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Research Paper

The application of molecular structural predictors of intestinal absorption to screening of compounds for transdermal penetration

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Abstract

Objectives The development of methods to predict the transport of molecules across biological membranes, without the need for time-consuming collection of experimental data, is a rapidly growing science. The use of structural characteristics of molecules has been investigated to predict the maximum transport rates of molecules across skin epidermal and intestinal membranes, known as maximum flux and maximum absorbable dose, respectively, although different approaches have been used. The aim of the present study was to determine whether the relationship between polar surface area and number of rotatable bonds of molecules and their permeability through intestinal membranes could be applied to the permeation of solutes through the epidermis following topical application.
Methods We used a published dataset of human epidermal maximum flux values for 182 solutes and stepwise regression to determine relationships between structural predictors and maximum membrane transport rates.
Key findings Results showed that diffusion processes occurring across intestinal and skin epidermal membranes cannot be estimated by the same solute molecular properties, as different combinations of partitioning and diffusion processes appear to be dominating in each type of membrane. The basis of these differences in terms of molecular weight dependence and the usefulness of polar surface area are discussed.
Conclusions Based on available literature, we concluded that transdermal penetration is poorly predicted by parameters derived from intestinal or Caco-2 model membranes. While this approach may be useful for small sets of structurally related compounds, it appears to have limited value for screening and selection of novel structures in the pharmaceutical industry.
Keywords maximum absorbable dose; maximum flux; membrane transport; polar surface area; rotatable bonds

Introduction

Meaningful prediction of the diffusion of solutes in biological systems, based on structural characteristics, particularly absorption across membranes, has been the goal of many in the pharmaceutical and risk-assessment industries. There is increasing industry pressure to reduce time expenditure on the screening and assessment of potential drug candidate molecules and reduce costs in the discovery, research and development phases of novel therapeutics by eliminating likely unsuitable candidates at very early stages.

In order to streamline the discovery and development processes, a number of in-vitro experimental models have been developed to aid in the rapid screening of drug candidates, such as Caco-2 monolayers for predicting intestinal absorption[1] and models for the assessment of transdermal absorption potential based on artificial membranes,[2] animal,[3,4] or human[5] skin. The ultimate time-saving methods, however, are based on computational models, with no experimental component, which have been suggested to mimic solute membrane transport processes based on molecular structure–permeability relationships (known as quantitative structure–activity relationships, or QSAR characteristics).[6,7] With the oral route traditionally being the preferred route for administration, it is not surprising that the most highly investigated of these computational models are those applicable to intestinal drug permeability.[8]

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A review by Lipinski *et al*. provided a guide to correlating physical properties such as lipophilicity, molecular weight and hydrogen bonding to successful drug development for orally administered compounds.[9] However, several other structural descriptors, including surface properties and number of rotatable bonds, have also been introduced into the absorption computational field.[10–12] One of these surface property descriptors, polar surface area (PSA), is also suggested to encode hydrogen-bonding information due to the strong correlation observed between these two descriptors.[13,14] There are a number of approaches to calculating PSA, depending primarily on the kind of molecular surface area used (van der Waals being the most common) or which atoms are considered to be polar, with the use of nitrogen, oxygen (although sometimes also sulphur) and attached hydrogen atoms the most common.[15,16] In addition to its successful application for the prediction of intestinal absorption in terms of maximum absorbable dose (MAD)[12,17,18] and the Caco-2 monolayer cell culture membrane model,[16] PSA has also been successfully used for the prediction of blood–brain barrier transport.[19–21] The number of rotatable bonds within a molecule has also been identified as a second valuable property, together with PSA, for predicting oral bioavailability in rats, with fewer (< 10) rotatable bonds and a PSA of less than 140 Å^2 (or 12 or fewer hydrogen bonding groups) giving a high probability of good oral bioavailability in the rat.[11] PSA has also been suggested to capture some solvation characteristics of a molecule, with a linear correlation found between PSA and the aqueous free energy of solvation in a recent analysis of 188 compounds by Iyer *et al*.[22]

At the beginning of this century, the transdermal route was vying with oral treatment as the most successful innovative research area in drug delivery, with around 40% of drug delivery candidate products under clinical evaluation related to transdermal or dermal systems.[23] More recently, in a 2008 review by Prausnitz and Langer, it was estimated that more than one billion transdermal patches were being manufactured annually.[24] This increasing interest has led to the growth of predictive methods for transdermal absorption. Previous solute physicochemical predictors of transdermal absorption potential have included lipophilicity (octanol:water partition coefficient),[25] molecular weight (MW),[26] various functional group contributions,[27] hydrogen acceptor and donor ability,[7] dipole properties and Hansen solubility parameters.[28] The reporting of transdermal penetration in terms of either permeability coefficient (K_p, cm/s), the rate with which a solute can move through a membrane, or maximum flux (J_{max}, mol/cm^2 per h), the maximum amount of a solute that can be delivered through a membrane per unit time, has great bearing on the relative importance of the predictors mentioned above.

Our analysis of literature data for solutes applied to human epidermal membranes in aqueous solution or as pure liquids used J_{max} rather than K_p.[26] The maximum flux provides the more clinically relevant answer for the pharmaceutical industry, where the aim is to optimise solute penetration, and to the risk assessors, whose aim is to determine potential maximum exposure. The concept of J_{max} through skin is similar to intestinal MAD in that both describe the maximum amount of a solute that is capable of passing through a biological membrane.

The aim of this study was to determine whether the relationships expressing solute permeability through intestinal membranes as functions of PSA and the number of rotatable bonds could similarly be applied to the permeation of solutes through the epidermis following topical application. We used our published database[26] of 182 solutes applied to human skin *in vitro* and performed correlations of this data with calculated PSA and rotatable bond parameters.

Materials and Methods

Rotatable bonds

Rotatable bonds were defined, according to Veber *et al*.[11] as any single bond, not in a ring, bound to a non-terminal heavy atom (i.e. non-hydrogen). The number of rotatable bonds (RB) was obtained by summation. C–N bonds were excluded from the count because of their high rotational energy barrier.

Polar surface area

The topological PSA (or TPSA, Å^2) was calculated using the fast calculation method described by Ertl *et al*.[15] from the summation of tabulated surface contributions of polar fragments, which those authors showed to be practically identical to those derived from the more laborious calculations of three-dimensional PSA.

Transdermal penetration

The maximum flux, J_{max} (mol/cm^2 per h), and permeability coefficient, K_p, values of solutes were taken from a previously published database of topically applied solutes in aqueous solution.[26] The complete database of 278 records encompassed solutes with an extremely wide range of physicochemical properties, with log octanol:water partition coefficient (log K_{ow}) values ranging from –5.7 to 8.7, molecular weight (MW), varying from 18 to 765 Da, melting point (Mpt) from 147 to 582 K and aqueous solubility (S_{aq}) from 6.9×10^{-10} mol/ml to completely miscible with water.

Maximum absorbable dose

MAD was calculated according to the method of Hilgers *et al*.[29] The relationship used is shown in equation (1):

$$MAD = S_{aq} \times K_a \times V_i \times TT_i \quad (1)$$

where K_a is the absorption rate constant (derived from first order kinetics, units s^{-1}), V_i is the intestinal volume and TT_i is the intestinal transit time. MAD, the product of these four parameters, has the units of mass. Aqueous solubilities were obtained from a published database[26] or SciFinder Scholar (CAS, American Chemical Society). K_a was calculated from published membrane permeability coefficient data, also using the method of Hilgers *et al*.[29] The values for V_i (20 ml) and TT_i (270 min) were obtained from Hilgers *et al*.[29]

Data analysis

Stepwise linear regressions between human skin permeability, in the form of both J_{max} and K_p values (and their logarithmic equivalents), and the variables MW, PSA and

RB were performed with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL).

Results and Discussion

Relationship between J_{max}, polar surface area and rotatable bonds

The correlation between log J_{max} and MW, one of the easiest structural relationships to derive, was reported by Magnusson *et al*. to have a regression coefficient (R^2) of 0.69 (Figure 1a).[26] The use of PSA or RB for the prediction of transdermal absorption potential would have to significantly improve on this simple relationship with MW in order to be of enough practical value to warrant time spent on the calculation of the parameters involved.

Our analysis showed a poor correlation between log J_{max} and PSA (Figure 1b, R^2 = 0.30) or RB (Figure 1c, R^2 = 0.20). Stepwise linear regression was performed to relate log J_{max} with the three parameters, MW, PSA and RB. PSA was eliminated as a significant parameter but the combination of MW and RB gave a similar correlation to that obtained with MW alone (0.70, compared to 0.69).[26] The resulting relationship is described in equation (2), with R^2 = 0.704).

$$\log J_{max} = -4.612 - 0.01578 \times MW + 0.082 \times RB \quad (2)$$

Figure 2 shows a plot of experimental literature values of log J_{max}[26] versus log J_{max} predicted by equation (1). The original work of Magnusson *et al*.[26] also identified the inclusion of the parameters melting point (K) and hydrogen bond acceptor ability as being able to increase their linear prediction correlation with log J_{max} to 0.76. This is interesting, given the view that PSA strongly correlates with hydrogen bonding ability.[13,14] Clearly the calculation of PSA and RB does not appear to offer any significant advantage over a simple prediction of transdermal penetration potential based on MW. We have recently reported that log J_{max} has a parabolic relationship with solute lipophilicity (as defined by the logarithm of the octanol–water partition coefficient) for phenolic solutes in which there is a similar MW and PSA.[30] We also found that a parabolic relationship exists between the maximum flux of steroids penetrating human epidermis and their lipophilicity.[31]

Relationship between K_p, polar surface area and rotatable bonds

Log K_p and K_p were poorly correlated with PSA and RB or their combination (all $R^2 < 0.3$). The dependence of log K_p on MW and lipophilicity reported by Potts and Guy[25] also appears to be less sensitive to the effects of surface property descriptors, which have been reported to be of value in intestinal absorption models.

Polar surface area and other membrane permeability measures

While a number of studies have reported significant linear correlations between Caco-2 monolayer,[10,17] intestinal,[12,32] blood–brain barrier,[21] pancreas and hepatic basal membrane[33] permeability and solute PSA, it has also been recognised that a sigmoidal relationship exists between PSA and the actual absorbed fraction after oral administration, arguably the more clinically relevant measure.[14,17,21] This finding suggests that while there may be value in using

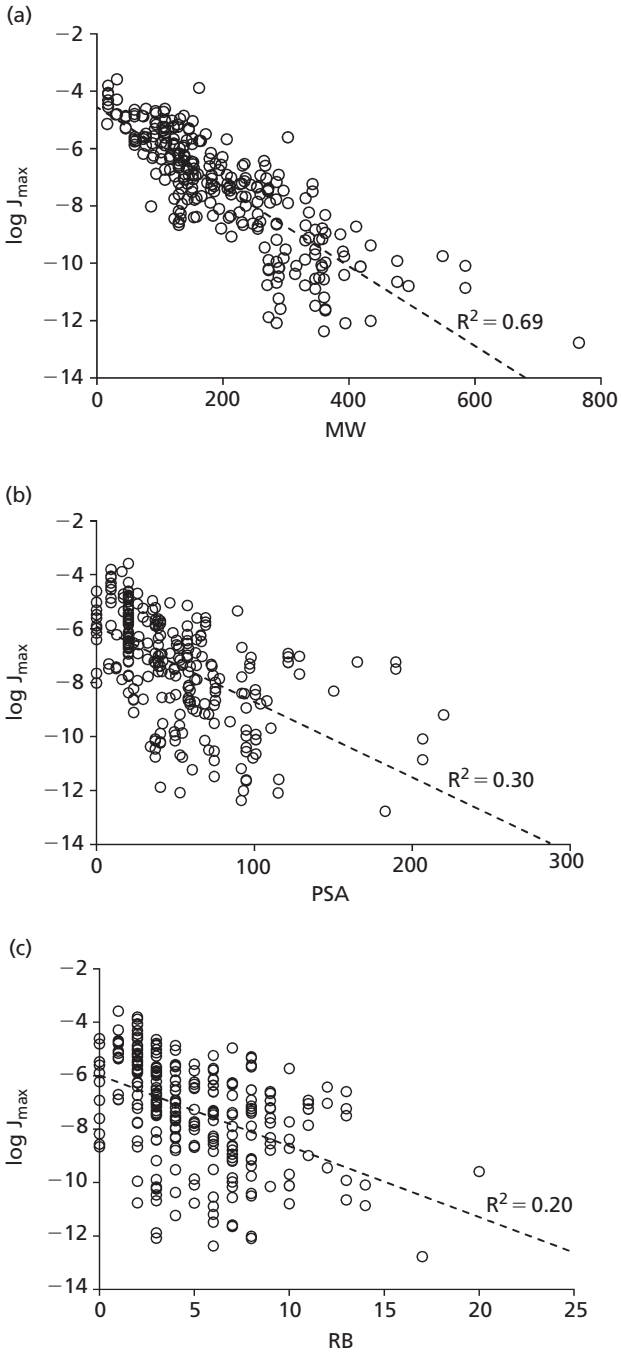

Figure 1 Relationships observed between log J_{max} and MW, PSA and RB. (a) log J_{max} and MW (R^2 = 0.688); (b) log J_{max} and PSA (R^2 = 0.301) and (c) log J_{max} and RB (R^2 = 0.20). Dashed lines represent linear regression best fits of data. Skin permeation data was taken from our literature set reported in Magnusson *et al*.[26] PSA was derived for compounds in this dataset by the method of Ertl *et al*.[15] as described above. Rotatable bonds were defined according to the method of Veber *et al*.[11] and RB was derived for compounds in this dataset by summation. MW, molecular weight; PSA, polar surface area; RB, number of rotatable bonds; J_{max}, maximum flux

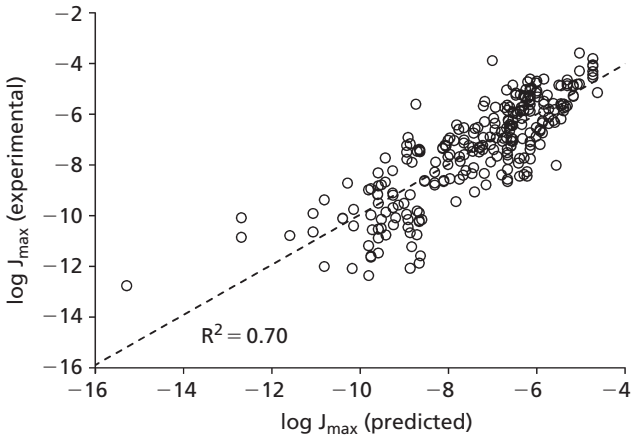

Figure 2 Relationship between experimental values of log J_{max} and predicted log J_{max}. Experimental J_{max} values from the literature[26] compared to predicted log J_{max} values using MW and RB, derived from stepwise linear regression, as shown in equation (2). The dashed line represents linear regression best fit of data ($R^2 = 0.70$). MW, molecular weight; RB, number of rotatable bonds; J_{max}, maximum flux

PSA as a screening tool to assess large drug databases to eliminate unlikely candidates, its ability to predict actual oral absorption data in humans may be limited. Similar to our current findings with transdermal absorption potential, the use of PSA in the prediction of pulmonary absorption has also been less successful. In contrast to intestinal and blood–brain barrier transport, pulmonary epithelium appeared to be more permeable to compounds with high molecular PSA when drugs were administered by intratracheal nebulisation in rats.[32] Data from the same study also showed that, despite the significant correlation (R^2 = 0.98) observed between PSA and the log of human intestinal absorption coefficients, the apparent relationship with the log of Caco-2 permeability was less well correlated (R^2 = 0.499). Indeed, the data show no significant relationship between the values presented for human intestinal absorption and Caco-2 monolayer permeability for the compounds tested, bringing into question the robustness of Caco-2 monolayer permeability as a surrogate measure of intestinal absorption potential.[32]

We therefore examined data from a number of studies of intestinal and Caco-2 permeability in relation to drug structure and determined the equivalent linear correlations with solute PSA. The results shown in Table 1 indicate that although significant relationships were observed in some studies, not all of the data indicated the existence of any meaningful relationships. The pooled Caco-2 membrane permeability data are shown in Figure 3, where a poor correlation with PSA was observed. This analysis suggests that although PSA may be an effective tool for screening for absorption potential for some groups of similar solutes, it is not suitable for use in such interpretation with groups of solutes with widely varying properties.

As part of the present study we examined the value of MW as a predictor of membrane permeability using the data from a number of intestinal absorption and Caco-2 membrane permeability studies. The data in Table 1 also show that MW offers little value in these estimations, except in the case of the work by Tronde *et al.*,[32] where a correlation of 0.94 was observed between the log of intestinal absorption and MW. It was also noted in this study that a significant linear correlation exists between PSA and MW (R^2 = 0.95) that was not present in any of the other studies and may therefore have been due to the selection of the eight compounds used in their study. Our most recent study, involving an examination of the solute structural determinants of pancreatic and hepatic permeabilities, showed that whilst PSA was important, MW was a non-significant contributor to the regressions.[33]

Table 1 Correlation between literature values for Caco-2 cell monolayer or intestinal membrane permeability and solute PSA or MW

Experimental model	R^2 (PSA)	R^2 (MW)	R^2 (PSA vs. MW)	Reference
Caco-2 monolayer	0.14	0.02	0.40	Hilgers *et al.*, 2003[29]
Caco-2 monolayer	0.50	0.37	0.95	Tronde *et al.*, 2003[32]
Caco-2 monolayer	0.25	0.06	0.00	Zhu *et al.*, 2002[34]
Caco-2 monolayer	0.75	0.00	0.31	Stenberg *et al.*, 2001[17]
Caco-2 monolayer	0.98	0.20	0.22	Palm *et al.*, 1998[10]
Human intestine	0.98	0.94	0.95	Tronde *et al.*, 2003[32]
Human jejunum	0.21	0.00	0.21	Winiwarter *et al.*, 2003[18]
Human jejunum	0.35	0.01	0.20	Winiwarter *et al.*, 1998[12]

Linear correlation coefficients (R^2); log permeability, cm/s $\times$ 10^{-6}. The correlation between polar surface area (PSA) and molecular weight (MW) for compounds used in these studies is also shown.

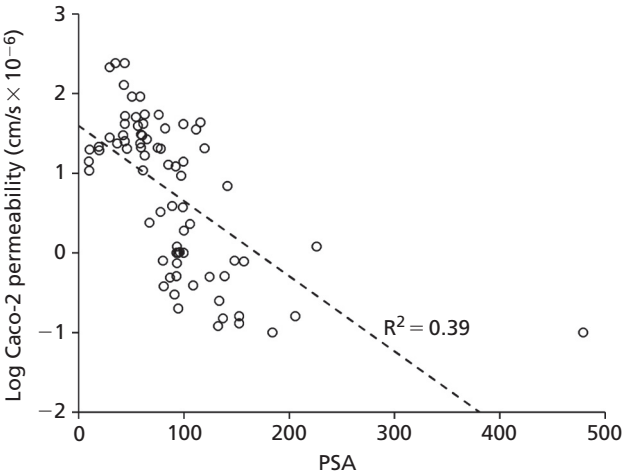

Figure 3 Relationship between Caco-2 monolayer permeability and solute PSA (R^2 = 0.41). Based on combined literature data.[16,32,34] PSA, polar surface area

Maximum membrane absorption measures

The intestinal absorption equivalent of J_{max} is the MAD, which predicts the theoretical dose of drug that could maximally be absorbed across rat intestine, based on consideration of intestinal permeability, solute solubility, intestinal volume and residence time.[29,34] MAD has been suggested to be a useful parameter to predict bioavailability

Table 2 Correlation observed between literature values for log maximum absorbable dose (log MAD) and solute PSA, log PSA or MW

Experimental model	R^2 (PSA)	R^2 (log PSA)	R^2 (MW)	Reference
Caco-2 monolayer	0.70	0.74	0.12	Hilgers *et al*., 2003[29]
Caco-2 monolayer	0.25	0.16	0.58	Tronde *et al*., 2003[32]
Caco-2 monolayer	0.02	0.02	0.17	Zhu *et al*., 2002[34]
Caco-2 monolayer	0.73	0.74	0.00	Stenberg *et al*., 2001[17]
Caco-2 monolayer	0.83	0.84	0.01	Palm *et al*., 1998[10]
Human intestine	0.05	0.02	0.46	Tronde *et al*., 2003[32]
Human jejunum	0.19	0.12	0.09	Winiwarter *et al*., 2003[18]
Human jejunum	0.04	0.02	0.59	Winiwarter *et al*., 1998[12]

Linear correlation coefficients (R^2). PSA, polar surface area; MW, molecular weight.

for the screening of early drug candidates for oral delivery[35] and outperformed Caco-2 cell permeability coefficients for a series of oxazolidinones, but with a tendency to underestimate absorption for high permeability, low solubility solutes.[29] Although there was no direct linear correlation between MAD and either bioavailability or Caco-2 permeability apparent in the data presented in that study, log–log transformation of the data produced an R^2 value of approximately 0.7. The use of PSA or log PSA to predict log MAD for these solutes gave R^2 values of 0.70 and 0.74, respectively, suggesting that the properties of molecules encompassed in the PSA parameter are significant in estimating maximum absorption potential.

We investigated whether MAD (calculated from intestinal and Caco-2 permeability data presented in the literature using the equation outlined by Hilgers *et al*.[29]) correlated better with PSA or log PSA than the permeability coefficients shown in Table 1. The results of these analyses indicated that neither PSA nor log PSA were robust predictors of MAD (Table 2), with correlations above 0.7 seen in only three of the eight datasets examined. Furthermore, the most robust predictor of transdermal J_{max}, MW, was of no value in the estimation of intestinal MAD values (Table 2).

Conclusions

This work shows that the diffusion processes occurring across intestinal and external epidermal membranes cannot be estimated by the same simple set of solute molecular properties, implying that different combinations of partitioning and diffusion processes may be dominating in each of these two types of membrane. The MW dependency of the maximum absorbable transdermal dose, J_{max}, suggests that a pore size restriction clearly exists in this membrane. The lack of correlation with molecular size for absorption across intestinal, the Caco-2 model membranes or pancreas and hepatic membranes suggests either that no pore size restriction exists within the MW size range of solutes studied, or that active transport processes are also involved in permeation through this membrane which mask the dependency of pure passive diffusion components on molecular size. The lack of robustness of the relationship between the log of membrane permeability and PSA in intestinal or Caco-2 models also implies that these molecular properties may only dominate in groups of similar compounds, limiting its usefulness in the screening and selection of novel structures in the pharmaceutical industry.

While the use of structural parameters to predict transdermal permeability is well known and of some value, the approach of selecting transdermal candidate compounds from predictions based on permeability through other membranes is, to our knowledge, a novel one. The rapid growth in the transdermal field has led to great interest in predictive methods and simple, accurate in-vitro techniques would be attractive. However, in this work, we have demonstrated that such a predictive goal cannot be achieved yet, based on available literature data. Better controlled, specifically designed investigations may be necessary to advance knowledge in the area.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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