Comparison of Diffusion and Permeability of Selected Drugs: Effect of Physicochemical Properties and Permeation Enhancers

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Accepted 20 May 2005
Revised 21 December 2004
Received 15 March 2004

An attempt was made to compare and assess the permeability characteristics of selected drug molecules by varying lipophilicity and molecular weight using *in vitro* diffusion assembly and everted small intestinal sac model. Apparent permeability coefficient, flux, permeability coefficient and enhancement ratio of selected drug molecules through both the models were compared. The permeation data was compared with physicochemical properties including solubility parameter of drug molecules. *In vitro* data was found to be higher than the data obtained from everted sac model. Permeation of drugs through everted sac model was significantly enhanced by incorporation of sodium taurocholate (1%), which could be correlated with *in vitro* diffusion data.

Oral route is the most preferred means of administering drugs for the reason of convenience and compliance. Development of an *in vitro* model for evaluating oral absorption of drug molecules would be most advantageous. It would help in faster evaluation of permeability characteristics of drug molecules. Another potential use of an *in vitro* model would be rapid screening of drug delivery systems such as microparticles, nanoparticles and liposomes for the effect of formulation on release of drug molecules.

In vitro models using live animal tissues would be of a great value in the assessment of permeability characteristics of drug molecules and thus leading to prediction of preliminary data as a rate and extent of drug absorption. This study was undertaken in order to compare and assess the permeability characteristics of selected drug molecules by varying lipophilicity and molecular weight using *in vitro* diffusion assembly[1] and everted sac model[2]. The aim of the study was to compare the data obtained from the above studies and to observe for any correlation. Enhancement ratio was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. Thus obtained data is compared with their physicochemical properties including solubility parameter (δ) calculated using Fedors and Hoy's method, to obtain a possible correlation. Diclofenac sodium, diltiazem hydrochloride, verapamil hydrochloride and salbutamol sulphate were the selected drug molecules.

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Diclofenac sodium was obtained as a gift sample from Bangalore Pharmaceutical Research Laboratories, Bangalore; diltiazem hydrochloride and verapamil hydrochloride were obtained as a gift sample from Micro Laboratories, Bangalore; salbutamol sulphate as a gift sample from Eros Pharma, Bangalore. All chemicals used were of analytical grade.

A spectrophotometric (Shimadzu UV/Vis 160 spectrophotometer) method of estimation was developed in pH 7.4 phosphate buffer. Aliquots of drug solution that were withdrawn were suitably diluted and analyzed at 237 nm 229 nm, 275 nm and 276 nm for diltiazem hydrochloride, verapamil hydrochloride, diclofenac sodium and salbutamol sulphate, respectively.

Phosphate buffered saline and pH 7.4 phosphate buffer were prepared by standard methods[5]. The pH of buffers was measured using a Digsun pH meter previously standardized with buffer tablets of pH 4 and 7. A modified form of Keshary-Chein[1] type in vitro permeation cell was used. Diffusion studies were carried out by taking various concentrations of drug dissolved in 10 ml of phosphate buffer pH 7.4. The solution was placed in the donor compartment having cellophane membrane between donor and receptor compartments. The receptive buffer, consisting of 100 ml of pH 7.4 phosphate

buffer, was placed in the receptor compartment and stirred continuously at 36-37°. Aliquots of 1 ml were withdrawn at 30 min interval for first 60 min thereafter every hour till 12h. For permeation enhancement studies 1% sodium taurocholate was added to the donor compartment and experiment was carried out as described above.

The modified Crane and Wilson's everted sac technique was adopted for studying the permeation of drug molecules[2,3]. Rabbits were fasted overnight, allowed free access to water and small intestine was carefully excised after sacrificing. Entire small intestine was repeatedly washed with pre-warmed phosphate buffered saline and aerated. Segments of small intestine ranging from 8.0-8.5 cm were carefully everted using a thin and smooth glass rod. The distal end of the segment was tied and proximal end was attached to a cannula. The segment was carefully suspended in 50 ml of donor solution maintained at 37±1° under constant stirring and aeration.

A sample of receptor solution, pH 7.4 phosphate buffer, was introduced into the serosal compartment of the segment. Permeation studies were carried out with varying concentrations of drug dissolved in 50 ml of phosphate buffer in the donor compartment. Aliquots were withdrawn from the serosal compartment at every 15 min interval for 4 h and amount of drug present was estimated spectrophotometrically.

Permeability was evaluated by factors including the permeability coefficient, apparent permeability coefficient (Papp), flux and enhancement ratio. Permeability coefficient, which is the velocity of drug passage through the membrane in cm/sec, flux the amount of material flowing through a unit cross sectional barrier in unit time; apparent permeability coefficient which represents absorption constant and enhancement ratio being a ratio of permeability coefficient in presence of permeation enhancer to a permeability coefficient in the absence of permeation enhancer indicating the extent of enhancement in the permeation of drug molecules

Apparent permeability coefficient[7,8] was obtained from the equation, Papp=dQ/dtx1/A.Co, where A is the area in cm^2, Co is the initial concentration; Flux (J)[9] was calculated using the equation, Flux J=-dM/S.dt, where S is the surface area and dM/dt is rate of permeation. Enhancement ratio (ER) is obtained using the formula, ER=permeability coefficient with enhancer/permeability coefficient of drug alone[10].

Absorption constants expressed as apparent permeability coefficient was calculated was found to be in the order of salbutamol sulphate>verapamil hydrochloride> diltiazem hydrochloride>diclofenac sodium, shown in Table 1. Flux was found to be in the order of diltiazem hydrochloride>verapamil hydrochloride>diclofenac sodium> salbutamol sulphate as shown in Table 1. On comparing the above data with physicochemical properties[13] of drug molecules, it was observed that molecular weight and pKa were found to be in the same order as that of apparent permeability coefficient. However, Log P and solubility parameter did not show any correlation with apparent permeability coefficient. Incorporation of 1% sodium taurocholate as permeation enhancer did not have any effect on diffusion of drug molecules as the flux and permeability coefficients were found to be same.

Permeation studies through everted sac rabbit showed apparent permeability coefficient in the order of diclofenac sodium>salbutamolsulphate>diltiazem hydrochloride> verapamil hydrochloride. A similar pattern of results were seen with flux of the drug molecules. Permeability of drug molecules was found to be significantly lesser than diffusion data shown in Table 1. Further, the permeation behavior was attempted to correlate with physicochemical properties of the drug molecules. In case of diclofenac sodium, the high permeability could be attributed towards its adequate lipophlicity[9], low molecular size[10] and its high solubility at elevated pH. Salbutamol sulphate, though it has a large molecular size, exhibited high permeation, which could be attributed to its lipophilicity, good aqueous solubility and undissociation in the medium. Low permeability of diltiazem hydrochloride could be due to its high hydrophilicity, which is evident from its aqueous solubility and its solubility parameter and verapamil hydrochloride in spite of its un dissociation in the medium, due to its low hydrophilicity and large molecular size did not exhibit good permeability.

Effect of permeation enhancers on permeability was obtained from calculation of the enhancement ratio. An increase in permeability by incorporation of permeation enhancer was observed in case of all drugs. Diltiazem hydrochloride was found to have the highest enhancement followed by verapamil hydrochloride, salbutamaol sulphate and diclofenac sodium. Incorporation of permeation enhancer seems to have favored the permeability of drugs that had poor permeability.

After incorporation of permeation enhancer, it was observed that the apparent permeability coefficient was equal to the diffusion coefficient. Though there was no correlation between permeation and diffusion, in presence of the permeation enhancer, permeation correlated with diffusion data

TABLE 1: PERMEABIITY CHARACTERISTICS, EFFECT OF PERMEATION ENHANCER AND PHYSICOCHEMICAL PROPERTIES.

Drug	Solubility parameter (δ)		Mol. weight	Log P	pKa	Flux (mg/sq. cm/min)		Permeability coefficient		Apparentpermeability coefficient (Papp)			ER
	Fedors method	Hoy's method				Everted sac $x10^{-3}$	*In vitro* $x10^{-2}$	Everted sac $x10^{-6}$	*In vitro* $x10^{-6}$	Everted sac $x10^{-5}$	Everted sac (Na-T) $x10^{-4}$	*In vitro* $x10^{-4}$	
Diltiazem hydro chloride	31.14	7.86	451.0	2.7	7.7	4.20	6.21	4.02	3.52	7.86	6.67	5.6	8.48
Verapamil hydro chloride	9.38	8.84	491.0	3.8	8.9	2.63	6.03	2.30	4.69	3.36	2.99	3.34	8.89
Salbutamol sulphate	9.80	7.33	576.0	0.1	9.3	4.24	4.75	11.5	7.04	8.96	4.73	3.83	5.28
Diclofenac sodium	11.33	11.78	318.1	1.5	4.2	8.12	5.38	5.75	3.52	9.28	5.61	4.75	6.04

Mol. Wt. refers to molecular weight, Papp is the apparent permeability coefficient, log P is the partition coefficient, pKa denotes dissociation constant and ER is the enhancement ratio

with correlation coefficient r^2 of 0.9624 and Pearson's correlation coefficient of 0.9262.

In an attempt to relate the diffusion and permeation with physicochemical properties of drug molecules it was observed that diffusion was dependent on molecular size, pKa followed by concentration gradient. Permeation also depended on molecular size followed by lipophilicity, pKa and aqueous solubility of drug but not affected by concentration gradient. Incorporation of permeation enhancer, 1% sodium taurocholate brought about enhancement in permeability but did not affect diffusion, which is expected considering that enhancers act by temporarily altering the permeability of lipid bilayers of biological membranes.

To conclude, the diffusion of drug molecules through *in vitro* diffusion model is considerably higher than permeation seen in everted sac model. Factors affecting diffusion include the concentration gradient, aqueous solubility, molecular weight of drug molecules while permeation was found to be dependent on lipophilicity to a great extent. However, incorporation of permeation enhancer greatly enhanced permeation through the biological membrane while no enhancement in the diffusion was observed. Increased permeation in presence of permeation enhancer resulted in a permeability value equal to diffusion in case of all the drug molecules. Preliminary studies have shown some degree of correlation between in vitro diffusion and everted sac model, further study needs to be carried out for evaluating and obtaining correlation between *in vitro* and *in situ* models.

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