The relation between molecular properties of drugs and their transport across the intestinal membrane

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ABSTRACT

The aim of this study was to investigate the relationship between the intestinal absorption of structurally diverse model drugs across the rat intestinal mucosa and their molecular properties. Permeability coefficients for 13 compounds were determined in anaesthetized rats. Drug solution in phosphate buffered saline (PBS) was perfused through the intestinal segment with flow rate of 0.21 ml/min and samples were taken from outlet tubing at different time points up to 90 min. The permeability values ranged from 1.6×10^{-5} to 2×10^{-4} cm/sec for atenolol and ibuprofen respectively. Molecular properties of drugs including the number of hydrogen bond donors and acceptors, log P, logD, topological polar surface area and number of rotatable bonds were considered. The results indicated that compounds which meet 10 or fewer number of rotatable bonds and topological surface area equal to or less than 140 A° have a high probability of good intestinal permeability and fraction of dose which is absorbed in human. Moreover the results indicated that lower number of hydrogen bond counts and higher logD and logP values are associated with higher permeability and bioavailabilty of drugs. Therefore the experimental and computational methods could be used for the prediction of intestinal drug permeability.

Keywords: F_a, Permeability, PSA, Rotatable bond, Hydrogen bond, LogP.

INTRODUCTION

The optimization of the biologic activity plays an important role in development of new chemical entities (NCEs). Therefore, one of the main aims for drug research is to gain sufficient understanding of the molecular properties that limit the intestinal drug absorption. Since the cost of drug development is many times larger than the cost of drug discovery, predictive methodologies which help selection of orally bioavailable drug candidates are of profound significance (1). Several in vitro and in situ models can be used early in drug development to determine the intestinal absorption of a drug (2, 3). Among these the single-pass intestinal perfusion (SPIP) technique in rat is the most frequently used technique which provides conditions closer to what is faced following oral administration (4). The physicochemical descriptors of drug molecules that are believed to influence the transport of drugs across the intestinal barrier have been previously used to predict the oral fraction absorbed in human. According to Lipinski (5), four parameters namely molecular weight, log P, the number of hydrogen bond donors and acceptors are thought to be associated with solubility and permeability of drugs which are the two basic requirements for any drug to have good pharmacokinetic parameters. In addition, other properties such as molecular flexibility and polar surface area have been recognized to have impact on oral bioavailability of drugs (6). The intestinal permeability values for 15 compounds (13 of them are passively transported and two are actively transported) in rats using SPIP technique have been determined in our lab and correlated with human absorption parameters. In this study the relevant molecular and physico-chemical properties were considered to investigate the relationship between the intestinal absorption of structurally diverse model drugs in rat and their molecular properties.

MATERIALS AND METHODS

Chemicals

Naproxen was provided from Sigma (St. Louis, MO, USA) and ketoprofen was from Wako (Osaka, Japan). Furosemide and hydrochloro-thiazide were provided by Shasun Chemicals and Drugs LTD. (Pondicherry, India). Propranolol was provided by ICI-Pharma (Madrid, Spain) and

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metoprolol was from Ciba-Geigy (Barcelona, Spain). Phenol red was purchased from Sigma chemical company (St. Louis, MO, USA). Acetonitrile and methanol were HPLC grade and obtained from Merck (Darmstadt, Germany). KH_2PO_4, NaH_2PO_4, Na_2HPO_4, Orthophosphoric acid, NaOH, NaCl, glacial acetic acid and triethylamine were purchased from Merck (Darmstadt, Germany) as well. Double distilled water was used during the entire HPLC procedure. All other chemicals were from Sigma Chemical Co. (St. Louis MO, USA).

Measurement of rat intestinal permeability coefficients

In situ permeation studies were performed using established methods adapted from the literature (7, 8). Briefly, male Wistar rats (weight, 200-250 g; age, 7-9 weeks) were maintained on 12 h light-dark cycle and fasted 12-18 h before experiment, but drinking water was readily accessible. The rats were anaesthetized using an intraperitoneal injection of pentobarbital (60 mg/kg) and placed on a heated pad to keep normal body temperature. By making a midline abdominal incision, a 10 cm section of the proximal rat jejunum was located gently with plastic tubing (1 mm i.d., 2.15mm o.d.), rinsed with saline (37^{o}C) and attached to the perfusion assembly which consisted of a syringe pump (Palmer, UK) and a 60 ml syringe was connected to it. Care was taken to handle the small intestine gently and to minimize the surgery in order to maintain an intact blood supply. The preparation time took less than 30 minutes. Blank perfusion buffer was infused for 10 min by a syringe pump followed by perfusion of compounds at a flow rate of 0.2 ml/min for 90 min. The perfusate was collected every 10 min in microtubes. The length of segment was measured at the end and finally the animal was euthanized with a cardiac injection of saturated solution of KCl. Samples were frozen immediately and stored at -20^{o}C until analysis. In all animal studies "Guide to the care and use of experimental animals" by Canadian Council on Animal Care, was followed (9).

Composition of perfusion solution

The composition of perfusion solution was as follows: 40 mM Na_2HPO_4 (anhydrous), 26 mM $NaH_2PO_4.2H_2O$ and 119 mM NaCl. Phenol red (0.7 mM) was added to the solution as a non-absorbable marker in each experiment. The pH of the prepared buffer was adjusted to 7.2. Preliminary experiments showed that there was not considerable adsorption of the compounds on the tubing and syringe. The stability of drugs was assessed by incubation at 37 ^{o}C for 2 hours. In the case of labile compounds, solutions were protected from light and air. There were no sign of degradation of compounds during this period of time.

Analytical methods

All samples were analyzed by the reverse-phase high performance liquid chromatography. For samples containing naproxen and ketoprofen, the mobile phase was a mixture of 19.9 % methanol, 27.9 % of acetonitrile, 51.8 % water and 0.4 % triethylamine (adjusted to pH 3.2) (10). The mobile phase for furosemide, antipyrine and hydrochlorothiazide samples consisted of 42% acetonitrile, 58% water, 0.9 % glacial acetic acid and 0.1% triethylamine (adjusted to pH 5.6) (11). Metoprolol and propranolol were analyzed using 55% methanol, 45% of 0.05 M KH_2PO_4 aqueous solution (adjusted to pH 6) and 0.2 % triethylamine as mobile phase. Detection wave-lengths were 270, 280 and 227 nm respectively. For other drugs the composition of mobile phases and detection wavelengths were as follows: piroxicam: 39 % acetonitrile, 61% sodium acetate 0.1 M and 0.05% triethylamine (adjusted to pH 2.6) λ=330 nm (12), atenolol: 10% acetonitrile, 90% phosphate buffer 0.67 molar (pH=7.4) and 0.2% triethylamine (adjusted to pH 3) λ=225 nm (13), cimetidine & ranitidine: 78% KH_2PO_4 0.05 M, 22% acetonitrile and 0.05% triethylamine (adjusted to pH 8) λ=229 nm, carbamazepine: 67% methanol, 33% water and 1% glacial acetic acid. λ=230 nm (14), phenol red: 45%KH_2PO_4 0.05 M and 55% methanol (adjusted to pH 2.6) λ=430 nm (15), ibuprofen: 85% Acetonitrile, 15% of 0.067 M phosphate buffer and 0.2% Orthophosphoric acid λ=254 nm (16). The mobile phases were filtered through sintered glass filter P5 (1-1.6 micron) (Winteg, Germany) and degassed in sonicator (Liarre, Italy) under vacuum and then were pumped in isocratic mode in all cases. The column used for all samples was Shimpack VP-ODS 5 µm 4.6 x 250 mm (Shimadzu, Kyoto, Japan) with a Shimpack VP-ODS 5 µm 4.6 x 50 mm guard column (Shimadzu, Kyoto, Japan). Finally the Reference Standards (RS) of compounds were used to quantitate the samples.

DATA ANALYSIS

Effective permeability coefficients (P_{eff}) were calculated from the steady-state concentrations of compounds in the collected perfusate which is considered to happen when the concentration of phenol red was at the steady state level. It was reached about 40 min after the beginning of the perfusion which is confirmed by plotting the ratio of the outlet to inlet concentrations (corrected for

water transport) versus time. The intestinal net water flux (NWF, µl/h/cm) was calculated according from the following equation:

$$NWF = \frac{(1 - [Ph.red_{(out)} / Ph.red_{(in)}]\ Q_{in}}{l}$$

Where [Ph.red $_{(in)}$] and [Ph.red $_{(out)}$] are the inlet and outlet concentrations of the non-absorbable, water flux marker phenol red. A negative net water flux indicates loss of fluid from the mucosal side (lumen) to the serosal side (blood). A positive net water flux indicates secretion of fluid into the segment (17).

P_{eff} was calculated using following equation according to the parallel tube model (18, 19):

$$P_{eff} = -Q \ln[C_{out}/C_{in}] \ /2\pi rl$$

In which C_{in} is the inlet concentration and C_{out} is the outlet concentration of compound which is corrected for volume change in segment using phenol red concentration in inlet and outlet tubing. Q is the flow rate (0.2 ml/min), r is the rat intestinal radius (0.18 cm) (18) and l is the length of the segment. It has been demonstrated that in humans at a Q_{in} of 2-3 ml/min, P_{eff} is membrane-controlled. In the rat model the Q_{in} is scaled to 0.2 ml/min, since the radius of the rat intestine is about 10 times less than that of human (17).

Calculation of the molecular descriptors for model drugs

Rotatable bonds

Molecular flexibility depends on the number of rotatable bonds in the molecule structure. It is obtained simply by counting the non-terminal, non-cyclic, single bonds except C-N amide bond (20).

Hydrogen bonding (HB) descriptors

These include a count of the number of potential HB donors and acceptors. Hydrogen bond donors were taken as any hetero atom with at least one bonded hydrogen and hydrogen bond acceptors were taken as any heteroatom without a formal positive charge, excluding halogens, pyrrole nitrogen, heteroaromatic oxygen and sulfur, and higher oxidation staes of nitrogen, phosphorus, and sulfur but including the oxygens bonded to them (6).

TPSA (Topological Polar Surface Area)

TPSA, captured as the Van der Waals surface area of all nitrogen and oxygen atoms plus their attached hydrogen atoms, was considered as an indicator for number of HB donors and acceptors (21). TPSA was calculated with a Web-based molecular descriptors calculator (http://www.molinspiration.com) using SMILES notations or chemical structure inputs.

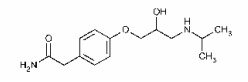
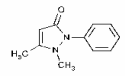
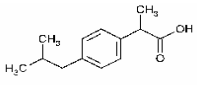
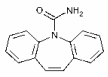
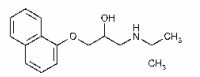
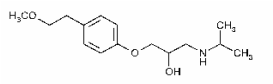
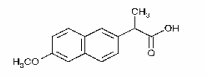
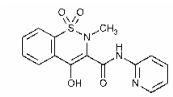
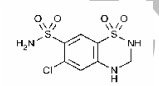
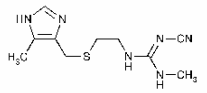
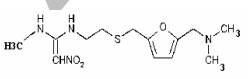
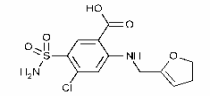
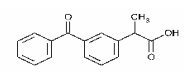
Octanol-water partition coefficients (log P)

log P values for tested compounds were taken from literature (22).

RESULTS AND DISCUSSION

In the present study, the relationship between molecular descriptors and permeability values in rats are discussed to evaluate properties which could be relevant for intestinal permeability. Table 1 shows the calculated molecular descriptors and obtained permeability coefficients for compounds tested in SPIP model. While molecular weight is the most convenient way to define molecular size, it may not be sufficient, because MW contains no information about the actual three-dimensional shape of the molecules (23). The scattered plot of permeability versus MW is shown in Fig.1 As it can be seen, there is no clear relationship between MW and permeability values, although the majority of tested compounds with high permeability have a MW lower than those of low permeability and bioavailability (MW< 270). The reason for the lack of influence of molecular weight on oral bioavailability of tested compounds is that the narrow MW range has been considered, where the correlation of the oral bioavailability with MW is reduced (6). Furthermore the probable success of molecular weight as a predictor of oral bioavailability for larger data sets might arise from the dependence of oral bioavailability on molecular weight related properties. An apparent molecular weight cutoff could be perceived because the MW-related properties, the number of rotatable bonds and corresponding molecular flexibility, are reduced below molecular weight of 500, and especially below molecular weight of 400, to the point that oral availability can reach high levels. On the other hand, hydrogen bonding could be used as a factor affecting permeability. Hydrogen bonding descriptors include the number of potential HB donors and acceptors. These descriptors neither describe the strength of the hydrogen bond nor accounts for the possibility of internal hydrogen bonds. However more sophisticated descriptors may be able to include these effects. For instance, Polar Surface Area (PSA) of a molecule encodes more hydrogen bonding information. It refers to surface area of the oxygen, nitrogen, sulfur and attached hydrogen atoms. It has been shown to correlate well with drug transport properties, such as collections of molecules or whole virtual combinatorial libraries, a new and fast methodology has been developed to calculate the PSA from fragment contributions. This method has been termed TPSA (Topological PSA). The new TPSA methodology has been validated by correlation with published data of various types of

Table 1. The chemical structures, rat intestinal permeabilities and molecular descriptors of tested compounds

Drug	Chemical structure	MW	No. HB_b	No. HB_{a+}	RB	Fa (%) (human)	P_{eff} (rat)(10^{-5}) (cm/sec)	TPSA	LogP*	LogD** pH=6.5
Atenolol		266	4	5	8	50	1.6	84.6	0.5	-2.6
Antipyrine		188	0	3	1	100	5.9	26.9	1.01	1.01
Ibuprofen		206	1	2	4	99	20	37.3	3.14	1.21
Carbamazepine		236	2	3	1	97	6.2	48.0	2.93	2.93
Propranolol		259	2	3	6	90	5.6	41.5	2.65	-0.38
Metoprolol		267	2	4	9	95	3.3	50.7	1.72	-1.48
Naproxen		230	1	3	3	100	11	46.5	2.86	0.51
Piroxicam		331	2	7	2	99	7.9	99.6	0.29	-1.13
Hydro-chlorothiazide		298	4	7	1	54	2.0	118.4	-0.15	-2.85
Cimetidine		252	3	6	8	79	4.8	88.6	0.79	0.31
Ranitidine		317	2	7	9	50	2.2	86.3	0.15	-6..48
Furosemide		331	4	7	5	61	3.3	118.7	0.74	-1.86
Ketoprofen		254	1	3	4	100	9.6	54.4	3.31	1.31

*, ** The logD and logP values for the listed compounds were taken from reference(22)
Mw: Molecular weight, **HB_d** and **HB_a**: HB donors and acceptors respectively, **RB**: number of rotatable bonds, **P_{eff}**: Effective permeability coefficients, **TPSA**: Topological Polar Surface Area, **log P**: Octanol-water partition coefficients, **log D**: Octanol-water distribution coefficients

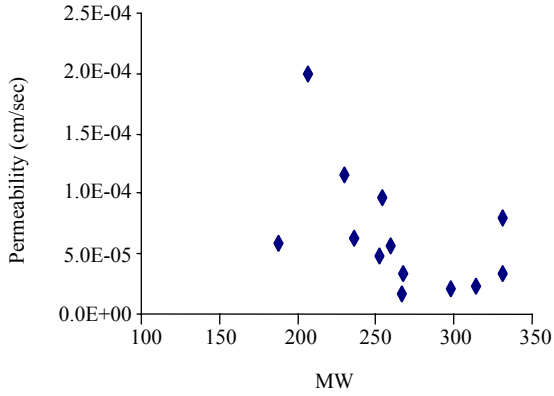


Figure 1. Plot of the molecular weight (MW) of the tested compounds vs rat intestinal permeability.

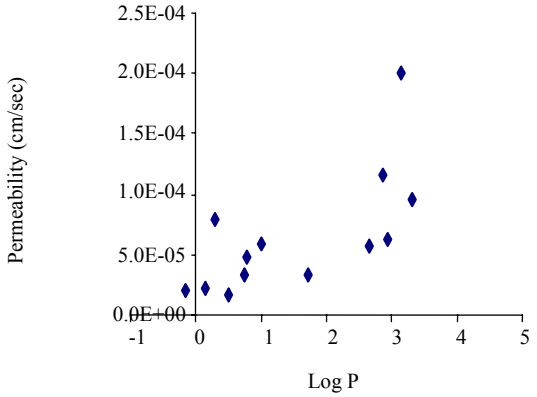


Figure 4. Plot of the logP values of the tested compounds vs rat intestinal permeabililty.

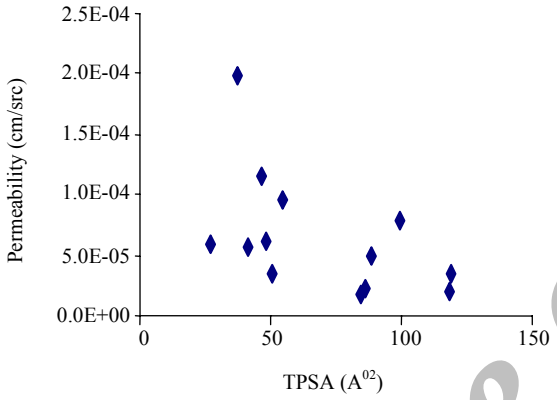


Figure 2. Plot of the topological polar surface area (TPSA) values of the tested compounds vs rat intestinal permeability.

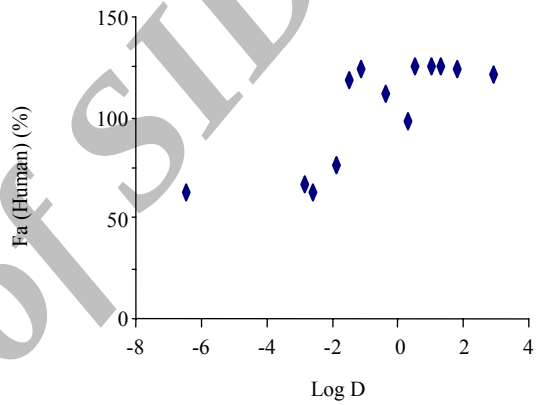


Figure 5. Plot of the logD (pH=6.5) values of the tested compounds vs Fraction of dose absorbed in human (Fa).

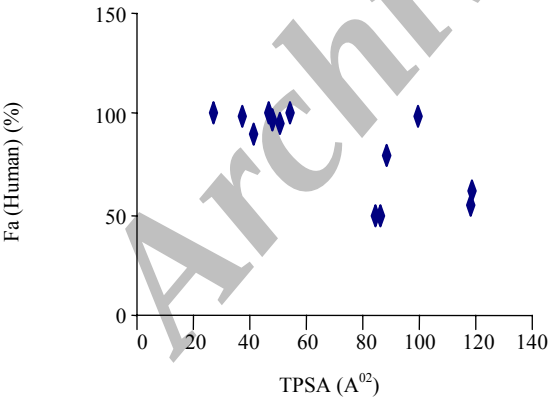


Figure 3. Plot of the topological polar surface area (TPSA) of the tested compounds vs fraction of dose absorbed in human (Fa).

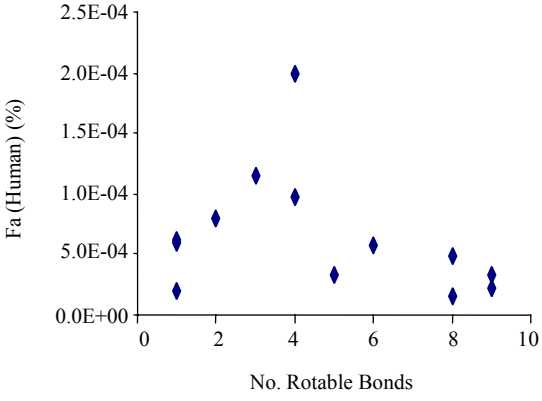


Figure 6. Plot of the number of rotatable bonds of the tested compounds vs rat intestinal permeability.

intestinal absorption, or blood-brain barrier penetration (1, 24-29). Current methodologies to calculate PSA, however, are relatively time consuming, because of the necessity to create a reasonable 3D molecular geometry and to calculate the surface itself. In order to enable virtual bioavailability screening of very large drug transport properties, including intestinal absorption, blood-brain barrier penetration, and Caco-2 cell permeability. All these datasets have been already studied by using 3D PSA. In all cases, the TPSA methodology performance is well and provides results of the same quality as the computationally much more demanding 3D PSA

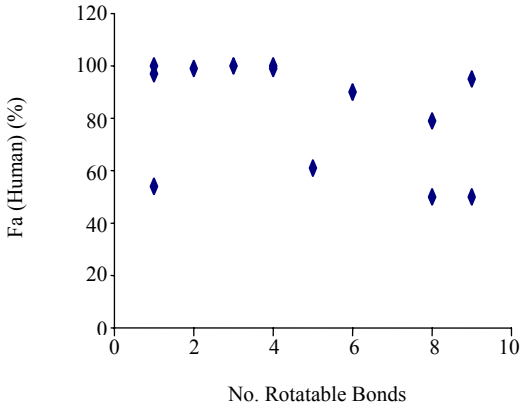


Figure 7. Plot of the number of rotatable bonds of the tested compounds *vs* Fraction of dose absorbed in human (Fa).

(21). Fig 2 indicates that the permeability values of diverse set of tested compounds were poorly described by TPSA. This may be caused by the lack of scaling of the atomic surface areas making up TPSA. That is, in the present model the surface area of each atom type that contributes to TPSA is given equal weight. However, the contribution to membrane permeability varies by the type of atom. From Fig.3 it is clear that drugs with a TPSA < 60 $Å^2$ will be completely absorbed ($F_a \geq$ 90), While drugs with a TPSA values more than that will be absorbed to a less degree. Of course all compounds which have been tested in this nvestigation have TPSA values less than 140 $Å^2$ and all have an acceptable $F_a \geq 40\%$ (6). This finding has been confirmed by other investigators using larger datasets. This suggests that when the polarity of the drug molecule decreases, the transcellular transport route starts to dominate and the fraction absorbed increases. The polar surface area and hydrogen bond count also tend to increases by increase in molecular weight. For most biological processes in which biological membranes are involved, the lipophilicity is a key descriptor (23). Particularly 1-octanol/water distribution coefficients at a selected pH (log D) and 1-octanol/water partition coefficients (log P), have received considerable attention in quantitative structure-activity relation (QSAR) approaches. This relies on the assumption that more lipophilic drugs will partition faster into the lipid cell membranes. However, it is well known that lipophilicity (as defined by the pH-partition hypothesis) only gives an approximate indication of drug absorption. As it is seen in Fig.4, a coarse correlation is found between octanol/water partition coefficients and permeabilities estimated in SPIP model. From a sigmoidal permeability-lipophilicity relationship in Caco-2 monolayers (30), it was assumed that compounds should have comparable MW. That means the position of a permeability lipophilicity curve on the lipo-philicity axis should be considered as a function of MW. Therefore it is proposed to define several lipophilicity ranges for a particular MW range, in such a way that each range represents a zone of different distribution behavior. Fig.5 shows that in general the percent absorption increases by increase in log D and tends to reach to a maximum. It has been shown that the molecular flexibility correlates with molecular weight, that is, larger compounds would be more flexible (6, 31). The relationship between molecular flexibility and permeability values for tested compounds is evaluated in Fig.6. The apparent dependence of oral bioavailability on rotatable bonds is shown in Fig. 7. As it can be seen the majority of compounds with six or fewer rotatable bonds meet $F_a\% \geq 90$ criterion. Regarding that all tested compounds have the number of rotatable bonds equal to or less than 10, and considering the relatively narrow range of MW for tested compounds (187 < MW<348), all compounds have satisfactory oral bioavailability. In fact in the MW range below 400, almost all of the compounds have 10 or fewer rotatable bonds, suggesting that there is a molecular weight threshold below which flexibility is sufficiently limited to have little impact on $F_a\%$.

CONCLUSION

In conclusion, the results suggested that experimental and computational methods could be used to predict the intestinal drug permeability and to facilitate the design of viable new drug candidates. Considering the molecular properties of model drugs, higher intestinal permeability and human fraction of dose absorbed are associated with reduced molecular flexibility, as measured by the number of rotatable bonds and lower polar surface area or lower hydrogen bond counts.

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