ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 56 (2001) 145–148

IL FARMACO

Physicochemical properties in pharmacokinetic lead optimization

Stefanie D. Krämer *, Heidi Wunderli-Allenspach

Institute of Pharmaceutical Sciences, Department of Applied BioSciences, ETH Zürich, Winterthurerstrasse. 190, CH-8057 Zürich, Switzerland

Abstract

The ADME (*a*bsorption, *d*istribution in the body, *m*etabolism and *e*limination from the body) profile of a drug determines its pharmacokinetics in the body. Modern drug design includes the modeling of pharmacokinetically favorable behavior. The pharmacokinetic parameters of most interest concern intestinal absorption, blood–brain barrier (BBB) passage and metabolism. Traditionally, experimental parameters such as partition coefficients and chromatographic capacity factors have been used for the estimation of intestinal absorption or BBB passage of newly synthesized compounds. Several studies have shown a sigmoidal relationship between intestinal absorption and lipophilicity. The latter is usually expressed by the apparent partition coefficient log *D* in a biphasic system at physiological pH or by the affinity to a lipophilic phase determined by chromatographic techniques. In contrast, structure-based descriptors need no experimental investigation of the compound studied. The most relevant descriptors give information on hydrogen-bonding characteristics and molecular volume. In recent years, attempts have been made to recognize substrates for multidrug resistance proteins by their structure characteristics without crucial success. There is evidence that multidrug resistance is not only driven by direct protein–substrate recognition, but also by the behavior of the compound in the lipid environment of the protein.

Keywords: Absorption prediction; Blood–brain barrier passage; Lead optimization; Multi-drug resistance; Pharmacokinetic; Physicochemical parameters

1. Pharmacokinetics — a key issue

The processes that determine the pharmacokinetic behavior of a drug compound are its *a*bsorption, *d*istribution in the body, *m*etabolism and *e*limination from the body (ADME). A prerequisite for complete absorption is the *l*iberation of a drug [1] (LADME). Up to now most effort has been put into the prediction of intestinal absorption and blood–brain barrier (BBB) passage. Only recently computer programs predicting first-pass metabolism have gained more interest, as bioavailability is a function of both absorption and metabolism [2]. The following survey will concentrate on the discussion of some models to predict absorption and BBB passage.

2. The absorption pathways

In vivo barriers that are relevant in drug therapy are epithelial, e.g. in the intestinal tract, and endothelial cell layers, e.g. the BBB. The different pathways that can be used by drug compounds to cross these barriers are shown schematically in Fig. 1. Passage can occur paracellularly between the cells and transcellularly through the cells. Though intestinal absorption via the paracellular way may be relevant for hydrophilic compounds with molecular weights lower than 200 [3], it is negligible at the BBB due to the occlusive network of tight junctions at the cell–cell contacts of the brain capillary endothelial cells [4]. Lipophilic compounds preferably use the transcellular pathway. The latter is commonly subdivided into ‘passive diffusion’ through the barrier, carrier-mediated permeation and endo- and trans-cytosis. Carriers are generally not of high relevance for drug uptake. However, multidrug resistance proteins, which cause efflux of drug compounds out of the barrier cells and, therefore, hinder their barrier passage, are of high significance for drug absorption and BBB passage.

* Corresponding author.

E-mail address: stefanie.kramer@pharma.anbi.ethz.ch (S.D. Krämer).

0014-827X/01/$ - see front matter
PII: S0014-827X(01)01028-X

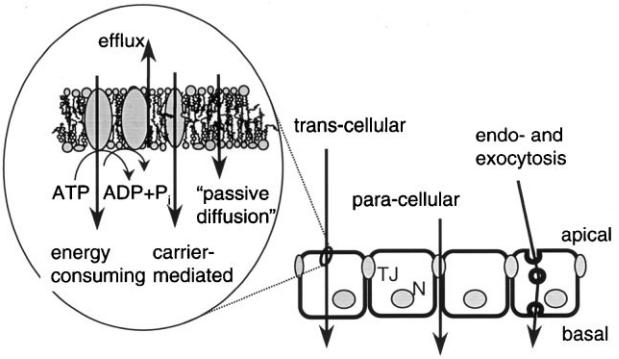

Fig. 1. Routes across in vivo barriers. Schematic epithelial or endothelial barrier with tight junctions (TJ) and nuclei (N). The magnification shows a sketch of the cell membrane consisting of a lipid bilayer and membrane proteins. For details see text.

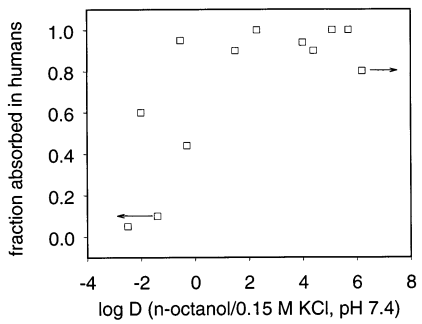

Fig. 2. Comparison between the fraction absorbed in humans and the *n*-octanol/aqueous log *D* at pH 7.4. log *D* was determined by the potentiometric titration technique (Sirius Analytical Ltd, Forest Row, UK). Data points with flashes could be lower or higher (log *D*). Redrawn from Ref. [9].

3. Experimental models to predict transcellular permeation

3.1. Partitioning in the n-octanol/aqueous system

It has been known for a century now that barrier permeation that is not carrier-mediated correlates with the lipophilicity of an organic compound [5,6]. A useful tool to express lipophilicity with a relatively high predictive value for membrane passage was empirically found in the biphasic *n*-octanol/aqueous distribution system [7]. Here, the partitioning of a compound is represented as the concentration ratio between the lipophilic (*n*-octanol) and the aqueous phase. When considering all the species of a compound, e.g. the ionized and unionized forms, the partition coefficient is called the distribution coefficient *D*. For compounds with acidic or basic characteristics *D* is pH dependent. The partition coefficient of one specific molecular species, e.g. a defined ionization species, and under ideal (Nernst) conditions [8] is called the true partition coefficient *P*. Lipophilicity is usually expressed on a logarithmic scale, i.e. log *D*. Fig. 2 shows the relationship of intestinal absorption in humans and log *D* in the *n*-octanol/0.15 M KCl system at pH 7.4 of 12 structurally divergent compounds with molecular weights between 259 and 644 [9]. The log D values were determined by the potentiometric titration technique [10].

3.2. Liposomes

In a few studies, lipid membranes instead of *n*-octanol were used in partition experiments for the prediction of in vivo barrier passage. Usually, phosphatidylcholine liposomes serve as model membranes. Balon et al. [11] applied the titration technique in a phosphatidylcholine liposomes/0.15 M KCl system to determine *D*, which they used to calculate the absorption potential AP, defined by Dressman et al. [1]. AP results from multiplication of *D* with a function of the solubility of the compound in the aqueous phase, the therapeutic dose and the average intestinal volume. AP correlated in a sigmoidal function with in vivo absorption in humans.

3.3. Immobilized artificial membranes (IAMs) and high-performance liquid chromatography (HPLC)

Besides partition experiments, chromatography is a common tool to measure the lipophilicity of a compound and finally estimate its in vivo absorption or BBB passage. In order to model a lipid membrane, lipid analoga were linked to silica surfaces to form IAMs. Such IAM columns are used in HPLC [12]. This technique is frequently applied; however, depending on its characteristics, the compound can also adsorb to uncapped silanol groups and lead to misinterpretation of the results [13].

3.4. Permeation across lipophilic phases

Recently, two promising techniques to measure permeation of compounds across either phospholipid or alkane phases have been introduced to predict 'passive diffusion' across in vivo barriers [14,15].

4. Permeation prediction from structural characteristics

4.1. General parameters describing lipophilicity and membrane passage

The discovery of the structural characteristics determining lipophilicity and lipid membrane passage made it possible to estimate barrier passage from a given two- or three-dimensional chemical structure of a compound. The most relevant characteristics are summa-

rized in Table 1. A more detailed delineation of lipophilicity can be found in Ref. [16]. Such characteristics are either treated in a purely quantitative manner, not regarding their orientation in the three-dimensional structure (see sections 4.2, 4.3, 5.1), or in a more sophisticated way based on vectors mapping the molecular surface in a three-dimensional system (sections 4.4, 5.2).

4.2. Hydrogen bonding characteristics

A simple approach to estimate barrier passage is the counting of the hydrogen-bond acceptors and donors of a compound, e.g. see Ref. [17]. The predictive force for compounds with a medium number of hydrogen bonding capacities (somewhere between 5 and 20) is not satisfactory. This approach is limited by the fact that such numbers also include intramolecular hydrogen bonds and hydrogen bonds that are not accessible for water molecules. In addition, the numbers contain no information on the strength of the possible hydrogen bonds with the aqueous environment. The first problem is overcome by the calculation of the polar surface area of an energy-minimized three-dimensional structure of the molecule.

4.3. Polar surface area

The polar surface area of a molecule is the surface area associated with hydrogen bond acceptors and hydrogen bond donors, i.e. nitrogen and oxygen atoms and hydrogen atoms bound to these heteroatoms. Polar surface areas calculated on the basis of energy-minimized structures reveal a negative sigmoidal relationship with barrier passage, e.g. see Ref. [17]. However, in this model the absorption of compounds with medium values for polar surface area also cannot be predicted reliably.

4.4. Volsurf

Some more recent models include information on the three-dimensional orientation of hydrophilicity and hydrophobicity in the molecule. One example is 'Volsurf', a computer program described by Crivori et al. [18]. The evaluation of the descriptors revealed that hydrogen-bonding capacities are less disadvantageous for membrane passage when they are not homogeneously distributed over the molecule (expressed as integy moment).

Table 1
Structure characteristics determining lipophilicity and lipid membrane passage

Structure characteristic	Influence on lipophilicity and membrane permeability
Hydrogen-bonding capacity	Measure for hydrophilicity; unfavorable for membrane passage
Molecular size	Measure for hydrophobicity; favorable for membrane passage
Flexibility	Flexibility can shield hydrogen-bonding capacities; favorable for membrane passage

5. Computational approaches based on experimental data

5.1. log P and log D calculations

The classical computational method to estimate barrier passage is the calculation of log P from additive values for molecular fragments [7,19–21]. Newer computer programs are able to estimate log D from the calculated log P and pK_a values of a compound (ACD, Advanced Chemistry Development Inc., Toronto, Canada; PALLAS, CompuDrug Chemistry Ltd, Budapest, Hungary). Such values may then be used for the prediction of barrier passage as described above.

5.2. Molecular lipophilicity potential (MLP)

MLP takes the three-dimensional arrangement of different lipophilic fragments of a compound into account [22].

5.3. Abraham equation

The solvation equation by Abraham and Chanda [23] uses two additional descriptors besides the acidic and basic hydrogen-bonding capacities and molecular size. They are descriptors for the molar excess refractivity R and the dipolarity/polarizability π of a compound. On applying the whole equation, R is favorable for barrier passage, whereas π is unfavorable. Hydrogen-bonding capacities and π of a compound are either determined experimentally in partition or chromatographic systems or are calculated from databases for molecule fragments. R and the molecular volume can easily be calculated. Gratton et al. published a promising correlation between predicted brain uptake data and experimentally determined values [24].

6. Aiming at prediction of multidrug resistance

Several attempts have been made to recognize typical structural characteristics of substrates for multidrug resistance proteins. However, none of them was successful in the prediction of drug efflux (for reviews see Refs. [25,26]). The key protein in multidrug resistance is P-glycoprotein (P-gp), which belongs to the ATP-binding cassette (ABC) transporter family. In recent years it has become evident that the activity of P-gp as ATPase

and as a drug efflux pump depends strongly on its lipid environment [27]. P-gp presumably recognizes its substrates and modulators out of the lipid membrane, as these compounds usually have a high membrane affinity. Based on this and on our own findings on drug–membrane interactions [28,29], we conclude that the criteria for being a P-gp substrate is not simply based on protein–substrate recognition, but also on substrate–lipid membrane interactions. Work is currently going on in our laboratory to understand better the relationships between drug–membrane interactions, P-gp activity and efflux phenomena.

7. Conclusions

There are several experimental and computational methods available today to estimate drug absorption and BBB passage. Although none of them is able to reflect transcellular permeation by so-called passive diffusion exactly, they give useful estimates for barrier permeation. Multidrug resistance phenomena are still not predictable, and may be the major reason for unexpected low barrier passage of many compounds. We need a better understanding of the permeation processes, including multidrug resistance phenomena, to develop more precise tools for the prediction of absorption and BBB passage.

References

[1] J.B. Dressman, G.L. Amidon, D. Fleisher, Absorption potential: estimating the fraction absorbed for orally administered compounds, J. Pharm. Sci. 74 (1985) 588–589.
[2] B. Testa, G. Cruciani, Structure–metabolism relations and the challenge of predicting biotransformation, in: B. Testa, H. van der Waterbeemd, G. Folkers, R. Guy (Eds.), Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies, VCH, Weinheim, 2001.
[3] H. Lennernäs, Does fluid flow across the intestinal mucosa affect quantitative oral drug absorption? Is it time for a reevaluation?, Pharm. Res. 12 (1995) 1573–1582.
[4] M.W. Brightman, J.H. Tao-Chen, Tight junctions of brain endothelium and epithelium, in: W.M. Pardridge (Ed.), The Blood–Brain Barrier. Cellular and Molecular Biology, Raven Press, New York, 1993, pp. 107–125.
[5] H. Meyer, Zur Theorie der Alkoholnarkose, Arch. Exp. Path. Pharmakol. 42 (1899) 109–118.
[6] E. Overton, Ueber die osmotischen Eigenschaften der Zelle in ihrer Bedeutung für die Toxikologie und Pharmacologie, Z. Phys. Chem. 22 (1896) 189–209.
[7] C. Hansch, A.J. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley Interscience, New York, 1979.
[8] W. Nernst, Verteilung eines Stoffes zwischen zwei Lösungsmitteln und zwischen Lösungsmittel und Dampfraum, Z. Phys. Chem. 8 (1891) 110–139.
[9] S.D. Krämer, Absorption prediction from physicochemical parameters, Pharm. Sci. Tech. Today 2 (1999) 373–380.
[10] A. Avdeef, Assessment of distribution–pH profiles, in: V. Pliska, B. Testa, H. van de Waterbeemd (Eds.), Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, 1996, pp. 109–139.
[11] K. Balon, B.U. Riebesehl, B.W. Müller, Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption, Pharm. Res. 16 (1999) 890–896.
[12] S. Ong, H. Liu, C. Pidgeon, Immobilized-artificial-membrane chromatography: measurements of membrane partition coefficient and predicting drug membrane permeability, J. Chromatogr. A 728 (1996) 113–128.
[13] C. Ottiger, H. Wunderli-Allenspach, Immobilized artificial membrane (IAM)–HPLC for partition studies of neutral and ionized acids and bases in comparison with the liposomal partition system, Pharm. Res. 16 (1999) 643–650.
[14] M. Kansy, F. Senner, K. Gubernator, Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes, J. Med. Chem. 41 (1998) 1007–1010.
[15] B. Faller, R. Wohnsland, Physicochemical parameters as tools in drug discovery and lead optimization, in: B. Testa, H. van der Waterbeemd, G. Folkers, R. Guy (Eds.), Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies, VCH, Weinheim, 2001.
[16] V. Pliska, B. Testa, H. van de Waterbeemd, Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, 1996.
[17] K. Palm, P. Stenberg, K. Luthman, P. Artursson, Polar molecular surface properties predict the intestinal absorption of drugs in humans, Pharm. Res. 14 (1997) 568–571.
[18] P. Crivori, G. Cruciani, P.A. Carrupt, B. Testa, Predicting blood-brain barrier permeation from three-dimensional molecular structure, J. Med. Chem. 43 (2000) 2204–2216.
[19] R.F. Rekker, H.M. De Kort, The hydrophobic fragmental constant; an extension to a 1000 data point set, Eur. J. Med. Chem. Chim. Ther. 14 (1979) 479–488.
[20] P. Broto, G. Moreau, C. Vandycke, Molecular-structures-perception, auto-correlation descriptor and SAR studies-system of atomic contributions for the calculation of the normal-octanol water partition-coefficients, Eur. J. Med. Chem. Chim. Ther. 19 (1984) 71–78.
[21] A.K. Ghose, G.M. Crippen, Atomic physicochemical parameters for 3-dimensional structure-directed quantitative structure–activity-relationships. 1. Partition coefficients as a measure of hydrophobicity, J. Comput. Chem. 7 (1986) 565–577.
[22] P. Gaillard, P.A. Carrupt, B. Testa, A. Boudon, Molecular lipophilicity potential, a tool in 3D QSAR: method and applications, J. Comput.-Aided Mol. Des. 8 (1994) 83–96.
[23] M.H. Abraham, H.S. Chadha, Applications of a solvation equation to drug transport properties, in: V. Pliska, B. Testa, H. van de Waterbeemd (Eds.), Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, 1996, pp. 311–337.
[24] J.A. Gratton, M.H. Abraham, M.W. Bradbury, H.S. Chadha, Molecular factors influencing drug transfer across the blood-brain barrier, J. Pharm. Pharmacol. 49 (1997) 1211–1216.
[25] G.D. Eytan, P.W. Kuchel, Mechanism of action of P-glycoprotein in relation to passive membrane permeation, Int. Rev. Cytol. 190 (1999) 175–250.
[26] J. Ferté, Analysis of the tangled relationships between P-glycoprotein-mediated multidrug resistance and the lipid phase of the cell membrane, Eur. J. Biochem. 267 (2000) 277–294.
[27] Y. Romsicki, J. Sharom, The membrane lipid environment modulates drug interactions with the P-glycoprotein multidrug transporter, Biochemistry 38 (1999) 6887–7896.
[28] S.D. Krämer, A. Braun, C. Jakits-Deiser, H. Wunderli-Allenspach, Towards the predictability of drug–lipid membrane interactions: the pH-dependent affinity of propranolol to phosphatidylinositol containing liposomes, Pharm. Res. 15 (1998) 739–744.
[29] S.D. Krämer, C. Jakits-Deiser, H. Wunderli-Allenspach, Free fatty acids cause pH-dependent changes in drug–lipid membrane interactions around physiological pH, Pharm. Res. 14 (1997) 827–832.