mobile phase.

The following standards were used: clonidine hydrochloride, moxonidine hydrochloride, guanfacine hydrochloride, efaroxan hydrochloride, idazoxan hydrochloride, rilmenidine hemifumarate, harmane, harmine, tizanidine hydrochloride, naphazoline hydrochloride, xylometazoline hydrochloride, tetrahydrozoline hydrochloride, oxymetazoline hydrochloride, maprotiline hydrochloride, citalopram hydrobromide, guanabenz, mianserin hydrochloride, venlafaxine hydrochloride, prazepam, levomepromazine hydrochloride, ziprasidone hydrochloride, fluoxetine hydrochloride, lorazepam, oxazepam, sertraline hydrochloride, agmatine sulfate, lamotrigine, levetiracetam, brimonidine tartrate, bromazepam, topiramate, carbamazepine, risperidone, clonazepam, clozapine, reserpine and olanzapine (Sigma–Aldrich, St. Louis, MO, USA); tramazoline hydrochloride (Zdravlje, Leskovac, Serbia); amiloride hydrochloride (Galenika, Belgrade, Serbia); viloxazine (Hemofarm, Vrsac, Srbija). Dodecane and DMSO were obtained from Sigma–Aldrich (St. Louis, MO, USA). The porcine Polar Brain Lipid (PBL) (catalog no. 141101C) was from Avanti Polar Lipids, Inc. (Alabaster, AL). Sodium chloride, J. T. Baker (Deventer, The Netherlands), sodium hydrogen phosphate, Merck (Darmstadt, Germany) and potassium dihydrogen phosphate, Merck (Darmstadt, Germany) were used for the preparation of the physiological buffer . The PAMPA 96-well plates (MultiScreen-HV, 0.45 μm, clear, non-sterile) (catalog no. MAHVN4510) were purchased from Merck Millipore.

2.2. Artificial membrane permeability assay

Effective permeability of 40 compounds (18 IRs/α-ARs ligands and 22 CNS drugs) was examined using PAMPA-BBB model, which was based on the BBB model described in literature (Di et al., 2003). The compounds were dissolved in DMSO (5 mg/mL) and diluted in physiological phosphate buffer at pH = 7.4 in order to obtain secondary stock solution (25 μg/mL). The donor wells were filled with 300 μL of secondary stock solution, while the acceptors were filled with 300 μL buffer solution. The filter membrane was wetted with 4 μL artificial membrane solution, which was made of PBL in dodecane (20 mg/mL). The acceptor filter plate was carefully put on the donor plate and left undisturbed for 18 h. At the end of incubation period the sandwich was carefully disassembled and the concentration of drug in the acceptor and the donor wells, as well as reference were determined by standard analytical method-HPLC using UV detector. In cases where compounds had weak UV absorbance, samples were transferred to Liquid Chromatography Mass Spectrometry (LC–MS).

Samples were analyzed in triplicate and the average of this three runs were reported. The following Eqs. (1) and (2) were used to calculate effective permeability of compounds taking into account iso-pH conditions and their membrane retention (Avdeef, 2003):

$$VaCa(t) + VdCd(t) = VdCd(0)(1 - R) \tag{1}$$

$$P_e = -\frac{2.303Vd}{A(t - \tau_{lag})}\left(\frac{1}{1 + r_v}\right)\log_{10}\left[1 - \left(\frac{1 + r_v^{-1}}{1 - R}\right)\frac{Ca(t)}{Cd(0)}\right] \tag{2}$$

where Va = acceptor volume (cm^3)-300 μL; Vd = donor volume (cm^3)-300 μL; A = filter area (cm^2)-25 cm^3; t = permeation time (s); $Ca(t)$ and $Cd(t)$ = the concentration of compound in acceptor and donor wells at time t (μM); $Cd(0)$ = the concentration of compound in donor well at time 0 (μM); τ_{lag} = time needed to saturate the membrane is relatively short compared to the total permeation time (approximately 20 min for unstirred plates) (Avdeef et al., 2001); R = mole fraction of compound retained by membrane; r_v = Vd/Va.