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ORIGINAL ARTICLE

SCREENING OF BLOOD-BRAIN BARRIER PENETRATION USING THE IMMOBILIZED ARTIFICIAL MEMBRANE PHOSPHATIDYLCHOLINE COLUMN CHROMATOGRAPHY AT THE PHYSIOLOGICAL PH

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Summary

In this study, 21 structurally diverse drugs with examined central nervous system penetration were used for prediction of blood-brain barrier penetration using HPLC-UV instrument. Drugs' chromatographic capacity factors were measured by the immobilized artificial membrane presented by phosphatidylcholine column. The correlation between chromatographic capacity factor, octanol-water partition coefficient (log P) and molecular polar surface area (PSA) were determined at the physiological pH. The correlation factor 0.6677 with respect to log P and 0.7199 in reference to PSA was assigned. The developed *in vitro* prediction method may be used as a screening tool for blood-brain barrier penetration of drugs with passive transport mechanism.

Key words: Blood-Brain Barrier; CNS penetration; Immobilized Artificial Membrane; HPLC; Prediction

INTRODUCTION

There is an increasing interest in predicting the process of passive penetration of drugs into the central nervous system (CNS). The passive penetration of different compounds is restricted by the continuous layer of the endothelium lining capillaries, commonly called the blood-brain barrier (BBB) [1,2]. The penetration of hydrophilic drugs into the brain is mainly restricted by the presence of this barrier. The lipophilic drugs including CNS-targeted drugs may enter the brain by transcellular passive diffusion. Some drugs may also cross the BBB by active process, involving influx and efflux transporters [3].

The pharmacological activity of all CNS drugs depends not only on receptor activity but also on the achieved compound concentration in the brain.

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Direct measurement of concentration of different drugs in CNS is difficult and time-consuming [4-6]. The compound BBB penetration potential may be assessed by *in silico*, *in vitro* and *in vivo* methods.

In silico methods include the assessment of physicochemical properties of different compounds, such as molecular polar surface area (PSA) [7] and octanol-water partition coefficient (log P) [8]. *In vivo* methods include microdialysis [9,10], cerebrospinal fluid sampling [11], nuclear magnetic resonance [12] and pharmacokinetic and tissue distribution studies [13-18]. *In vitro* methods use isolated brain capillaries, measurement of parallel artificial membrane permeability assay (PAMPA) [19] and the immobilized artificial membrane (IAM) chromatography [3]. The IAM chromatography may be successfully used for predicting the extent of passive penetration of compounds into the brain tissue.

It was the aim of the present study to upgrade and validate a computational BBB permeation model published by Yoon et al. (2006) for the physiological pH 7.4. Improvement of this already published method for the physiological pH is really important because of different ionization of tested compounds and due to this subsequent different penetration of BBB. The second change with respect to the model published by these authors [3] is using a different type of column. The utility of the IAM phosphatidylcholine column chromatography for the prediction of the BBB penetration potential was attested for 21 structurally unrelated compounds. Brain permeation data of these compounds were found in literature.

MATERIALS AND METHOD

Chemicals

Atenolol, β-estradiol, caffeine, cefuroxime, chlorpromazine, cimetidine, corticosterone, desipramine, enalapril, hydrocortisone, ibuprofen, imipramine, lomefloxacin, loperamide, nadolol, piroxicam, progesterone, promazine, propranolol, testosterone and p-toluidine were purchased from Sigma Aldrich (Steinheim, Germany). Acetonitrile gradient grade LiChrosolv was purchased from Merck (Darmstadt, Germany). KH_2PO_4, Na_2HPO_4, KCl, and NaCl were purchased from Lachema (Neratovice, The Czech Republic). Water was reverse osmosis pure.

Apparatus

The HPLC system consisted of a P200 gradient pump (Spectra-Physics Analytical, Fremont, USA), a 7125 injection valve – 10 µl loop (Rheodyne, Cotati, USA), an UV1000 detector (Spectra-Physics Analytical, Fremont, USA) and a CSW Chromatography Station 1.5 software (DataApex, Praha, Czech republic).

Software Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 5.1.0, 2008, ChemAxon (http://www.chemaxon.com).

Chromatographic conditions

For analyses an IAM.PC.DD 2 (150 x 4.6 mm; 12 µm) column (Regis Technologies, Morton Grove, IL) was used. The mobile phase was 80% PBS and 20% acetonitrile (*v/v*) with pH adjusted to 7.4 using Na_2HPO_4. The phosphate-buffered saline (PBS) was prepared with 2.7 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, and 8.1 mM Na_2HPO_4. The final pH was established in final 20% organic mobile phase. It was delivered isocratically at a flow rate of 1 ml/min. The absorbance was measured at 210 nm. All chromatograms were obtained at 37°C.

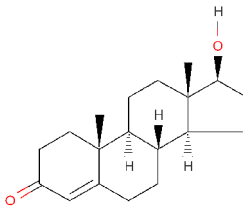
The drugs were dissolved in mobile phase in situ; the concentrations of therapeutic drugs in samples were 10 µg/ml. The samples were measured in triplicates.

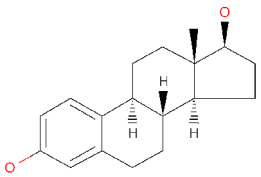
RESULTS & DISCUSSION

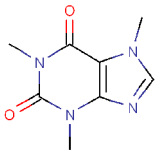
Twenty-one structurally diverse reference drugs were used for development of a screening method for determination of the BBB penetration potential. The compounds, classified in literature as "high brain penetration" (CNS+) drugs were testosterone, imipramine, desipramine, chlorpromazine, promazine, β-estradiol, caffeine, ibuprofen, propranolol, progesterone and p-Toluidine (Table 1).

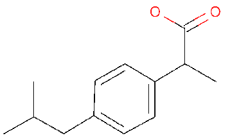
The compounds with "low brain penetration" (CNS-) were loperamide, cefuroxime, enalapril, lomefloxacin, piroxicam, nadolol, atenolol, hydrocortisone, cimetidine and corticosterone [3,19] (Table 2). These literature classifications were based on the measurement of the rate by which the drug enters the brain.

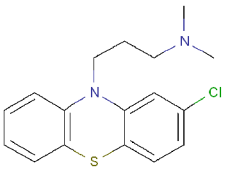
Table 1. Formulas of tested drugs (CNS+).

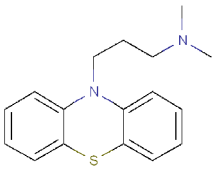

testosterone

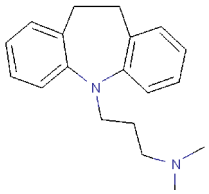

β-estradiol

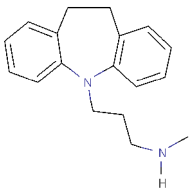

caffeine

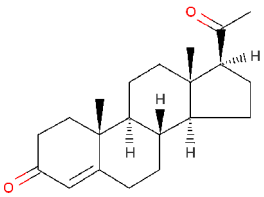

ibuprofen

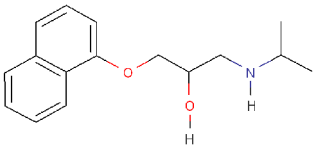

chlorpromazine


promazine


imipramine


desipramine


progesterone


propranolol

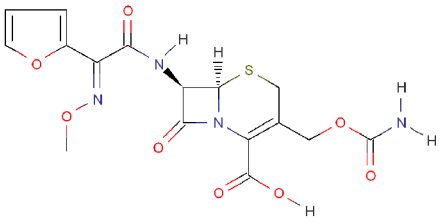
The most important coefficient for determination of IAM partition was k_{IAM} (IAM capacity factor), which was calculated as

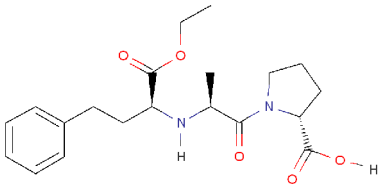
$$k_{IAM} = (t_r - t_0)/t_0$$

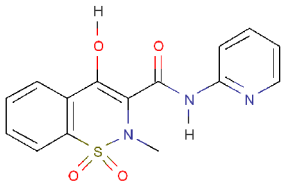
where t_r is the retention time of the drug and t_0 is the hold up time of the column.

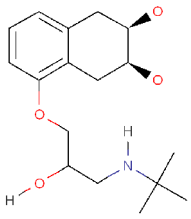
In this study, the k_{IAM} was determined for twenty-one reference drugs (Table 1,2). The k_{IAM} values were determined with the mobile phase at pH 7.4 although Yoon et al. (2006) recommended using the mobile phase at pH 5.5. The differentiation between CNS+ and CNS – drugs was most successful when the power function of the capacity factors (k_{IAM}/MWn) was set at n = 4 at pH 5.5.

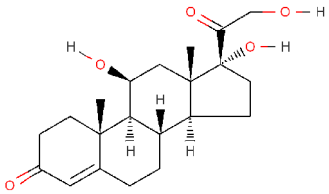
Table 2. Formulas of tested drugs (CNS-).

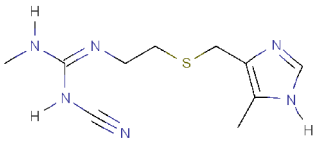

cefuroxime

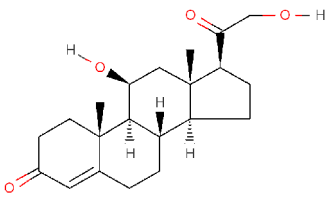

enalapril

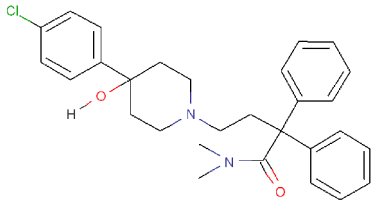

piroxicam

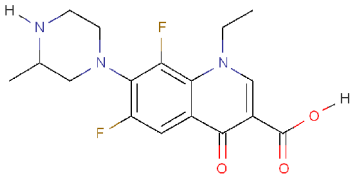

nadolol

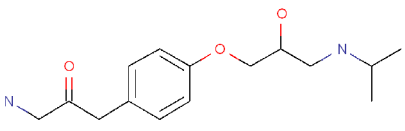

hydrocortisone


cimetidine


corticosterone


loperamide


lomefloxacin


atenolol

Our experiment was however done with the mobile phase of higher pH because of the need of similar environment to that in the human body. And the change of the pH range may significantly change the state of chemical ionization of many drugs. Chemical ionization is a very important factor which may markedly change the possibility of a molecule to penetrate through the BBB.

Prediction of CNS penetration at the pH 7.4 potential based on IAM partition coefficients:

According to Yoon et al. (2006) the assortment of drugs which can cross the BBB (CNS+) and those which do not penetrate into the brain (CNS-) was made based on k_{IAM} corrected by the molecular weight (MW).

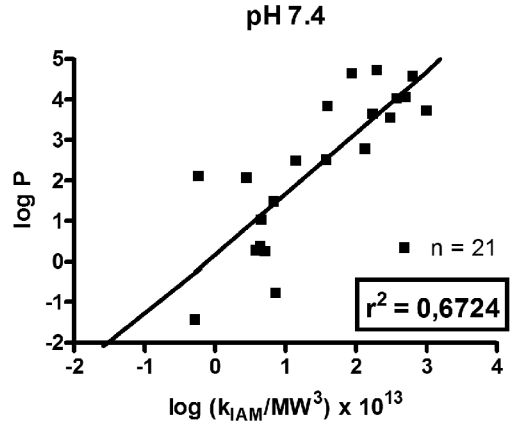
In this study, the k_{IAM} was determinated for 21 reference drugs. The k_{IAM} values were determined at the mobile phase pH 7.4. The differentiation between CNS+ and CNS- drugs (permeability – P_m) was made based on k_{IAM} corrected by the molecular size with the power function at 3, 4 and 5 (Figure 1,2,3,4).

$$P_m = k_{IAM} / MW^n$$

The assortment at pH 7.4 was the most successful with the power function of the molecular weight set at 4. The formula, which is given below was designed.

$$X = k_{IAM} / MW^4 \times 10^{10}$$

Figure 3 and 4 illustrated the correlation between log P and PSA and k_{IAM} / MW^4. Good correlation was observed with the correlations coefficient (r^2) being 0.6677 and 0.7199 at physiological pH 7.4.



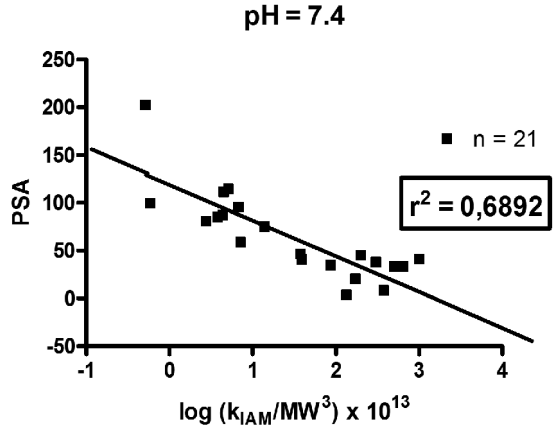
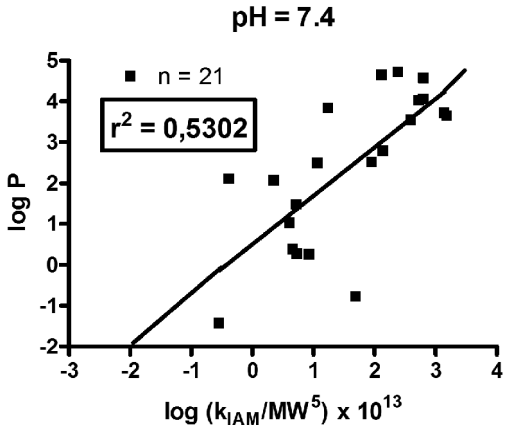


Figure 1. Correlation between log P and k_{IAM} / MW^3 and PSA k_{IAM} / MW^3 and determined at the mobile phase pH of 7.4.



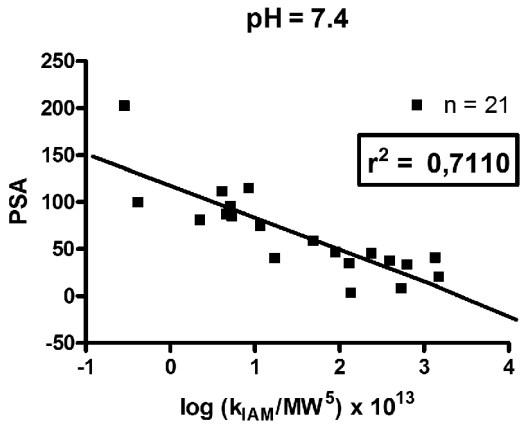


Figure 2. Correlation between log P and k_{IAM} / MW^5 and PSA k_{IAM} / MW^5 and determined at the mobile phase pH of 7.4

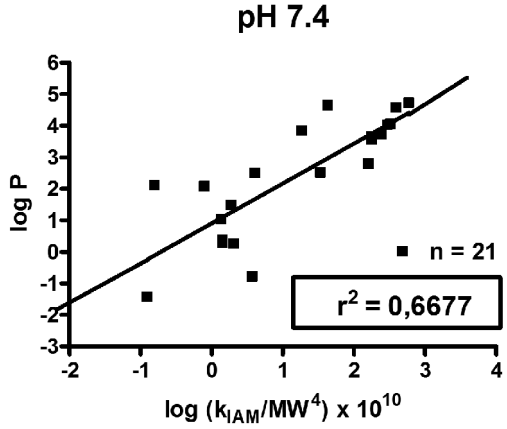


Figure 3. Correlation between log P and k_{IAM} / MW^4 determined at the mobile phase pH of 7.4.

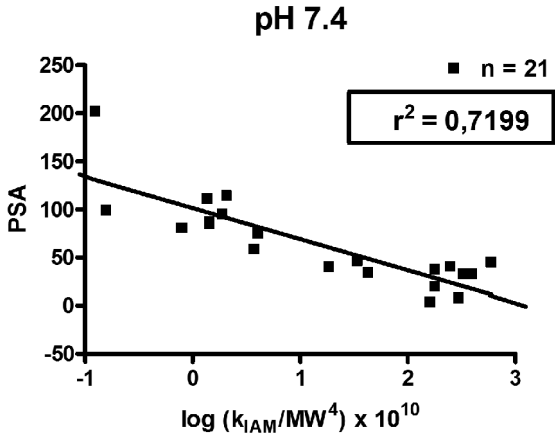


Figure 4. Correlation between polar surface area (PSA) and k_{IAM}/ MW^4 determined at the mobile phase pH of 7.4.

Moreover, predicted constants – Log P, PSA and MW of tested compounds were calculated by using a special software Marvin. If considered Log P, it can be clearly seen that all substances are more soluble in water than in octanol. In case of PSA (sum of surfaces of polar atoms: oxygens, nitrogens and attached hydrogens, in a molecule), it is a parameter very useful for prediction of drug transport properties [20] (Table 3).

The PSA has been previously shown to correlate with human intestinal absorption [20,21]. When PSA is applied to a larger and more diverse compound set, however, outliers become more frequent [22,23]. In this study, a good correlation was observed between PSA and k_{IAM}/ MW^4 with the correlation being 0.7199 at the mobile phase pH of 7.4.

Table 3. Values of the Human physicochemical parameters (pK_a, Log P and PSA) and different k_{IAM} coefficients of 21 structurally unrelated drugs at the pH 7.4.

Compound		MW	Rt	Log P	PSA	pK_a	k_{IAM}^3 (x 10^{10})	k_{IAM}^4 (x 10^{13})	k_{IAM}^5 (x 10^{13})
cefuroxime	**CNS-**	424	1.82	-1.44	201.89	3.15	0.525	0.13	0.292
enalapril	**CNS-**	376	1.71	2.10	98.77	3.18/5.19	0.593	0.16	0.420
lomefloxacin	**CNS-**	352	2.90	2.06	80.29	5.65/8.70	2.822	0.80	2.278
piroxicam	**CNS-**	331	3.45	1.02	110.81	4.27	4.560	1.38	4.163
nadolol	**CNS-**	309	3.01	0.37	86.53	9.76	4.458	1.44	4.669
atenolol	**CNS-**	266	2.24	0.26	84.58	9.67	3.842	1.44	5.430
hydrocortisone	**CNS-**	362	5.55	1.47	94.83	12.74	6.892	1.90	5.259
cimetidine	**CNS-**	252	2.39	0.24	114.19	6.92	5.239	2.08	8.551
caffeine	**CNS+**	194	1.99	-0.79	58.44	1.50	7.269	3.75	49.827
corticosterone	**CNS-**	346	8.90	2.48	74.60	-	14.114	4.08	11.789
loperamide	**CNS-**	477	57.33	4.71	44.98	9.41	39.712	8.32	17.454
ibuprofen	**CNS+**	206	5.67	3.83	40.13	4.86	38.454	18.67	90.615
propranolol	**CNS+**	259	21.30	2.50	46.07	9.67	88.550	34.19	132.004
progesterone	**CNS+**	314	56.07	4.63	34.14	-	136.085	43.34	138.023
testosterone	**CNS+**	288	64.12	3.54	37.30	-	202.291	70.24	243.889
imipramine	**CNS+**	280	89.71	4.01	7.68	9.19	309.802	110.00	395.155
desipramine	**CNS+**	266	95.09	3.64	19.85	10.02	383.326	143.29	541.758
p-Toluidine	**CNS+**	107	4.08	2.78	3.24	5.46	174.562	163.14	1524.694
promazine	**CNS+**	284	153.76	4.04	32.98	9.20	511.985	180.28	634.777
chlorpromazine	**CNS+**	319	274.48	4.56	32.98	9.19	647.342	203.29	636.139
ß-estradiol	**CNS+**	272	267.90	3.71	40.46	10.33	1019.084	372.58	1377.439

Rt … retention time, units are min.

The CNS- drugs showed evident inability to bound to the phosphatidylcholine column and have X values less than 0.50, whereas the CNS+ drugs bound much better and their X values were higher than 1.00.

CONCLUSIONS

As a result, the study was performed to specify the method publicized by Yoon et al. (2006) for another pH and another column. The pH value was 7.4, the physilological pH, which is important for chemical ionization of many drugs. The correlation for this pH range was good (0.6677 with log P and 0.7199 with PSA). This study indicates that in vitro IAM capacity factors (k_{IAM}/MW^4) may be used to classify drugs as CNS- and CNS+ with a success at the pH range 7.4.

ACKNOWLEDGEMENT

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