Correlating Partitioning and Caco-2 Cell Permeability of Structurally Diverse Small Molecular Weight Compounds

Mehran Yazdanian,[1,2] Susan L. Glynn,[1] James L. Wright,[1] and Amale Hawi[1]

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INTRODUCTION

The Caco-2 cell model for determination of cellular transport properties is used with increasing regularity in industry and academia as a surrogate marker for *in vivo* intestinal permeability in humans (1–5). The routine use of this model as a screen for selection of new chemical entities for drug discovery and development has renewed the interest in establishing a general relationship between *in vitro* cellular permeability and physical chemical parameters for structurally and physicochemically diverse sets of compounds.

The ability of a drug molecule to permeate cell membranes by passive diffusion is primarily dependent on its partitioning into the membrane lipid bilayer. The most frequent single physical chemical parameter that has been used for the prediction of cellular permeability is octanol/water partitioning (3,6–10). Correlating this parameter with Caco-2 cell permeability coefficients has yielded mixed results. For example, Hilgers et al. reported a sigmoidal relationship whereas Artursson and Karlsson observed a poor linear correlation (3,10). Using other physical chemical parameters, simple linear relationships between Caco-2 cell permeability coefficients and hydrogen bonding capacity (11,12), molecular surface properties (13), or capacity factors from immobilized artificial membranes (IAM) columns (14) have been reported. However, in all of these investigations, the small size of the data sets as well as the homologous nature of most of the compounds studied make the selection of a single or combined physical chemical factors for predicting Caco-2 cell permeability infeasible.

In this paper, the potential for using an easily measurable physical parameter such as oil/water partitioning as predictor of Caco-2 cellular permeability was investigated. Distribution coefficients in three distinct solvent systems: octanol, hexadecane, and propyleneglycol dipelargonate (PGDP) were determined. Octanol was chosen since it has been traditionally used to measure the lipophilicity of drugs as a predictor of solute/membrane partitioning and hence cellular permeability. Hexadecane was used due to its suggested conformational similarities to the cell membrane interior (15). PGDP was used as a model solvent to mimic the lipidic end of phospholipids present in the lipid bilayer (16). The relationship between *in vitro* Caco-2 cell permeability and distribution coefficient for a large set of structurally diverse, small molecular weight is presented. Compounds with a variety of physical chemical characteristics, transported via both transcellular and paracellular transport routes have been included in this data set in order to have the greatest utility in the process of compound selection and optimization in drug discovery and development.

EXPERIMENTAL SECTION

Materials

Acebutolol, acetylsalicylic acid, acyclovir, alprenolol, aminopyrine, atenolol, caffeine, chlorothiazide, chlorpromazine, cimetidine, clonidine, corticosterone, desipramine, diazepam, dopamine, estradiol, ganciclovir, griseofulvin, hydrochlorothiazide, hydrocortisone, indomethacin, labutalol, mannitol, methyl scopolamine, metoprolol, nadolol, nicotine, pindolol, phenytoin, pirenzepine, progesterone, propranolol, salicylic acid, scopolamine, sucrose, sulfasalazine, terbutaline, testosterone, timolol, and uracil were purchased from Sigma, St. Louis, MO. Dexamethasone, urea, and warfarin were purchased from Aldrich, Milwaukee, WI. Bremazocine was purchased from Research Biochemicals International, Natick, MA. Ganciclovir, meloxicam, nevirapine, piroxicam, ranitidine, and telmisartan were obtained from Boehringer Ingelheim Pharm. Inc., Ridgefield, CT. Zidovudine was provided by Pharmatec Int. (West Orange, NJ). [^{14}C]Acetylsalicylic acid, [^{3}H]acyclovir, [^{14}C]aminopyrine, [^{14}C]caffeine, [^{3}H]chlorpromazine, [^{3}H]corticosterone, [^{3}H]desipramine, [^{3}H]dexamethasone, [^{3}H]diazepam, [^{3}H]dopamine, [^{3}H]estradiol, griseofulvin, [3H]hydrocortisone, [^{14}C]indomethacin, [^{14}C]mannitol, [^{3}H]nicotine, [^{3}H]phencyclidine, [^{14}C]phenytoin, [^{3}H]pirenzepine, [^{14}C]progesterone, [^{14}C]salicylic acid, [^{14}C]sucrose, [^{14}C]testosterone, and [^{14}C]uracil were purchased from New England Nuclear, Boston, MA. [^{3}H]bremazocine, [^{3}H]cimetidine, [^{3}H]clonidine, [^{3}H]scopolamine, [^{3}H]methyl scopolamine, [^{14}C]urea were obtained from Amersham Corporation, Arlington Heights, IL. [^{3}H]nevirapine was synthesized at Boehringer Ingelheim Pharm. Inc.'s radiosynthesis laboratory.

Caco-2 Cells

Caco-2 cells, originating from a human colorectal carcinoma, were obtained from American Tissue Culture Collection, Rockville, MD. The cells were grown at 37°C in an atmosphere of 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum, 1% non-essential amino acids, penicillin (100 Units/ml), and streptomycin (100 μg/ml). All culture media and reagents were from Gibco BRL Products, Gaithersburg, MD. Confluent cell monolayers were subcultured every 7 days by treatment with 0.25% trypsin containing 1 mM EDTA.

[1] Department of Pharmaceutics, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut 06877.

[2] To whom correspondence should be addressed. (e-mail: myazdanian@bi-pharm.com)

ABBREVIATIONS: HBSS, Hank's Balanced Salt Solution; PBS, Phosphate buffered saline, pH 7.4; PGDP, Propyleneglycol dipelargonate; $P_{Caco\text{-}2}$, Caco-2 cell permeability coefficient (cm/sec); D_{oct}, Octanol/PBS distribution coefficient; D_{hex}, Hexadecane/PBS distribution coefficient; D_{pgdp}, PGDP/PBS distribution coefficient.

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Permeability Studies

Caco-2 cells were seeded at a density of 80,000 cells/cm^2 in 6-well plates on polycarbonate filters (Costar, Transwell® cell culture inserts, diameter 24.5 mm, pore size 3.0 μm) coated with rat tail collagen type I. The cells were allowed to grow and differentiate for up to 25 days. Cells of passage numbers 23 to 50 were used throughout.

Prior to permeability experiments, the culture medium was replaced with the transport medium, Hank's Balanced Salt Solution (HBSS), pH 7.4, and equilibrated for 30 minutes at 37°C. Drug solutions were prepared in HBSS at a final concentrations of 0.01 to 0.1 mM. All permeability experiments were performed in an incubator at 37°C and an atmosphere of 5% CO_2. The pH of the transport medium did not change by more than 0.2 pH units over the duration of a typical experiment (2 hours). Radiolabelled isotopes were diluted in ethanol and known aliquots were evaporated in vials to which cold drug solutions (prepared as described above) were added to give a final radioactive concentration of ~0.15 μCi/ml.

To initiate permeability experiments, the apical side of the monolayers received 1.5 ml of drug solutions. The plates were then placed on an orbital shaker (Titer Plate Shaker, Lab-Line Instruments) at ~100 rpm (considered medium stirring rate). The amount of solute permeated was determined by either moving the inserts to new wells containing fresh medium or taking a sample from the basolateral side and replacing it with fresh medium at discrete time intervals. Transport rates were then determined by plotting the cumulative amount permeated as a function of time. Samples were either analyzed by HPLC (Hewlett Packard, HP 1090) or counted on a liquid scintillation counter (Packard, Tri-Carb, A2700).

All experiments were performed under "sink" conditions where the concentration of the solute in the receiver side was less than 10% of the dose applied at all time points, hence minimizing the diffusion of the solute back from the receiving to the donor side. The apparent permeability of coefficient, $P_{Caco\text{-}2}$, was then determined according to equation 1:

$$P_{Caco\text{-}2} = J/AC_o \quad (1)$$

where J was the rate of appearance of the drug in the receiver chamber, C_o was the initial concentration of the solute in the donor chamber, and A was the surface area of the filter. Mass balance was calculated for each experiment. In all cases the mass balance was more than 90%. Mannitol permeability was used to assess the integrity of Caco-2 cell monolayers. Transwell® plates were used for permeability experiment when mannitol leakage was less than 0.5% per hour. Over a 22 month period where Caco-2 cell permeability values for the drugs in the data base were measured, the mannitol permeability coefficients averaged $3.8 \pm 1.2 \times 10^{-7}$ cm/sec (N = 102). Permeability coefficients were determined at least in triplicates and the mean and standard deviations were reported.

Distribution Coefficient Measurement

Distribution coefficients (D) for the compounds were determined as follows: For radiolabelled compounds, approximately 0.5 μCi of the solute was added to a vial and the solvent was evaporated. To this vial equal volumes of the oil phase (octanol, hexadecane, or PGDP) and phosphate buffered saline (PBS), pH 7.4, were added and the vial was vortexed for 30 seconds and rotated end-over-end for 2 hours at 37°C. The vial was then centrifuged at 800 rpm for 3 minutes and let stand at 37°C for another 2 hours to allow for total separation of phases. Samples from both oil and buffer phases were taken and counted on a liquid scintillation counter. For non-radiolabelled compounds a known stock solution of each compound in organic or aqueous solution was prepared. The volume of the organic to aqueous phase was changed to allow for detection of the solute by HPLC. Distribution coefficient was determined from the ratio of the concentration of the compound in oil to that in the buffer phase. For some hydrophilic non-radiolabelled compounds such as nadolol and ranitidine, distribution coefficients in hexadecane and PGDP could not be measured due to analytical detection limitations. The measurements were done at least in triplicates for each compound. The mean and standard deviations are reported.

RESULTS AND DISCUSSION

Caco-2 Cell Permeability Coefficients

The Caco-2 cell permeability coefficients for 51 structurally diverse compounds with molecular weights in the range of 60 to 515 and different net charges at pH 7.4 have been measured. The results are presented in order of decreasing permeability coefficients in Table 1 along with the values for percent of oral dose absorbed in humans for compounds obtained from the literature (3,17–22).

The numerical values for Caco-2 cell permeability coefficients determined in this study were similar to those reported by Artursson and Karlsson (3) but varied as much as two orders of magnitude from those reported by Stewart et al. for the same compounds (5). The differences in the permeability coefficients reported from various laboratories is attributed to variations in cell culture conditions such as passage number, type of medium, and days in culture, as well as the experimental setups used for their determination (23).

Similar to the sigmoidal relationship first shown by Artursson and Karlsson in 1991, the data in this study also follow a sigmoidal pattern where compounds with the lowest and highest permeabilities form two distinct plateau regions separated by a steep line (Figure 1). Compounds in this data set with very low Caco-2 cell permeability, $P_{Caco\text{-}2}$ less than about 0.4×10^{-6} cm/sec, exhibited very poor oral absorption whereas compounds with high cellular permeability, $P_{Caco\text{-}2}$ larger than $\sim 7 \times 10^{-6}$ cm/sec had excellent oral absorption.

Cell Permeability and Partitioning

Octanol/PBS, hexadecane/PBS, and PGDP/PBS (D_{oct}, D_{hex} and D_{pgdp}) distribution coefficients at pH 7.4 for the compounds in the data set were measured and are presented in Table 1. Figure 2 shows the relationships between Caco-2 cell permeability and D_{oct}, D_{hex} and D_{pgdp}.

Although the range of absolute values of distribution coefficients for the three solvents vary, the trend in the profiles are very similar. It can be readily seen that the more lipophilic compounds in all the systems have in general higher Caco-2 cell permeability coefficients. For example, when log D_{oct},

Table 1. Caco-2 Cell Permeability Coefficient, Percent Oral Absorption, and Distribution Coefficients for 51 Compounds

Compound	MW	$P_{caco-2} \times 10^6$ (cm/sec)[a]	% Absorbed (references)	Log D_{oct}	Log D_{hex}	Log D_{pgdp}	Charge
Griseofulvin	352.8	36.6 ± 5.3 (6)	Irregular (17)	2.47 ± 0.06	0.81 ± 0.02	1.96 ± 0.01	0
Aminopyrine	231.3	36.5 ± 2.9 (6)	100 (18)	0.63 ± 0.06	−1.04 ± 0.02	−0.11 ± 0.03	0
Piroxicam	331.4	35.6 ± 6.7 (6)	100 (19)	−0.07 ± 0.04	−1.52 ± 0.35	−0.07 ± 0.05	−
Diazepam	284.8	33.4 ± 2.5 (6)	100 (17)	2.58 ± 0.06	1.37 ± 0.05	2.36 ± 0.16	0
Caffeine	194.2	30.8 ± 1.5 (6)	100 (19)	0.02 ± 0.02	−2.25 ± 0.09	−0.64 ± 0.04	0
Nevirapine	266.3	30.1 ± 7.3 (9)	>90 (20)	1.81 ± 0.05	−0.49 ± 0.09	0.97 ± 0.03	0
Phenytoin	252.3	26.7 ± 2.7 (6)	90 (19)	2.26 ± 0.02	−2.13 ± 0.01	1.37 ± 0.01	+
Alprenolol	249.3	25.3 ± 7.0 (6)	93 (3)	1.38 ± 0.05	0.22 ± 0.20	0.72 ± 0.06	+
Testosterone	288.4	24.9 ± 3.3 (6)	100 (3)	2.91 ± 0.04	0.60 ± 0.03	2.11 ± 0.11	0
Phencyclidine	248.4	24.7 ± 3.0 (9)	—	1.31 ± 0.09	1.23 ± 0.25	1.07 ± 0.09	+
Desipramine	266.4	24.4 ± 2.3 (6)	>95 (21)	1.57 ± 0.07	0.54 ± 0.09	1.05 ± 0.04	+
Metoprolol	267.4	23.7 ± 1.3 (6)	95 (3)	0.51 ± 0.10	—	—	+
Progesterone	314.5	23.7 ± 1.3 (6)	—	3.48 ± 0.08	2.38 ± 0.05	3.02 ± 0.12	0
Salicylic acid	138.1	22.0 ± 0.5 (3)	100 (3)	−1.44 ± 0.01	−4.39 ± 0.11	−0.82 ± 0.04	−
Clonidine	230.1	21.8 ± 3.0 (9)	100 (17)	0.78 ± 0.06	−1.88 ± 0.04	0.08 ± 0.06	+
Propranolol	259.3	21.8 ± 3.1 (6)	90 (3)	1.55 ± 0.02	−0.31 ± 0.02	0.77 ± 0.06	+
Corticosterone	346.5	21.2 ± 3.7 (6)	100 (3)	1.78 ± 0.01	−1.60 ± 0.09	0.90 ± 0.03	0
Warfarin	308.3	21.1 ± 8.0 (6)	98 (3)	0.64 ± 0.01	−2.13 ± 0.02	0.18 ± 0.04	−
Indomethacin	357.7	20.4 ± 2.9 (6)	100 (20)	1.00 ± 0.03	−1.58 ± 0.07	0.34 ± 0.03	−
Chlorpromazine	318.9	19.9 ± 0.9 (3)	Erratic (19)	1.86 ± 0.10	0.67 ± 0.19	0.82 ± 0.16	+
Meloxicam	351.4	19.5 ± 6.2 (6)	90	0.03 ± 0.04	−1.00 ± 0.33	−0.45 ± 0.11	−
Nicotine	162.2	19.4 ± 4.9 (6)	100 (18)	0.41 ± 0.02	−0.69 ± 0.10	−0.37 ± 0.09	+
Estradiol	272.4	16.9 ± 1.2 (3)	Rapidly metabolized (18)	2.24 ± 0.04	0.20 ± 0.01	1.89 ± 0.04	0
Pindolol	248.3	16.7 ± 1.5 (6)	95 (17)	0.19 ± 0.05	2.13 ± 0.06	1.05 ± 0.17	+
Telmisartan	514.6	15.1 ± 0.6 (3)	90	2.41 ± 0.05	0.34 ± 0.23	1.40 ± 0.35	−
Hydrocortisone	362.5	14.0 ± 2.6 (6)	89 (3)	1.48 ± 0.01	−2.87 ± 0.02	0.22 ± 0.01	0
Timolol	328.4	12.8 ± 0.5 (3)	72 (17)	0.03 ± 0.04	—	—	+
Dexamethasone	392.5	12.2 ± 1.8 (6)	100 (3)	2.16 ± 0.11	−0.02 ± 0.20	0.70 ± 0.03	0
Scopolamine	303.4	11.8 ± 0.7 (6)	100 (18)	0.21 ± 0.02	−2.05 ± 0.04	0.57 ± 0.03	+
Dopamine	153.2	9.33 ± 3.48 (6)	—	−0.80 ± 0.03	−2.54 ± 0.12	−1.20 ± 0.13	+
Labetalol	316.4	9.31 ± 0.66 (3)	90 (17)	1.24 ± 0.03	—	—	+
Acetylsalicylic acid	180.2	9.09 ± 0.19 (4)	100 (3)	−2.25 ± 0.04	−3.59 ± 0.01	−2.95 ± 0.04	−
Bremazocine	351.9	8.02 ± 0.22 (6)	—	1.66 ± 0.05	−0.25 ± 0.02	0.55 ± 0.04	0
Zidovudine	267.2	6.93 ± 0.17 (6)	100 (17)	−0.58 ± 0.05	—	—	0
Urea	60.1	4.56 ± 0.23 (6)	—	−1.64 ± 0.02	−3.47 ± 0.03	−2.70 ± 0.04	+
Uracil	112.1	4.24 ± 0.19 (6)	—	−1.11 ± 0.01	−3.89 ± 0.14	−2.26 ± 0.14	+
Nadolol	309.4	3.88 ± 0.48 (6)	—	0.68 ± 0.03	—	—	+
Sucrose	342.3	1.71 ± 0.37 (6)	—	−3.34 ± 0.07	−4.41 ± 0.16	−3.65 ± 0.06	0
Cimetidine	252.3	1.37 ± 0.34 (3)	95 (17)	−0.36 ± 0.11	−3.32 ± 0.06	−2.04 ± 0.07	+
Methyl Scopolamine	318.5	0.69 ± 0.01 (3)	—	−1.14 ± 0.01	−3.47 ± 0.03	−2.70 ± 0.06	+
Hydrochlorothiazide	297.7	0.51 ± 0.02 (3)	90 (17)	−0.12 ± 0.05	−1.82 ± 0.26	−1.01 ± 0.03	+
Atenolol	266.3	0.53 ± 0.07 (5)	50 (3)	−1.29 ± 0.12	—	—	+
Acebutalol	336.4	0.51 ± 0.02 (3)	90 (17)	−0.09 ± 0.03	−2.18 ± 0.02	−1.87 ± 0.15	+
Terbutaline	225.3	0.47 ± 0.08 (3)	73 (3)	−1.07 ± 0.01	−3.27 ± 0.07	−0.51 ± 0.02	+
Ranitidine	314.4	0.49 ± 0.06 (6)	50 (20)	−0.12 ± 0.28	—	−1.57 ± 0.38	+
Pirenzepine	424.3	0.44 ± 0.05 (6)	Poor (17)	−0.46 ± 0.01	−3.97 ± 0.16	−2.21 ± 0.02	+
Mannitol	182.2	0.38 ± 0.12 (102)	16 (3)	−2.65 ± 0.08	−4.30 ± 0.23	−3.75 ± 0.40	0
Ganciclovir	255.2	0.38 ± 0.07 (6)	3 (19)	−0.10 ± 0.23	—	—	0
Sulfasalazine	394.4	0.30 ± 0.02 (3)	13 (3)	−0.42 ± 0.04	−1.51 ± 0.28	−0.93 ± 0.04	−
Acyclovir	225.2	0.25 ± 0.03 (9)	20 (17)	−0.35 ± 0.05	−3.87 ± 0.34	−2.44 ± 0.01	0
Chlorothiazide	295.7	0.19 ± 0.02 (6)	Dose dependent (19)	−1.15 ± 0.06	−2.19 ± 0.23	−1.98 ± 0.24	+

[a] Number of Caco-2 cell monolayers used.

log D_{hex}, and log D_{pgdp} are higher than approximately 0.5, −1.0, and 0 respectively, all of the compounds had high cellular permeability coefficients independent of the net charge of the compounds at pH 7.4. However, for compounds below these thresholds values, the distribution coefficients alone did not appear to be an indicator of the compound's cellular permeability. Such shortcomings in using a single physical chemical factor for correlation with cellular permeability have been reported previously (3). Some studies have used parameters and descriptors to account for hydrogen bonding, size, and

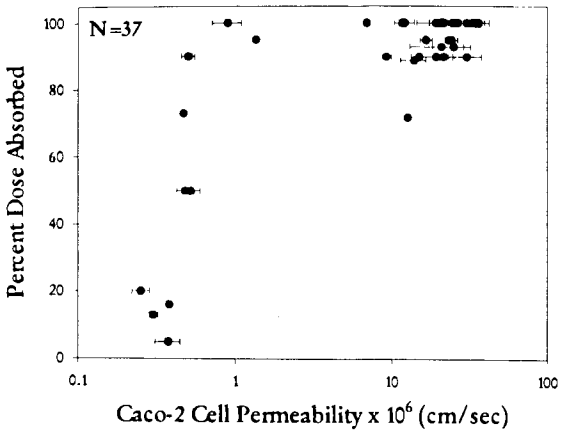

Fig. 1. Caco-2 cell permeability coefficient vs. percent oral absorption for 37 small molecular weight compounds.

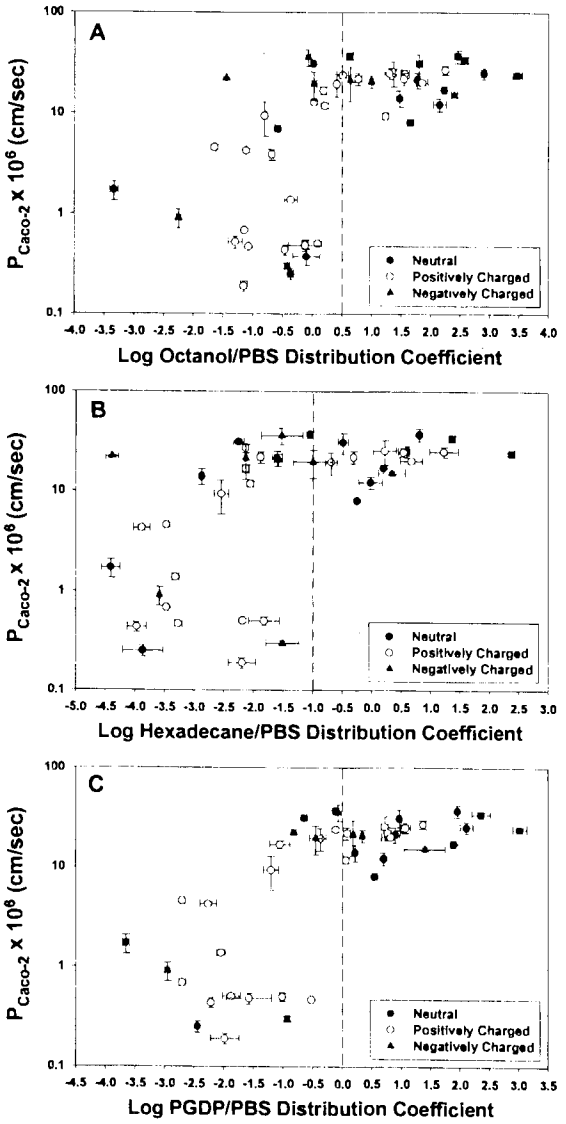

Fig. 2. Caco-2 cell permeability coefficient vs. log D_{oct} (A), log D_{hex} (B), and log D_{pgdp} (C).

shape of compounds to improve this correlation. These are discussed briefly below.

It has been shown that accounting for the hydrogen bonding ability of some small peptide-like compounds by subtracting the measured values of partitioning coefficients between octanol/water and pure alkane/water can lead to good linear correlations between cellular permeability and partitioning (7). Since log D_{oct} or log D_{pgdp} may reflect the outer lipophilic phospholipid rich part of the cell membrane whereas log D_{hex} can account for partitioning into interior non polar regions of the membrane, then the degree of hydrogen bonding capacity can be similarly calculated, as Δlog D, from the difference between log D_{hex} and either log D_{oct} or log D_{pgdp}. A very poor correlation was observed between Δlog D and Caco-2 cell permeability coefficients of our large structurally diverse data set.

A few studies have used simple linear mathematical models to correlate permeability coefficients with partitioning parameters by taking into account the effect of size e.g. molecular weight (6,10). This did not have an effect on the relationship between log P_{caco-2} and distribution coefficients for octanol, hexadecane, or PGDP for our data set. Estimation of molar volumes for the compounds using the LeBas additive method as another size descriptor did not improve this correlation either (22).

Recently, polar Van der Waals surface areas calculated using dynamic molecular surface properties of a series of homologous beta adrenoreceptor antagonists were shown to linearly correlate with the cellular permeability (13). We used this parameter as reported by Palm et al., for a few of the compounds in our data base; alprenolol, atenolol, diazepam, mannitol, metoprolol, pindolol, and sulfasalazine and found a linear correlation (r^2 = 0.90) (24). Whether the calculation of this parameter which accounts for both shape and flexibility of molecules for all of our large structurally diverse data set can improve this correlation is yet to be determined but it is beyond the scope of this paper, in particular, since such calculations require the use of very powerful computational techniques.

It is important to note that in correlating Caco-2 cell permeability data with distribution coefficients, a major assumption has been that passive diffusion was the major route of cellular transport for the compounds used in this study. Future studies will focus more on possible effects of various transport systems and efflux pumps on correlating permeability coefficient values with partitioning parameters.

CONCLUSIONS

Caco-2 cell permeability coefficients for 51 structurally diverse small molecular weight compounds were measured and correlated to distribution coefficients in three distinct solvent systems: octanol, hexadecane, and (PGDP). There were no simple mathematical correlations between Caco-2 cell permeability coefficients and partitioning values for all solvent systems. In general, however, the more lipophilic compounds had higher cellular permeability coefficients. It was possible to determine an approximate distribution coefficient value above which all compounds were highly cell permeable for all the solvent systems. These were in logarithmic units 0.5, −1.0, and 0 for octanol, hexadecane, PGDP solvent systems respectively. Below these distribution coefficients, as the compounds became

more hydrophilic, there was absolutely no trend in the data. This study reinforces the tenet that single physical parameters such as partitioning values can only be used to qualitatively determine Caco-2 cell permeability, and cannot be used as surrogate markers of *in vivo* intestinal permeability.

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