Drug Liposome Partitioning as a Tool for the Prediction of Human Passive Intestinal Absorption

K. Balon,[1] B. U. Riebesehl,[1,3] and B. W. Müller[2]

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Purpose. Appropriate physicochemical parameters are desired for the prediction of passive intestinal drug absorption during lead compound selection and drug development.

Methods. Liposome distribution coefficients measured titrimetrically and solubility data at pH 6.8 were used to characterize 21 structurally diverse ionizable drugs covering a range from <5% to almost complete absorption.

Results. A sigmoidal relationship was found between the percentage of human passive intestinal absorption and a new absorption potential parameter calculated from liposome distribution data and the solubility-dose ratio. In contrast, the human absorption data did not correlate with an octanol-based absorption potential or partitioning data alone. Poor correlations were found between liposome and octanol partitioning of ionic species or nonionic bases indicating the profound differences of the partitioning systems.

Conclusions. Liposome distribution coefficients of ionizable drugs derived by a pH-metric titration were successfully used to calculate a parameter that correlates with the percentage of passive intestinal absorption in humans. Profound differences between liposome and octanol partitioning were found for a highly diverse set of species. This titration technique may serve to generate liposome partitioning data for the selection and optimization of lead compounds and in drug development.

KEY WORDS: liposomes; octanol; partitioning; solubility; absorption; phospholipids.

INTRODUCTION

The implementation of combinatorial chemistry and high throughput screening in drug research has led to a much higher number of active drug candidates. The new screening methods tend to suggest also compounds with properties critical to absorption such as low solubility, high lipophilicity and high molecular weight (1). Therefore bioavailability surrogate parameters are utilized for selecting lead compounds with a promising bioavailability. Caco-2 cell permeability has been used successfully as an *in vitro* absorption parameter. Unfortunately Caco-2 cell cultures tend to show variable permeability and require laborious cultivation (2). Another disadvantage is the often observed steep correlation between permeation and absorption even on logarithmic scales. Physicochemical parameter-based estimation methods are attractive because of their throughput capacity, reproducibility and because they do not involve cumbersome cell cultivation. Some of the limitations associated with these estimations include their inability, to predict active transport as well as to catalyze enzymatic degradation of drugs. Many attempts have been made for correlating the *in vivo* absorption with drug lipophilicity. 1-octanol has been extensively used as it appeared to give the best correlations among the bulk solvents showing phase separation (3). However, good correlations were only found within homologous series of compounds. Octanol can interact with solutes by hydrophobic and H-bond interactions but fails to mimic the pronounced interfacial character of the bilayer structure of biomembranes and ionic interactions between membrane phospholipids and solutes. Historically it was hypothesized that only neutral molecules can penetrate through membranes (4). Therefore, attention was paid to the partition coefficients of the neutral forms of ionizable compounds. Numerous well-absorbed drug substances appear as ions at the absorptive sites in the small intestine. The relatively low partition coefficients in octanol for the ionic species cannot explain the absorption of these drugs. Studies with membrane lipid vesicles (5) and immobilized artificial membrane (IAM) columns (6) have shown important differences in interactions between drugs with phospholipids and octanol, especially of the ionic forms. Liposomes made from phospholipids are a partition system with characteristics similar to those of biomembranes. The Sirius pH metric logP titration method now allows the convenient and automated determination of liposome partitioning. Drug-liposome interactions are characterized by hydrophobic, H-bond and ionic interactions. High partition coefficients of ionic species are a combined effect of direct ionic interaction between drug and phospholipids and also interactions with the lipophilic part of the bilayer (7). The highly ordered bilayer structure of the phospholipid vesicles mimics also the impact of the membrane bilayer structure on the ability of a drug to permeate (8). A flexible molecule may be more suited for membrane penetration than a rather bulky molecule. A linear correlation between octanol and liposome logP for small neutral molecules has been proposed (9). Another publication describes a correlation between the octanol and liposome logP of the neutral species of ionizable drugs (10). Phagocytosis of solid drug through the intestinal mucosa is quantitatively negligible. Prior to absorption the administered single dose has to be able to dissolve in the intestinal fluid. Consequently, a factor for the solubility/dose relationship has to be included in the equation toward prediction for oral absorption.

MATERIALS AND METHODS

Materials

The chemical structures of the drug substances used in this investigation are given in Fig. 1. Acetylsalicylic acid(1), acyclovir (acycloguanosine)(2), allopurinol(3), amiloride hydrochloride(4), atenolol(5), diclofenac sodium(6), famotidine(7), furosemide(9), ibuprofen(10), miconazole nitrate(11), paromomycin sulfate(15), and propranolol hydrochloride(16) were purchased from Sigma Chemie Vertriebs GmbH (Deisenhofen, Germany). Fluoxetine hydrochloride(8), moxonidine(12), nizatidine(13), olanzapine(14), and xipamide(19) were supplied by Lilly Forschung GmbH (Hamburg, Germany).

[1] Lilly Forschung GmbH, Pharmaceutical Product Development, Wiesingerweg 25, D-20253 Hamburg, Germany.

[2] Department of Pharmaceutics and Biopharmaceutics, Christian Albrecht University, Gutenbergstrasse 76, D-24118 Kiel, Germany.

[3] To whom correspondence should be addressed. (e-mail: riebesehl_bernd@lilly.com)

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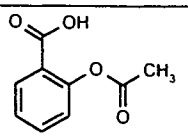
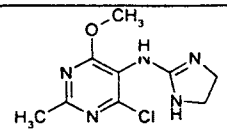
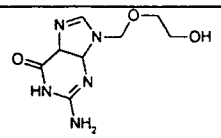
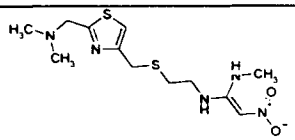
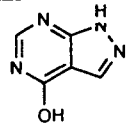
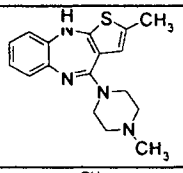
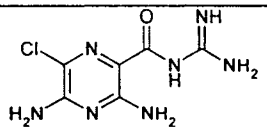
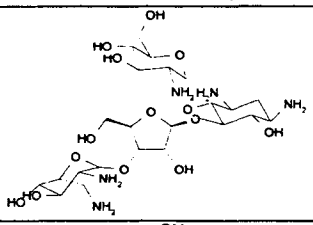
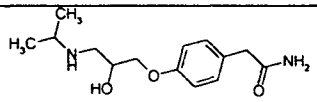
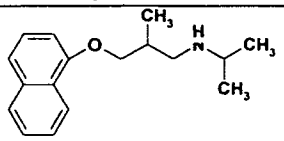
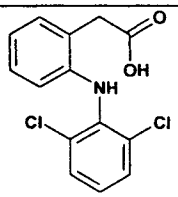
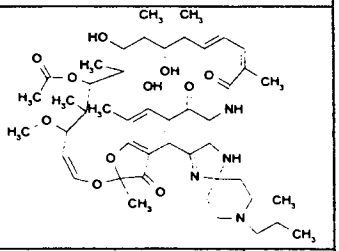
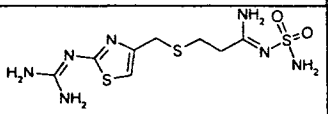
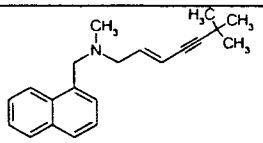
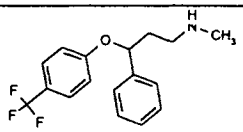
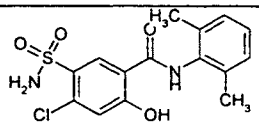
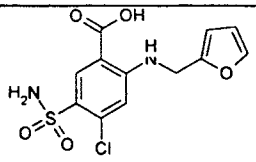
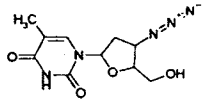
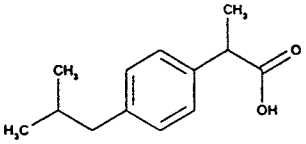
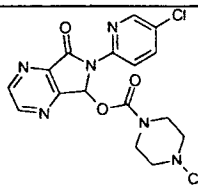
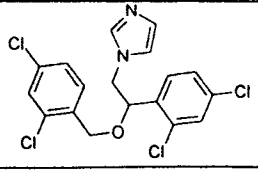
1 Acetylsalicylic acid		12 Moxonidine	
2 Acyclovir		13 Nizatidine	
3 Allopurinol		14 Olanzapine	
4 Amiloride		15 Paromomycin	
5 Atenolol		16 Propranolol	
6 Diclofenac		17 Rifabutine	
7 Famotidine		18 Terbinafine	
8 Fluoxetine		19 Xipamide	
9 Furosemide		20 Zidovudine	
10 Ibuprofen		21 Zopiclone	
11 Miconazole			

Fig. 1. Structures of drug substances.

Rifabutine(17) was supplied by Pharmacia Spa. (Italy). Terbinafine hydrochloride(18) was supplied by Novartis Pharma AG (Basel, Switzerland). Zidovudine(20) was supplied by The Wellcome Foundation Ltd. (London, UK). Zopiclone(21) was supplied by Rhône-Poulenc Rorer GmbH (Köln, Germany). Potassium chloride, potassium dihydrogenphosphate, 1-octanol, HCl-Titrisol® and KOH-Titrisol® were purchased from Merck KGaA (Darmstadt, Germany). Polysorbate 80 was purchased from Caelo (Hilden, Germany). Epikuron 200® was supplied by Lucas Meyer GmbH (Hamburg, Germany). Epikuron 200 is a purified soybean phosphatidylcholine with a phospholipid content of >92% consisting of glycerophosphocholin esters with various mainly unsaturated fatty acids, <3% lyso-phosphatidylcholine, <2% other phosphatidylcholines, <0.8% water, <2% oil and <0.2% α-tocopherol. With 56 – 60% of total fatty acids, linoleic acid is the most commonly encountered acid.

Experimental

Liposome Preparation

Sonicated Small Unilamellar Vesicles (S-SUV) were prepared as follows: 1.6 grams of phospholipid were dissolved in a small amount of methanol in a 200 ml round bottom flask. A solid phospholipid film was formed by vacuum evaporation of methanol at 50°C using a rotary evaporator. As determined by thermogravimetry, the resulting film contained less than 5% volatiles after 30 min vacuum drying. The phospholipid film was dispersed with 14.4 ml of a 0.15 M potassium chloride solution, resulting in a phospholipid concentration of 100 mg/ml; 15 ml of this phospholipid dispersion was sonicated for 20 min. in a 20 ml vial using a Bandelin Sonopuls HD70 sonifier equipped with a TT13 sonotrode (Bandelin Electronic GmbH, Berlin, Germany) at 50% amplitude setting with argon purge and cooling with ice water.

Determination of Partition Coefficients

Titrations were performed on PCA101® and GLPKA® automatic titrators (Sirius Analytical Instruments Ltd., Forest Row, UK). The processing of titration data was carried out using the PKALOGP® version 5.01 software. Four titrations at different ratios of phospholipid to water and phospholipid to drug were carried out for each compound to evaluate the partitioning of both the neutral and the ionized species (see Table I). Titrations were carried out at 37°C at an ionic strength of 0.15 M potassium chloride as acidimetric assay starting at pH 10.5. Zopiclon had to be titrated alkalimetrically starting at pH 3.5 respecting its instability under alkaline conditions. Olanzapine had to be dissolved at pH 3.5 before starting the assay at pH 10.5. The determination of octanol/water partition coefficients by pH-metric titration is described in detail elsewhere (11–12).

Table I. General Assay Protocol for $logP_{suv}$ Titrations

[Lipid]:[Drug][a]	ml Lipid	ml H_2O	Lipid/Water[b]
3	0.025	20	0.0013
10	0.1	15	0.0067
40	0.2	8	0.0250
100	1	20	0.0500

[a] [Lipid]:[Drug]: molar ratio of phospholipid to drug.
[b] (Lipid/Water): volume ratio of phospholipid to water.

Solubility

Solubility was determined in Simulated Intestinal Fluid (USP XXIII) at pH 6.8 without addition of pancreatin (KH_2PO_4 6.8 g = 0.05 M, polysorbate 80 0.01 g, KOH qs pH 6.8, distilled water ad 1000.0). The surface tension was adjusted with polysorbate 80 to a value of approximately 40 dyn/cm as reported for human gastric juice (13). The polysorbate 80 concentration was below the CMC (14). The procedure used for the solubility determination gives ranges of solubility from 0.02–10 mg/ml. The solubilities given in Table II are the mean values for the respective solubility ranges.

RESULTS AND DISCUSSION

Liposome Versus Octanol Partitioning

Figure 2 shows the comparison of partitioning of neutral and anionic species of acids in the liposome and octanol-water system. A linear correlation with a low slope of 0.37 and an intercept at 2.2 was found for the neutral acids. This implies that the liposomal systems is less discriminating than octanol and that especially compounds with a low octanol lipophilicity may show unexpected high partitioning to phospholipid bilayers. In contrast, the partitioning of the neutral forms of the most lipophilic acids diclofenac and ibuprofen is almost as high in the liposomal system as in the octanol system. The logP values of the neutral species of the acids ranged from log −1.8 to 4.2 in the octanol and only from log 1.7 to 4.3 in the liposomal system. A poor correlation with a low slope of 0.33 and an intercept at 2.6 was found for the anionic species. Here the partitioning of the anions was higher by at least two orders of magnitude in the liposomal system except for rifabutine. A consequence of the considerable anion partitioning in the liposomal system is also a small difference between the partition

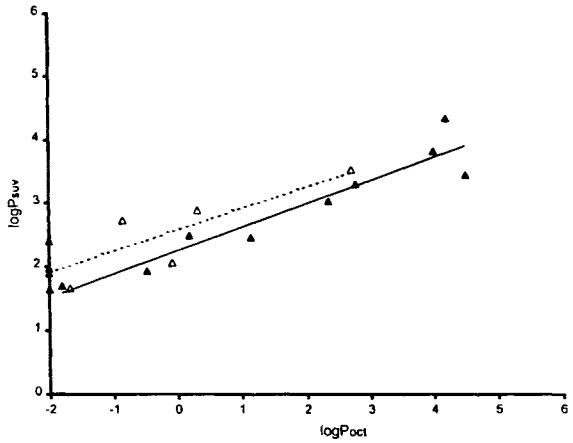


Fig. 2. Liposome versus octanol partitioning of the acids. ▲ - neutral forms ($logP_{SUV} = 0.37\ logP_{Oct} + 2.2$, $R^2 = 0.89$), △ - anionic forms ($logP_{SUV} = 0.33\ logP_{Oct} + 2.6$, $R^2 = 0.72$). The solid line represents the correlation for the neutral forms. The dotted line represents the correlation for the anionic forms.

Table II. Properties of Drug Substances

No.	Compound	pK_a 37°C	log P_{suv} neutral	Error	log P_{suv} ion	Error	logP_{Oct} neutral	Error	log P_{Oct} ion	Error	log D_{suv} pH6.8	logD_{Oct} pH6.8	Solubility pH6.8 [mg/ml]	Dose [mg]	AP_{SUV}	AP_{Oct}	Intestinal absorption %
1	Acetylsalicylic acid	3.41	2.4	0.1	1.6	0.1	1.1	0.1	−2.0	0.1	1.6	−2.0	0.8	500	1.2	−2.4	90 (19)
2	Acyclovir	2.16 9.04	1.7	0.1	2.0	0.1	−1.8	0.1	−2.0	0.1	1.7	−1.8	0.8	200	1.7	−1.3	20 (20)
3	Allopurinol	9.00	2.5	0.1	2.7	0.1	0.2	0.1	−0.9	0.2	2.5	0.1	0.4	300	2.0	−0.6	90 (21)
4	Amiloride[a]	8.35	1.8	0.1	1.6	0.1	0.1	0.1	−0.7	0.2	1.6	−0.6	0.1	5	2.3	0.4	50 (22)
5	Atenolol	9.25	2.2	0.1	1.0	0.2	0.5	0.1	−1.5	0.3	1.0	−1.3	10.0	50	2.7	0.4	56 (23)
6	Diclofenac	4.01	4.3	0.1	2.9	0.1	4.2	0.1	0.3	0.1	2.9	1.4	0.8	50	3.5	2.3	99 (24)
7	Famotidine	6.56 11.02	2.3	0.1	1.7	0.1	−0.8	0.1	−0.2	0.1	2.1	−1.0	0.8	40	2.8	−0.5	45 (25)
8	Fluoxetine	9.62	3.0	0.1	2.2	0.1	4.5	0.1	1.5	0.1	2.1	1.9	2.5	30	3.5	3.3	80 (26)
9	Furosemide	3.61 10.24	3.0	0.1	1.9	0.1	2.6	0.1	−2.0	0.1	1.9	−1.0	0.8	40	2.6	−0.1	65 (22)
10	Ibuprofen[a]	4.45	3.8	0.1	2.1	0.1	4.0	0.1	−0.1	0.1	2.1	1.6	2.5	200	2.6	1.6	80 (27)
11	Miconazole	6.12	3.7	0.3	2.9	0.3	4.9	0.1	1.2	0.3	3.6	4.5	0.02	250	1.9	3.1	25 (22)
12	Moxonidine[b]	7.36	1.8	0.1	1.3	0.1	0.9	0.1	−0.2	0.1	1.5	0.4	0.8	0.3	4.3	3.1	99 (28)
13	Nizatidine	2.44 6.75	3.0	0.4	2.8	0.4	−0.2	0.1	−0.9	0.1	2.9	−0.3	10.0	300	3.8	0.5	99 (25)
14	Olanzapine	5.44 7.80	3.7	0.1	2.7 2.3	0.1 0.1	3.0	0.1	0.0	0.1	3.0	2.0	0.02	10	2.7	1.7	75 (29)
15	Paromomycin	5.99 7.05 7.57 8.23 8.90	1.7	0.1	1.2	0.1	−2.0	0.1	−2.0	0.1	−0.6	−2.0	10.0	250	0.4	−1.0	3 (22)
16	Propranolol	9.14	3.2	0.1	2.5	0.1	3.4	0.1	0.5	0.1	2.5	1.2	7.5	80	3.9	2.9	99 (30)
17	Rifabutine	6.90 9.37	3.4	0.1	3.5 2.7	0.1 0.1	4.5	0.1	2.7 2.0	0.3 0.1	3.2	4.3	0.1	150	2.4	2.8	53 (31)
18	Terbinafine	7.05	5.0	0.2	3.0	0.2	6.0	0.1	2.3	0.1	4.6	5.5	0.02	250	2.9	3.5	80 (32)
19	Xipamide	4.58 10.47	3.3	0.1	1.7	0.1	2.8	0.1	−1.7	0.3	1.7	0.5	0.8	20	2.7	1.5	70 (22)
20	Zidovudine	9.45	1.9	0.1	2.4	0.1	−0.5	0.1	−2.0	0.1	1.9	−0.7	7.5	100	3.2	1.6	90 (33)
21	Zopiclone	6.76	1.8	0.2	1.4	0.2	1.5	0.1	−0.8	0.2	1.6	1.3	0.1	8	2.1	1.5	80 (34)

[a] All data at 25°C.
[b] SUV data at 25°C for Dioleoylphosphatidylcholine-liposomes.

$$AP_{SUV} = \log\left(\text{Distribution } pH_{6.8} \times \frac{\text{Solubility } pH_{6.8} \times V}{\text{Dose}}\right)$$

coefficients of neutral and anionic species. The differences were smaller for the hydrophilic acids than for the more lipophilic acids. For the three hydrophilic weak acids, acyclovir, allopurinol and zidovudine, the liposome partitioning of the anionic species were even higher than that of the neutral form. An even worse correlation was found between the distribution coefficients of the acids at pH 6.8 in both systems (Fig. 4). Figure 3 shows the comparison of partitioning of neutral and cationic species of bases in the liposome and octanol-water system. A poor correlation with a low slope of 0.33 and an intercept at 2.2 was found for the 13 neutral bases. The lipophilicity ranged between −2.0 and 6.0 in the octanol and between 1.0 and 5.0 in the liposomal system. Again the differences between neutral and ion partitioning in the liposomal system were small. In most of the cases, the difference was less than one log unit. Generally, the partition coefficients of the neutral forms are higher for liposomes than for octanol. Only for the neutral forms of the most lipophilic bases with a logP of 4 and higher the octanol partitioning exceeded the liposome partitioning. A poor correlation with a low slope of 0.37 and an intercept at 2.0 was found for the cationic species. A correlation between octanol and liposome distribution coefficients of the bases at pH 6.8 was also poor (Fig. 4). A general similarity between octanol and liposome partition coefficients of nonionized acids and bases as reported by Avdeef *et al.* could not be verified for all of the compounds discussed here. Such a similarity could be seen for propranolol and the most lipophilic acids ibuprofen and diclofenac. With regard to the flat and erratic correlations between liposome and octanol partitioning, this coincidence of similar partitioning coefficients may be limited to this particular range of lipophilicities. Furthermore, a linearity of correlation between octanol and phospholipid systems for small nonionic molecules as reported by Gobas *et al.* is not representative for more complex drug molecules (9,15).

Correlations with Human Intestinal Absorption

For the correlation of physicochemical parameters with human intestinal absorption data, a new type of Absorption Potential (AP_{SUV}) based on liposome partitioning (Eq. 1) was

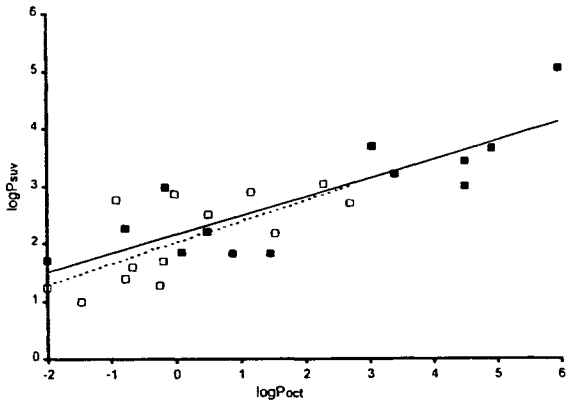


Fig. 3. Liposome versus octanol partitioning of the bases. ■—neutral forms ($logP_{SUV}$ = 0.33 $logP_{Oct}$ + 2.2, R^2 = 0.69), □—cationic forms ($logP_{SUV}$ = 0.37 $logP_{Oct}$ + 2.0, R^2 = 0.49). The solid line represents the correlation for the neutral forms. The dotted line represents the correlation for the cationic forms.

calculated from distribution, solubility, mean single oral dose, and the volume of the intestinal fluids. In contrast to the Absorption Potential introduced by Dressman *et al.* (16), the distribution coefficient at pH 6.8 is used instead of the partition coefficient. The Absorption Potential results for liposomes and octanol ($AP_{Octanol}$) and the references for the human absorption data are listed in Table II. pH 6.8 is considered to be a relevant pH for the fasted small intestine where most of the absorption takes place (17). Therefore, distribution coefficients were calculated for pH 6.8 and solubility was determined at pH 6.8. Figure 5 (a) shows the noncorrelation of intestinal absorption with liposome distribution at pH 6.8 ($logD_{SUV}$pH 6.8). Most of the drugs tested had a $logD_{SUV}$ of 1.5 to 3 absorption scattered between 20 and 100%. In contrast, a sigmoidal correlation of human intestinal absorption with AP_{SUV} was found (Fig. 5b). The high absorption of acetylsalicylic acid (1) and allopurinol (3) appears to be reached even with low AP_{SUV} values. This may be explained by their particularly low molecular weight, enabling additional paracellular diffusion through membranes (18). No correlations were found between $logD_{Oct}$ or $AP_{Octanol}$

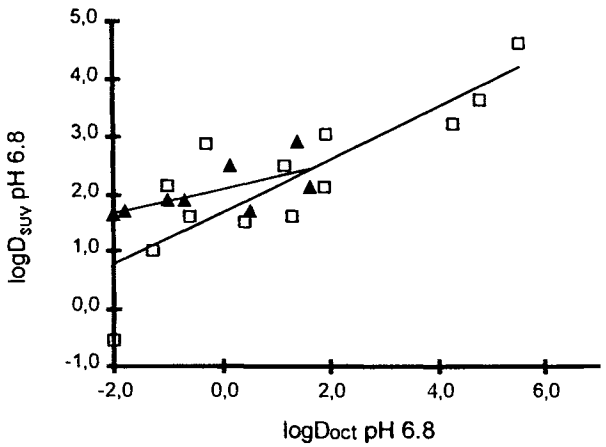


Fig. 4. Liposome versus octanol distribution at pH6.8. ▲ - Acids ($logD_{SUV}$ pH 6.8 = 0.22 $logD_{Oct}$ + 2.1, R^2; = 0.45), □—Bases ($logD_{SUV}$ pH 6.8 = 0.45 $logD_{Oct}$ + 1.7, R^2 = 0.71).

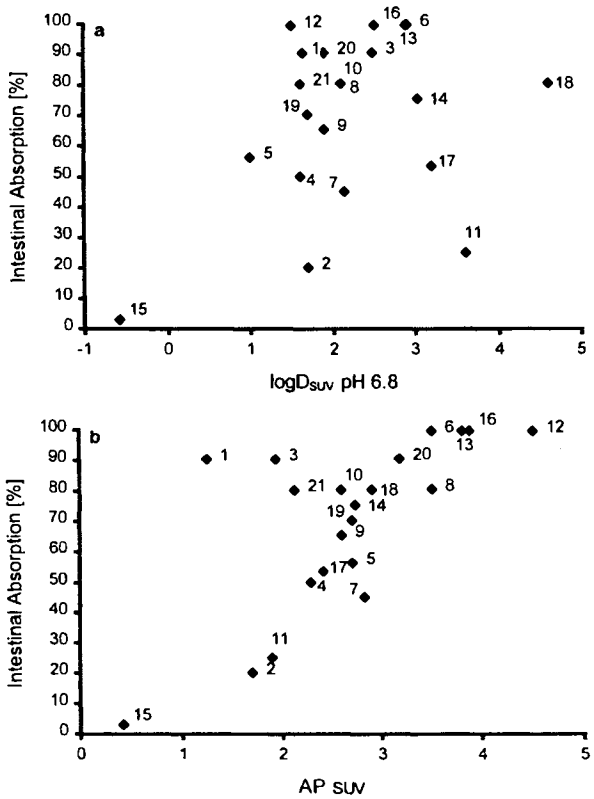


Fig. 5. (a) Intestinal Absorption versus $logD_{SUV}$ pH 6.8. The numbers in the graph refer to the substance numbers in Table II. Number 8 and 10 have identical values of absorption and logD. (b) Intestinal Absorption versus AP_{SUV}. The numbers in the graph refer to the substance numbers in Table II.

and the percentage of intestinal absorption (Fig. 6a and 6b). Particularly the low percentage of absorption of miconazole(11) and rifabutine(17) could not be predicted correctly using the octanol logD values. Their bulky lipophilic moiety may hinder, particularly, their integration in ordered phospholipid bilayers as it is expressed by lower liposome than octanol logD values. The most striking difference between the two systems is the much higher partitioning of ionic molecules in the liposomal system. This is first of all an effect of interactions between ionic drug molecules and the ionic functional groups in the phospholipid bilayer. It results in a higher affinity to membranes even of hydrophilic compounds than would be estimated from the low partitioning of drugs into organic bulk solvents. Another important factor for prediction of passive intestinal absorption is the solubility of the drug at intestinal pH. The prediction of intestinal absorption by logD values alone fails. Octanol as well as liposome distribution results do not correlate with the percentage of intestinal absorption as shown in Figs. 5a and 6a. A sufficient solubility relative to the single oral dose is a prerequisite for intestinal absorption. It could be shown that a high solubility to dose ratio may outweigh a low lipophilicity resulting in a high percentage of absorption. On the other hand even highly lipophilic compounds can be poorly absorbed when the solubility to dose ratio is small as, e.g., for miconazole[11] and rifabutine[17]. These two together with terbinafine [18] approach the main field of data points (Figs. 6b, 5b) or the

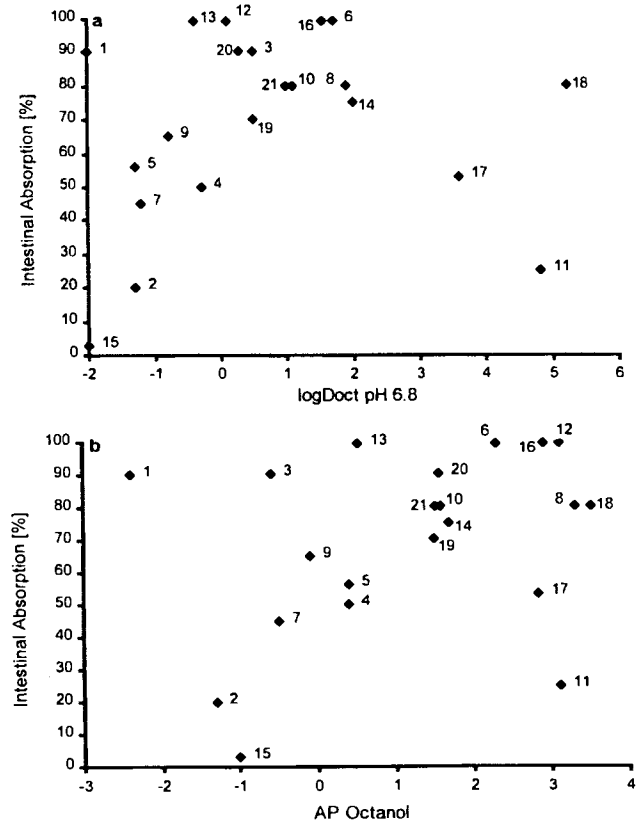


Fig. 6. (a) Intestinal Absorption versus $logD_{Oct}$ pH 6.8. The numbers in the graph refer to the substance numbers in Table II. (b) Intestinal Absorption versus $AP_{Octanol}$. The numbers in the graph refer to the substance numbers in Table II.

center line of data points when the correlation of absorption is changed from simple partitioning to the absorption potential.

CONCLUSIONS

Drug partitioning in the liposomal system can be measured conveniently by an automated potentiometric titration technique. It could be shown that octanol partitioning is poorly correlated with interactions between ionizable drugs and phospholipid bilayers. Liposome partitioning of structurally diverse acids and bases is characterized by much higher partitioning of ionic species compared to octanol so that the differences in logP between neutral and ionic species are smaller. Partitioning of hydrophilic compounds was found to be increased and partitioning of the neutral forms of highly lipophilic compounds to be similar or decreased compared to 1-octanol. As most drugs are ionizable and many exist in the ionic state at intestinal pH, this result is of relevance for *in vitro-in vivo* correlations with lipophilicity data. Liposome distribution coefficients of ionizable drugs have been successfully used to calculate a parameter, AP_{SUV}, that correlates with the percentage of passive intestinal absorption in humans. In contrast, the human absorption data did not correlate with an octanol-based absorption potential or partitioning data alone. This technique may serve for selection and optimization of lead compounds and in drug development. Limitations of this model are its inability to account for membrane passage via paracellular routes or any active transport mechanisms.

REFERENCES

1. C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Del. Rev.* **23**:3–25 (1997).
2. S. Yee. *In vitro* permeability across Caco-2 cells (colonic) can predict *in vivo* (small intestinal) absorption in man—fact or myth. *Pharm. Res.* **14**:763–766 (1997).
3. K. J. Schaper. Absorption of ionizable drugs: nonlinear dependence on logP, pKa and pH-quantitative relationships. *Quant.Struct.-Act.Relat.* **1**:13–27 (1982).
4. P. A. Shore, B. B. Brodie, and C. A. M. Hogben. The gastric secretion of drugs: A pH partition hypothesis. *J. Pharmacol. Exp. Ther.* **119**:361–369 (1957).
5. C. J. Alcorn, R. J. Simpson, D. E. Leahy, and T. J. Peters. Partition and distribution coefficients of solutes and drugs in brush border membrane vesicles. *Biochem. Pharmacol.* **45**:1775–1782 (1993).
6. F. Barbato, M. I. la Rotonda, and F. Quaglia. Interactions of nonsteroidal antiinflammatory drugs with phospholipids: comparison between octanol/buffer partition coefficients and chromatographic indexes on immobilized artificial membranes. *J. Pharm. Sci.* **86**:225–229 (1997).
7. H.-Y. Cheng, C. S. Randall, W. W. Holl, P. P. Constantinides, T.-L. Yue, and G. Z. Feuerstein. Carvedilol-liposome interaction: evidence for strong association with the hydrophobic region of the lipid bilayers. *Biochim. Biophys. Acta* **1284**:20–28 (1996).
8. G. V. Betageri, Y. Theriault, and J. A. Rogers. NMR Study of the interaction of beta-blockers with sonicated dimyritoylphosphatidylcholine liposomes in the presence of praseodymium cation. *Membrane Biochem.* **8**:197–206 (1989).
9. F. A. P. C. Gobas, J. M. Lahittete, G. Garofalo, W. Y. Shiu, and D. MacKay. A novel method for measuring membrane-water partition coefficients of hydrophobic organic chemicals: comparison with 1-octanol-water partitioning. *J. Pharm. Sci.* **77**:265–272 (1988).
10. A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, and K. Y. Tam. pH-metric logP 10: Determination of vesicle membrane–water partition coefficients of ionisable drugs. *Pharm. Res.* **15**:209–215 (1998).
11. A. Avdeef. pH-Metric logP. Part 1: Difference plots for determining ion-pair octanol-water partition coefficients of multiprotic substances. *Quant. Struct.-Act. Relat.* **11**:510–517 (1992).
12. A. Avdeef. pH-metric logP-2. Refinement of partition coefficients and ionisation constants of multiprotic substances. *J. Pharm. Sci.* **82**:183–190 (1993).
13. P. Finholt and S. Solvang. Dissolution kinetics of drugs in human gastric juice—the role of surface tension. *J. Pharm. Sci.* **57**:1322–1326 (1968).
14. L. S. C. Wan and P. F. S. Lee. CMC of Polysorbates. *J. Pharm. Sci.* **63**:136–137 (1974).
15. G. Camenisch, G. Folkers, and H. van de Waterbeemd. Review of passive drug absorption models: historical background, recent developments and limitations. *Pharm. Acta Helv.* **71**:309–327 (1996).
16. J. B. Dressman, G. L. Amidon, and D. Fleisher. Absorption potential: Estimating the fraction absorbed for orally administered compounds. *J. Pharm. Sci.* **74**:588–589 (1985).
17. J. B. Dressman, and V. A. Gray. Change of pH intestinal fluid. *Pharmacopeial Forum* **22**:1943–1945 (1996).
18. H. Lennernäs. Does fluid flow across the intestinal mucosa affect quantitative oral drug absorption? Is it time for a reevaluation? *Pharm. Res.* **12**:1573–1582 (1995).
19. K. Hartke (ed). *DAB10 Kommentar* Wiss. Verl.-Ges. Stuttgart, 1991.
20. P. de Miranda, and M. R. Blum. Pharmacokinetics of acyclovir after intravenous and oral administration. *J. Antimicrob. Chemother.* **12** Suppl. B:29–37 (1983).
21. H. Breithaupt and M. Tittel. Kinetics of allopurinol after single intravenous and oral doses. *Eur. J. Clin. Pharmacol.* **22**:77–84 (1982).
22. F. von Bruchhausen (ed.). *Hagers Handbuch der pharmazeutischen Praxis, 5. Aufl.*, Springer Verlag Berlin, 1993.

23. W. F. Frishman. Atenolol and timolol, two new systemic b-adrenoceptor antagonists. *N. Engl. J. Med.* **306**:1456–1462 (1982).
24. J. E. F. Reynolds, (ed), *Martindale, The Extra Pharmacopoeia, 31st Edition*, The Royal Pharmaceutical Society London, 1996.
25. K. Lauritsen, L. S. Laursen, and J. Rask-Madsen. Clinical Pharmacokinetics of drugs used in the treatment of gastrointestinal diseases (Part1). *Clin. Pharmacokinet.* **19**:11–31 (1990).
26. L. Lemberger, R. F. Bergstrom, R. L. Wollen, N. A. Farid, G. G. Enas, and G. R. Aronoff. Fluoxetine: Clinical pharmacology and physiological disposition. *J. Clin. Psychiatry* **46**:14–19 (1985).
27. R. F. N. Mills, S. S. Adams, E. E. Cliffe *et al.* The metabolism of ibuprofen. *Xenobiotica* **3**:589–598 (1973a).
28. R. A. Theodor, H.-J. Weimann, W. Weber *et al.* Absolute bioavailability of moxonidine. *Eur. J. Drug Metab. Pharmacokinet.* **16**:153–159 (1991).
29. B. Fulton, and K. L. Goa. Olanzapine A review of its pharmacological properties and therapeutic efficacy in the management of schizophrenia and related psychoses. *Drugs* **53**:281–298 (1997).
30. P. A. Routledge and D. G. Shand. Clinical pharmacokinetics of propranolol. *Clin. Pharmacokinet.* **4**:73–90 (1979).
31. M. H. Skinner, and T. F Blaschke. Clinical pharmacokinetics of rifabutin. *Clin. Pharmacokinet.* **28**:115–125 (1995).
32. J. C. Jensen. Clinical pharmacokinetics of terbinafine. *Clin. Exp. Dermatol.* **14**:110–113 (1989).
33. H. D. Langtry and D. M. Campoli-Richards. Zidovudin. *Drugs* **37**:407–450 (1989).
34. A. N. Wadworth and D. McTavish. Zopiclone A review of its pharmacological properties and therapeutic efficacy. *Drugs & Aging* **3**:441–459 (1993).