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Human Jejunal Effective Permeability and Its Correlation with Preclinical Drug Absorption Models

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Abstract

This review focuses on intestinal permeability measurements in humans and various aspects of in-vivo transport mechanisms. In addition, comparisons of human data with preclinical models and the blood-brain barrier is discussed.

The regional human jejunal perfusion technique has been validated by several crucial points. One of the most important findings is that there is a good correlation between the measured human effective permeability values and the extent of absorption of drugs in humans determined by pharmacokinetic studies. We have also shown that it is possible to determine the effective permeability (P_{eff}) for carrier-mediated transported compounds, and to classify them according to the proposed Biopharmaceutical Classification System (BCS). Furthermore, it is possible to predict human in-vivo permeability using preclincal permeability models, such as in-situ perfusion of rat jejunum, the Caco-2 model and excized intestinal segments in the Ussing chamber. The permeability of passively transported compounds can be predicted with a particularly high degree of accuracy. However, special care must be taken for drugs with a carrier-mediated transport mechanism, and a scaling factor has to be used. It is also suggested that it is possible to roughly estimate the permeability of the blood–brain barrier using measurements of intestinal permeability, even if the quantitative role of efflux of P-glycoprotein(s) in-vivo still remains to be clarified.

Finally, the data obtained in-vivo in humans emphasize the need for more clinical studies investigating the effect of physiological in-vivo factors and molecular mechanisms influencing the transport of drugs across the intestinal and as well as other membrane barriers. It is also important to study the effect of anti-transport mechanisms, such as efflux by P-glycoprotein(s), and gut wall metabolism, for example CYP 3A4, on the bioavailabaility.

Tests of intestinal permeability have been widely applied for the last 20 years by measuring differential urinary excretion of orally administered test substances, such as lactulose, polyethylene glycols and ^{51}Cr-EDTA, which provide a specific index of intestinal permeability (Sundquist et al 1980). However, this approach to assessing intestinal permeability is affected by intestinal transit, metabolism (if any) and the renal elimination rate, which means that it does not reflect true intestinal mucosal permeability. It has also been pointed out that the transport route of these markers during their passage across the intestine is still unclear (Lennernäs et al 1994; Lennernäs 1995). These studies provide a value which is influenced by the overall intestinal absorption process and systemic elimination for those marker substances under different physiological conditions. Accurate intestinal permeability for drugs and nutrients is difficult to study in-vivo in man. Previously, such experiments were performed using open intestinal perfusion systems but they have drawbacks such as high flow rate, low recovery and lack of control of the fluid composition (Phillips & Summerskill 1966; Sladen & Dawson 1970; Modigliani & Bernier 1971; Lennernäs et al 1992). Recently, new experimental techniques have been introduced for perfusions in both the proximal small intestine and colorectal regions (Knutson et al 1989; Lennernäs et al 1992, 1995d). These techniques are based on a single-pass perfusion of a segment between two balloons, and have recently been developed and validated in human jejunum and rectum (Knutson et al 1989; Lennernäs et al 1992, 1995d). These two perfusion approaches are considered to be the most accurate and direct estimation of the local absorption rate in man, i.e. the transport velocity or effective permeability across the intestinal barrier, expressed in cm s^{-1}. The rate (mass/time) and extent of drug absorption (mass/dose) from the intestinal lumen in-vivo are influenced by the dose/dissolution ratio, chemical degradation or metabolism in the lumen, luminal complex binding, intestinal transit, and effective permeability (P_{eff}) across the intestinal mucosa. The extent of drug absorption (M_t/Dose), i.e. the fraction of drug which has disappeared from the intestinal lumen during a certain residence time, assuming no luminal reactions, at any time t is (Amidon et al 1995):

$$M(t)/Dose = \int_0^t\int\int A \cdot P_{eff} \cdot C_{lumen} \cdot dAdT \qquad (1)$$

where A is the available intestinal surface area, P_{eff} is the average value of the effective intestinal permeability along the intestinal region where absorption occurs, and C_{lumen} is the free reference concentration of the drug in the intestinal lumen (Flynn at al 1974; Komiya et al 1980; Ho et al 1983; Amidon et al 1995; Lennernäs et al 1997a,b). From equation 1 and Fig. 1 it is apparent that P_{eff} is one of the key variables controlling the overall absorption rate and extent, and it is possible to use this value regardless of the transport mechanism of the drug (Amidon et al 1995).

It could be considered that P_{eff} reflects transport to the membrane (aqueous permeation e.g. diffusion and convection

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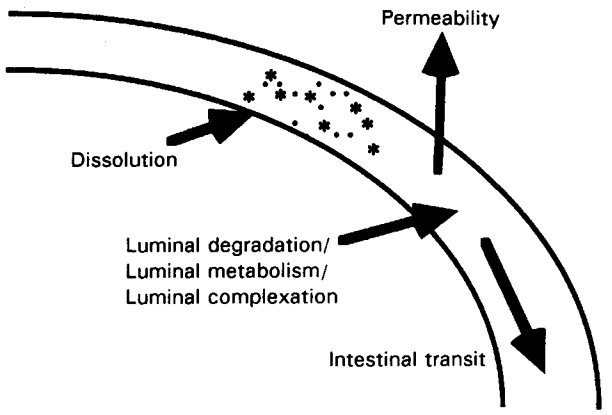

FIG. 1. Factors affecting oral drug absorption in general including: dose/solubility ratio, complex binding, chemical and enzymatic degradation, intestinal transit and effective intestinal permeability.

to the membrane), cell mucosa permeation including mucin and membrane translocation processes (passive or active transcellular transport or passive paracellular diffusion/convection), and perhaps transport through the cytosol, basolateral membrane, interstitial fluid and capillary wall to the blood (Flynn at al 1974; Komiya et al 1980; Ho et al 1983; Amidon et al 1995; Lennernäs et al 1995c). The view that P_{eff} reflects transport into portal vein, also includes metabolism occurring in the cytosol of the enterocyte. Metabolism by CYP 3A4 and cytosolic-localized peptidases will particularly influence the fraction reaching the portal vein (Wang et al 1989; Kolars et al 1991; Lennernäs et al 1995c; Wu et al 1995; Burton et al 1996; Samanen et al 1996). Therefore it is suggested that epithelial P_{eff} more accurately reflects the apical membrane diffusion for passively transported drugs (Lande et al 1994; Fagerholm & Lennernäs 1995; Lennernäs et al 1997a,b). Recently, it has also been reported that the permeability over epithelial cells is determined by the largest resistance which is usually considered to be the apical brush–border membrane (Lande et al 1994, 1995). Therefore, intestinal perfusion models, which measure the disappearance of the drug from the perfusion solution, directly reflect the transport across the apical epithelial cell membrane. This means that intestinal P_{eff} represents transport into the cytosol of the enterocyte. This view is certainly the case for passive transcellular diffusion where the apical membrane is rate-limiting. However, considering rapid transport for di- and tripeptides by means of the oligopeptide carrier, it seems likely that the basolateral membrane is the slowest step in the overall transport from lumen to portal blood.

Drug metabolism in the cell cytosol may influence the measured permeability if it is based on appearance of intact drug on the receiver side of the membrane, as in the Caco-2 and Ussing-chamber models. For the perfusion methodology, which is based on disappearance, a special concern is peptides, since they might be metabolized by membrane-bound enzymes (such as peptidases and esterases) in the brush-border. These enzymatic processes will increase the disappearance rate of the drug from the intestinal lumen during a perfusion experiment, and give an inaccurately high P_{eff} value. Therefore, it is crucial that metabolic processes are accounted for in any detailed model of the drug transport mechanism across the mucosal membrane as mentioned earlier (Amidon et al 1995; Lennernäs et al 1995c, 1997a,b). Furthermore, the carrier-mediated efflux transport of drugs by P-glycoproteins located in the apical membrane of mature enterocytes will give a lower P_{eff} value even if the passive diffusion across the lipid membrane phase is high (Thiebaud et al 1987; Croop et al 1989; Burton et al 1993; Benet et al 1996; Hidalgo & Li 1996; Lennernäs et al 1996b).

A third process that has been reported to affect the disappearance rate is binding to the intestinal tissue. P_{eff} is measured at a steady state in the perfusion system, so that the binding process that may influence (non-steady state) drug permeation is quasi steady state, and does not contribute to the measured permeability (Amidon et al 1995; Lennernäs et al 1995c). It is also unlikely that the binding capacity will be high enough to affect the mass balance transport for most drugs. However, it may be true for drugs with extremely high distribution volumes since this indicates extensive tissue binding.

It is apparent that these human intestinal-perfusion experiments are useful for generating knowledge on the direct in-vivo membrane transport. In addition, these techniques might be used to study the first-pass effect of drugs in the liver, drug metabolism in intestinal tissue by measuring the metabolites in the outlet perfusate, in-vivo dissolution of drugs, local pharmacological studies of drugs, nutrient absorption, biological mechanisms of different gastrointestinal diseases, food-drug interactions, and intestinal secretion of drugs and endogenous compounds (Ahrenstedt et al 1990; Knutson et al 1990; Lennernäs et al 1992, 1993a,b; Lindahl et al 1996; Bönlökke et al 1997).

This review will focus on permeability measurements in man emphasizing the different in-vivo transport mechanisms. In addition, I will discuss the correlation of our human data to preclinical models and the blood–brain barrier.

Regional Perfusion of the Proximal Small Intestine in Man In-vivo

This new technique has been developed to perform single-pass perfusion experiments in human subjects in-vivo in the fasted state (10-h fasting) (Knutson et al 1989; Lennernäs et al 1992). The perfusion instrument (Loc-I-Gut, Synectics AB, Sweden) is a 175-cm long and sterile polyvinyl tube (external diameter 5·3 mm), with six inner channels and is distally provided with two elongated latex balloons, placed 10-cm apart (Knutson et al 1989; Lennernäs et al 1992). The tube was inserted and positioned in the human proximal jejunum under the guidance of a fluoroscopic technique. Air (24–32 mL) was inflated into the two balloons, creating a 10-cm long jejunal segment (Fig. 2). The positioning of the tube usually takes 1 h, and the perfusion rate is between 2 and 3 mL min^{-1}. A more detailed description of the positioning procedure and the perfusion technique can be found elsewhere (Knutson et al 1989; Lennernäs et al 1992).

Theoretical Considerations and Data Analysis

Drug transport across the membrane of the perfused jejunal region (i.e. absorption) is the difference between the mass

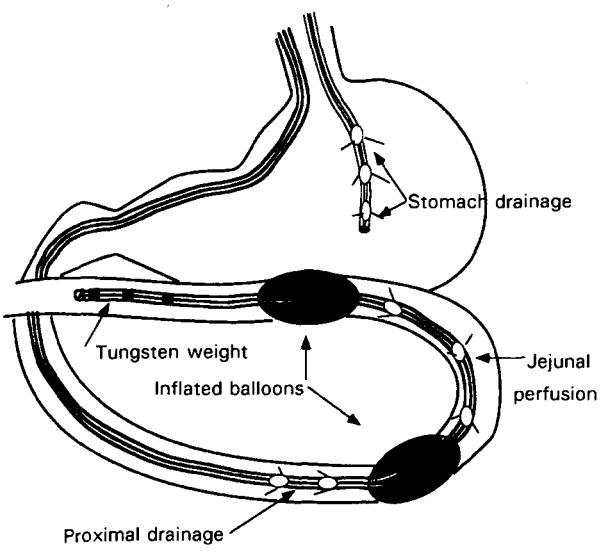

FIG. 2. The multichannel tube system with double balloons enabling segmental jejunal perfusion in man. The balloons are filled with air when the proximal balloon has passed the ligament of Treitz. Gastric drainage is obtained by a separate tube.

entering and leaving the intestinal segment:

$$dM/dt = Q_{in}C_{in} - Q_{out}C_{out} = Q(C_{in} - C_{out}) \quad (2)$$

where C_{in} and C_{out} are the inlet and outlet drug concentrations, respectively, and Q is the flow through the tube. The mass balance relationship has previously been proposed to describe the transport rate of the drug across the whole mucosal barrier (absorbed mass) according to Fick's first law (Flynn et al 1974; Amidon et al 1995; Lennernäs et al 1995c):

$$dM/dt = A \cdot P_{eff}(C_{ref}^{lumen} - C_{ref}^{blood}) \quad (3)$$

where A is the surface area of the membrane, P_{eff} is the effective permeability, and the reference concentrations are on the two opposite sides of the intestinal mucosa C_{ref}^{lumen}, C_{ref}^{blood}. Based on the discussion in the previous section where the apical cell membrane was considered as the barrier with the lowest permeability, and therefore is considered to be the rate-limiting step in the overall mucosal transport, the following relationship is given (Lande et al 1994; Fagerholm & Lennernäs 1995; Lande et al 1995; Lennernäs et al 1995c, 1997a,b):

$$dM/dt = A \cdot P_{eff}(C_{ref}^{lumen} - C_{ref}^{cytosol}) \quad (4)$$

However, since it is not possible to measure the concentration of a drug in the cytosol of the enterocyte or in the portal vein in man, we used the peripheral venous blood as the in-vivo reference on the receiver side. For example, we have shown that the peripheral plasma concentration is about one-hundredth the luminal concentration for antipyrine. These data indicated that it is valid to assume sink conditions, as antipyrine is a drug with a small volume of distribution (approximately 40 L in a 70-kg person) and is assumed to be evenly distributed in the body (Danhof et al 1982; Lennernäs et al 1992). In addition, we have reported a similar value for the liver extraction of fluvastatin, a CYP 2C9 substrate, after jejunal perfusion and intravenous administration, which further underlines the mass balance between disappearance and appearance rates of the investigated drug obtained by single-paas perfusion with Loc-I-Gut (Lindahl et al 1996).

P_{eff} and other variables were calculated from the steady-state level in the perfusate leaving the intestinal segment. The compounds of interest in the perfusate within the intestinal segment achieved equilibrium, when the concentrations of the solute and the [^{14}C]PEG 4000 in the outlet perfusate reached a plateau (at 50–60 min). Based on a residence-time distribution analysis, we have reported that the hydrodynamics within the perfused jejunal segment was best described by a well-mixed model (Lennernäs et al 1992, 1997a,b). P_{eff} was calculated using the outlet concentration as a reference concentration:

$$P_{eff} = \frac{Q_{in} \cdot (C_{in} - C_{out})}{C_{out} \cdot 2\pi R l} \quad (5)$$

where Q_{in} is the inlet perfusate rate, C_{in} and C_{out} are the inlet and outlet perfusate concentrations of the drug, respectively, R is the radius (R = 1·75 cm) and l is the length of the jejunal segment (10 cm). Q_{in} is the perfusion flow rate entering the jejunal segment. In all experiments the stability and adsorption of the drugs were carefully assessed (at 37°C for 180 min) and no degradation or adsorption were detected for any of the drugs investigated.

Effective Human Jejunal Permeability for Various Drugs

This regional jejunal perfusion approach to performing direct intestinal permeability studies in man has been validated according to criteria such as those shown in Table 1, including mass balance of the transport of antipyrine across the intestinal barrier (Fig. 1), physiological sink conditions of the drug concentrations between luminal and plasma compartments, the described hydrodynamics of the perfusion solution within the jejunal segment according to the well-stirred model, and the ability of the apical membrane in the jejunal mucosa to discriminate the passive diffusion of hydrophilic compounds with molecular weights between 18 and 350 Da. In addition, the functional viability of the mucosa was demonstrated by the rapid transmucosal transport of D-glucose and L-leucine from the regional jejunal segment, and complete recovery (> 95%) of PEG 4000 (a non-absorbable volume marker) in the perfusate leaving the jejunal segment (Lennernäs et al 1992, 1993a, 1994, 1997a,b; Fagerholm et al 1995, 1997).

We have determined the effective jejunal permeability (P_{eff}) for several drugs with different physicochemical properties and transport mechanisms in the proximal jejunum in man (Lennernäs et al 1992, 1993a, 1994, 1997a,b; Fagerholm et al 1995, 1997; Lindahl et al 1996). In our work with the Biopharma-

Table 1. The validation criteria of the regional jejunal perfusion method in man.

1.	Mass balance of antipyrine across the intestinal barrier.
2.	Physiological sink conditions.
3.	Complete recovery of the non-absorbable volume marker [^{14}C]-PEG 4000 in the outlet perfusate sample.
4.	The hydrodynamics can be described according to a well-mixed model.
5.	Molecular size selectivity of the jejunal membrane.
6.	Good prediction of the extent of drug absorption in-vivo in the P_{eff}-value.
7.	Carrier-mediated transport across the perfused jejunal segment.

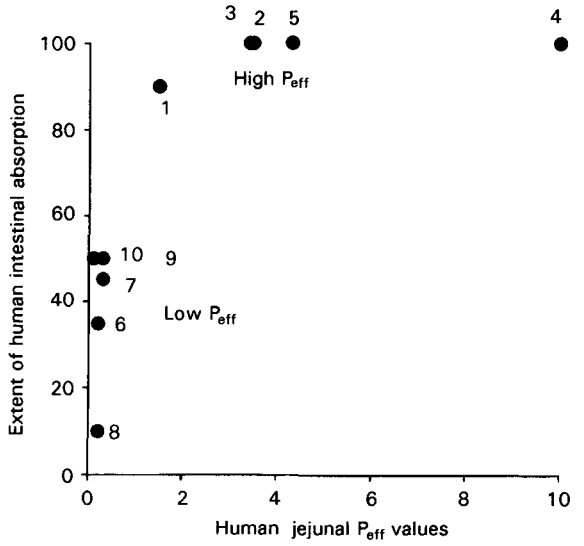

FIG. 3. The relation between extent of intestinal absorption and the measured human jejunal permeability value. 1. Metoprolol, 2. antipyrine, 3. L-dopa, 4. naproxen, 5. carbamazepine, 6. atenolol, 7. terbutaline, 8. enalaprilat, 9. furosemide, 10. hydrochlorothiazide. Permeability data were obtained from Lennernäs et al 1992, 1993, 1994, 1995a,b, 1997a; Fagerholm et al 1995, 1997; Lindahl et al 1996.

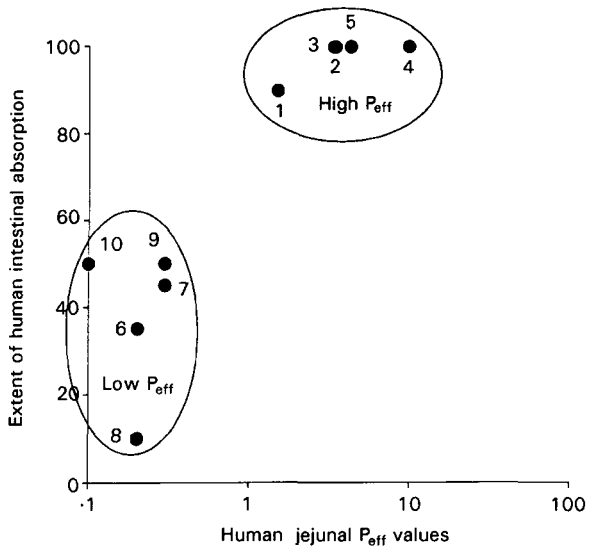

FIG. 4. The relation between extent of intestinal absorption and the measured human jejunal permeability value on a logarithmic scale. 1. Metoprolol, 2. antipyrine, 3. L-dopa, 4. naproxen, 5. carbamazepine, 6. atenolol, 7. terbutaline, 8. enalaprilat, 9. furosemide, 10. hydrochlorothiazide. Permeability data was obtained from Lennernäs et al 1992, 1993, 1994, 1995a,b, 1997a,b; Fagerholm et al 1995, 1997; Lindahl et al 1996.

ceutical Classification System (BCS) we have determined the P_{eff} value for 16 different drugs (Lennernäs et al 1995a, b, 1996b c). The relationship between measured human jejunal P_{eff} values and the extent of intestinal absorption (f_a) is characterized by a steep curve in the permeability range 0·5–1·5 10^{-4} cm s^{-1} (Fig. 3). Estimates of the extent of absorption (f_a) were recalculated from published pharmacokinetic studies (Fig. 3) (Lennernäs et al 1992, 1993a, 1994, 1995a,b, 1997a; Fagerholm et al 1995, 1997; Lindahl et al 1996). The extent of absorption (f_a) is defined as all processes from dissolution of the solid dosage form, to the intestinal transport of the drug into the intestinal tissue, i.e. across the apical membrane of the enterocyte. This is the general definition of extent of absorption, and does not include metabolic first-pass effects in the gut and liver or biliary excretion in the liver (E_G and E_H). The bioavailability (F) of a compound is a consequence of all these processes as shown (Lennernäs & Regårdh 1993; Lennernäs et al 1993b; Amidon et al 1995; Wu et al 1995; Benet et al 1996; Lindahl et al 1996):

$$F = fa(1 - E_G)(1 - E_H) \quad (6)$$

Furthermore, it is assumed that the major absorption of these drugs occurs in the proximal region of the small intestine when orally administered in an immediate-release product (Fig. 3) (Lennernäs et al 1992, 1993, 1994, 1997; Amidon et al 1995; Fagerholm et al 1995, 1997; Lindahl et al 1996). This means that the P_{eff} value measured in the proximal human jejunum is a good approximation of the permeability for the small intestine. Furthermore, based on the relationship in Fig. 3, the extent of absorption of drugs following oral administration is well predicted from the measured human P_{eff} value, i.e. when chemical or enzymatic stability in the lumen and complexation or dissolution can be excluded as potential factors affecting drug absorption in-vivo (Fig. 3). For instance, a drug with a high P_{eff} value might have a low extent of intestinal absorption when administered as a high dose in relation to its solubility (Amidon et al 1995). It is interesting to note that transformation of the permeability axis into a logarithmic scale results in a division of P_{eff} values of these drugs into two categories (low and high P_{eff}) (Fig. 4). Furthermore, it is apparent (Fig. 3) that the critical permeability range for the border between low and high permeability is between 0·5 and 1·5 10^{-4} cm s^{-1}. The accuracy of estimating extent of absorption in-vivo from the measured P_{eff} will be high for drugs that have their P_{eff} below or above this critical range. Therefore, it is crucial that more clinical determinations of the jejunal P_{eff} in man for drugs in the range, 0·5–1·5 10^{-4} cm s^{-1}, are carried out to characterize the steep part of this curve.

The drugs shown in Fig. 3 are also classified in accordance with the proposed Biopharmaceutical Classification System (BCS) for oral immediate-release products, i.e. based on the qualitative variables solubility (S) and permeability (P_{eff}) (Amidon et al 1995). The long-term goal of the human permeability project is to obtain quantitative values of the jejunal effective permeabilities (P_{eff}). This human permeability database will be regarded as one part of the recently proposed Biopharmaceutical Classification System (BCS) for oral immediate-release products (Amidon et al 1995). The major advantage of the BCS is that it will identify the key variables controlling drug absorption from immediate-release products, and thereby make it possible to classify drugs and simplify drug regulations. The following drugs have been investigated at Uppsala University within the BCS project: naproxen, metoprolol, ketoprofen, atenolol, furosemide, hydrochlorothiazide, carbamazepine, desipramine, α-methyldopa, (*R*,*S*)-verapamil, cimetidine, propranolol, lisinopril, losartan, amoxicillin and amiloride.

Studies of Transport Mechanisms In-vivo in Human Jejunum

The physiological mechanisms controlling intestinal absorption rates and transport routes of small drugs (500 Da) in man in-vivo and in commonly used absorption models are still not fully established (Lennernäs 1995; Uhing & Kimura 1995a,b; Schwartz et al 1995). More studies of these mechanisms in-vivo and validation of in-situ/in-vitro models are therefore necessary (Diamond 1995; Lennernäs 1995; Turner & Madara 1995). In addition, they are important for the understanding of the complex mechanisms determining the transport of nutrients, and different permeability markers used for diagnostic purposes, and for prediction of the extent of oral absorption of new candidate drugs in-vivo (Diamond 1995; Lennernäs 1995).

The general hypothesis for membrane transport has been that sufficiently lipophilic compounds are transported by transcellular diffusion, while hydrophilic compounds (those not transported by carrier-mediated processes) are transported by diffusion or convection via the paracellular route (Chadwick et al 1977; Taylor 1986; Pappenheimer & Reiss 1987; Hollander et al 1989; Taylor et al 1990; Artursson et al 1993). A mechanism whereby nutrients regulate the tight junction permeability has been discussed during the last decade (Pappenheimer 1987, 1993; Pappenheimer & Reiss 1987; Pappenheimer et al 1994). Nutrients and Na^+ have been reported to activate the Na^+-nutrient cotransporter in the apical enterocyte membrane, and thereby increase tight-junction permeability and net fluid absorption. During such conditions, intestinal transport of hydrophilic solutes (with molecular weights up to 5500 Da) through the opened tight junctions has been significantly enhanced (Pappenheimer & Reiss 1987; Gisolfi et al 1992; Sadowski & Meddings 1993; See & Bass 1993; Pappenheimer et al 1994). However, these results do not agree with our human data obtained by employing the regional perfusion technique (Lennernäs et al 1994; Nilsson et al 1994; Fagerholm et al 1995, 1997; Lennernäs 1995). D-Glucose and L-leucine in luminal concentrations of 50–80 and 20–60 mM, respectively (above their K_m values for carrier-mediated small intestinal transport) neither stimulated intestinal water absorption nor enhanced the P_{eff} for a range of different compounds (180–4000 Da, log D = 0·4). Fine et al (1993) reported similar in-vivo results which showed that carrier-mediated D-glucose absorption does not increase the passive permeability of the human jejunal mucosa to solutes with molecular radii between 2·6 Å (urea, 60 Da) and 4·0 Å (mannitol, 182 Da). The same group reported an almost identical investigation where luminal D-glucose (120 mM) increased the permeability of L-xylose (150 Da, molecular radius 3·4 Å, hydrophilic and passively transported) (Fine et al 1994). Other groups which have used different in-vivo approaches in intact animals have also reported that nutrient absorption, i.e. activation of the Na^+-nutrient cotransporter, did not increase the intestinal permeability for the nutrients or other small marker compounds (Schwartz et al 1995; Uhing & Kimura 1995a, b). It is therefore essential that further studies on this proposed mechanism are carried out using in-vivo, in-situ and in-vitro techniques to establish the quantitative importance of the potential regulation of paracellular absorption by the Na^+-nutrient cotransporter (Diamond 1995; Lennernäs 1995). A hypo-osmolar solution at the luminal side of the intestinal epithelium stimulates water absorption which might in parallel increase the uptake of compounds that are totally or partly transported with water (solvent drag) (Ochsenfahrt & Winne 1974; Winne 1974a,b; Hunt et al 1991; Karino et al 1982; Hirasawa et al 1984; Powell 1987). It has been suggested that the transport route is paracellular for both intestinal water and hydrophilic compounds under such conditions, but conclusive evidence is lacking (Pappenheimer & Reiss 1987; Fine et al 1993, 1994; Lennernäs 1995; Schwartz et al 1995). In three in-vivo studies in man, we stimulated water absorption using hypotonic solutions (170–180 mOsm L^{-1}), but the jejunal P_{eff} was not increased for hydrophilic compounds with molecular weights between 200 and 4000 Da (Lennernäs et al 1994; Nilsson et al 1994; Fagerholm et al 1995, 1997; Lennernäs 1995). These results suggested that quantitative paracellular absorption does not occur for any compound greater than 200 Da in the human small intestine in-vivo (Lennernäs et al 1994; Fagerholm et al 1995; Lennernäs 1995). This is consistent with the small area of the paracellular route, which is estimated to be about 0·01% of the total available surface area, and suggests molecular weight/pore size ratios greater than unity (Madara & Pappenheimer 1987).

Numerous attempts have been made to predict the passive transmucosal diffusion of drugs across the intestinal mucosa, or other membranes, from the drugs physicochemical properties (Overton 1899; Lien 1970, 1974; Martin 1981; Stein 1986; Walter & Gutknecht 1986; Cooper & Kasting 1987; Hollander et al 1988; Taylor et al 1990; Palm et al 1996; van de Waterbeemd et al 1992; Waterbeemd et al 1996; Testa et al 1996). These predictions have shown various degrees of success. Recently, drug transport across the membranes in the Caco-2 model and the blood-brain barrier have been predicted very precisely using conformational analysis followed by an estimation of the polar surface area (van de Waterbeemd et al 1992; Palm et al 1996).

Furthermore, it has been reported by Chikhale et al (1994) that the hydrogen bonding capacity of small peptides is one of the major factors determining the permeability across the Caco-2 monolayer and the blood-brain barrier in-vitro and in-situ, in rats (Conradi et al 1992; Chikhale et al 1994; Burton et al 1996; Samanen et al 1996). Consequently, there is a need for studies of the relationship between measured physicochemical properties for compounds with a wide range of chemical structures and in-vivo permeability across biological membranes. We are currently applying a multivariate analysis of such physicochemical properties to ascertain how well they predict the human effective intestinal permeability in-vivo for drugs with different structures (Fig. 5) (Winiwarter et al, unpublished). We have found a linear relationship between log D (pH 6·5) and the in-vivo P_{eff} value for four drugs (Lindahl et al 1996). This is interesting since other comparisons between log D and in-vitro permeability have suggested a sigmoidal relationship (Waterbeemd et al 1996; Burton et al 1996). These kinds of relationships demonstrate the importance and difficulties of trying to integrate aspects of drug absorption, as well other biopharmaceutic or pharmacokinetic variables, much earlier within the discovery and design process for new drugs (Gumbleton & Sneader 1994; Smith et al 1996).

The transport rates for drugs transported by various carrier-

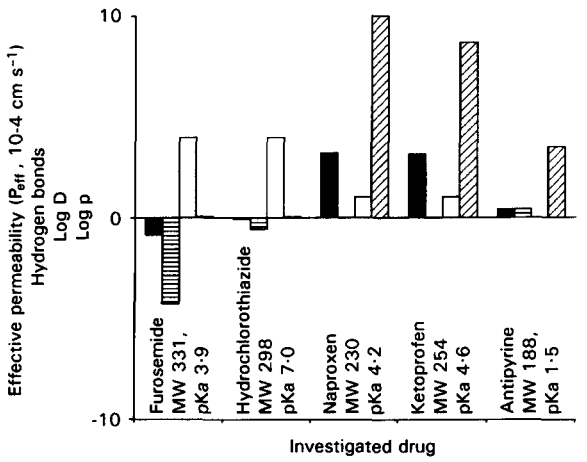

FIG. 5. The relation between some physicochemical variables and the measured human jejunal permeability value for some drugs. ■, Log p; ▤, Log D (oct/H_2O 7·4), □, H-bond, ▨, P_{eff}. Permeability data was obtained from Lennernäs et al 1992, 1993, 1994, 1995a; Fagerholm et al 1995, 1997; Lindahl et al 1996.

mediated mechanisms have been investigated in human jejunum for L-dopa, α-methyldopa and (*R*, *S*)-verapamil (Lennernäs et al 1993a, 1996b,c; Nilsson et al 1994). L-Dopa (log D, pH 6·5 approx. 2; 197 Da) and α-methyldopa (log D, pH 6·5 approx. 2; 211 Da) have been shown to be transported by a carrier-mediated process for amino acids (Merfeld et al 1986; Osiecka et al 1987; Hu & Borchardt 1990; Lennernäs et al 1993a; Nilsson et al 1995). The drug α-methyldopa is classified as a low-permeability drug with a P_{eff} value of about $0{\cdot}1 \times 10^{-4}$ cm s^{-1} at an inlet perfusate concentration of 6·7 mM. This P_{eff} value is approximately one-thirtieth that of L-dopa ($3{\cdot}4 \pm 1{\cdot}0 \times 10^{-4}$ cm s^{-1} at a luminal concentration of approximately 2·0–2·5 mM) (Lennernäs et al 1993a, 1996c; Nilsson et al 1994). The lower transport rate of α-methyldopa compared with that of L-dopa is probably due to a lower affinity to, or transport capacity of, the amino acid transport carrier. The small value for jejunal P_{eff} also means that the passive transmucosal diffusion of α-methyldopa is low. A small change in the chemical structure gives a marked alteration in the permeability for carrier-mediated transport through the mechanisms for large neutral amino acids, which is most probably due to a very narrow structure specificity of this amino acid carrier.

Verapamil is a well-known substrate for P-glycoprotein (Ford & Hait 1990). The human P_{eff} values for each enantiomer of (*R*,*S*)-verapamil (log D, pH 6·5 approx. 2; 455 Da) are similar (about $5–6 \times 10^{-4}$ cm s^{-1}, which predicts rapid transport across the human jejunal mucosa of both enantiomers (Fig. 3) (Lennernäs et al 1996b). Furthermore, it also indicates that the efflux mechanism, mediated by the P-glycoprotein in the apical membrane of the mature enterocytes on the tip of villi, might not affect the quantitative transport and extent of absorption of any of the enantiomers of (*R*,*S*)-verapamil in the human small intestine at a luminal concentration of 375 mg mL^{-1} (0·8 mM). This luminal concentration in the jejunum is assumed to occur in man following an oral dose of 100 mg (dissolved in 300 mL gastrointestinal fluid). Our clinical observations clearly suggest that not all compounds that are substrates for P-glycoproteins lead directly to reduced bioavailability (Lennernäs et al 1996a). More research is needed regarding the role of P-glycoprotein in quantitative drug transport across the human intestinal mucosa in-vivo (Benet et al 1996; Burton et al 1996; Fricker et al 1996; Tsuji & Tamai 1996). For example, the interaction of different factors needs to be investigated urgently at different luminal concentrations throughout the intestinal tract. Factors of potential importance include passive membrane diffusion, the concentration of the drug freely available for the transport protein, metabolism, interaction with metabolites formed inside the enterocyte, and the affinity and transport capacity of the P-glycoproteins involved. It is also essential that mechanisms behind the inhibition and induction of P-glycoproteins in the intestinal tissue are studied. It is also unclear how P-glycoproteins can transport compounds with a wide range of structure and functions (Burton et al 1993). Therefore, it is surprising that it has been reported that P-glycoproteins can distinguish slight differences in the structures of steroids. Fundamental knowledge of the dynamic mechanism of the efflux pump located in the intestinal mucosa will contribute to a better understanding of its role in biopharmaceutics and pharmacokinetics i.e. intestinal absorption and bioavailability of drugs. It would also be particularly interesting to investigate the potential inhibitory effect that some pharmaceutical additives, and detergents, such as Tween 80, might have on the P-glycoproteins in-vivo in man (Woodcock et al 1992; Nerurkar et al 1996).

The Correlation of Human Jejunal Permeabilities with Preclinical Permeability Models

P_{eff} has been measured by a single-pass perfusion approach in anaesthetized rats in-situ (thiobutabarbital Na^+) at a perfusion flow rate of 0·2 mL min^{-1}, which is one-tenth that for man (Lennernäs et al 1992; Fagerholm et al 1996). The viability of the rat perfusion model was assessed by testing the physiological function of the rat intestine during perfusions. For instance, PEG 4000 labelled with ^{14}C, an established non-absorbable compound, was used to indicate intact jejunal barrier. Further validation of the model was obtained by investigating the carrier-mediated cotransport of Na^+/D-glucose. This Na^+/D-glucose cotransporter is a membrane protein that is crucial for the membrane transport of these two compounds. Antipyrine was included as a marker for passive transcellular absorption. Antipyrine is also used as an indicator of extensive changes of mesenteric blood flow (Winne et al 1984). For passively transported compounds the rank order was the same in perfused proximal jejunal segments of both man and rat. The human P_{eff} estimates for drugs transported by passive diffusion were on average 3·6 times higher in man in-vivo than in rat in-situ, irrespective of the permeability classification of the drug (Fig. 6) (Fagerholm et al 1996; Lennernäs et al 1996a, 1997b). Plausible reasons for the lower P_{eff} value in the rat model are differences in effective absorptive area within the perfused segment, and species differences affecting partitioning into the membrane (K), diffusion coefficient (D_m) and diffusion distance (Brasitus & Dudeja 1985; Stein 1986; Fagerholm et al 1996). The anaesthesia will certainly also contribute to somewhat slower passive diffusion across the jejunal barrier. Carrier-mediated transported compounds, such

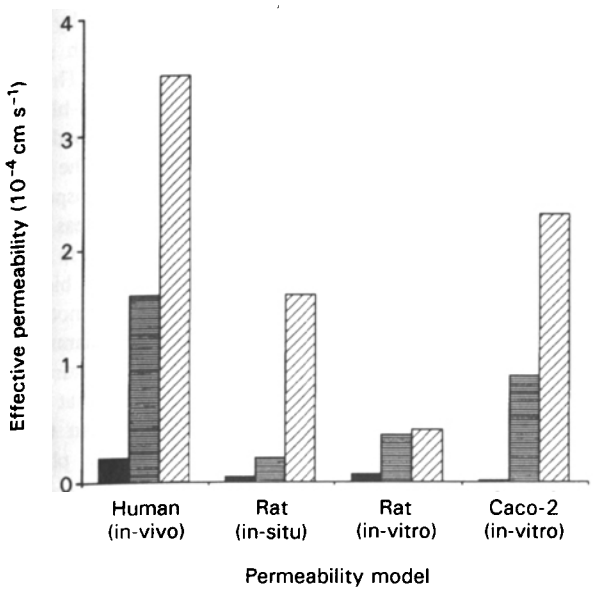

FIG. 6. The effective permeability values of atenolol, metoprolol and antipyrine in four different permeability models. These three drugs are transported across the intestinal barrier by passive diffusion. ■, Atenolol; ▤, metoprolol; ▨, antipyrine. Permeability data were obtained from Fagerholm et al (1996) and Lennernäs et al (1996a, 1997b).

as L-dopa and D-glucose, deviated from this linear relationship between the two models, which clearly demonstrates that each carrier-mediated transported drug needs to be carefully investigated to elucidate the transport mechanisms involved and to obtain a scaling factor for directly comparing the different species and models (Fig. 7) (Fagerholm et al 1996; Lennernäs et al (1996a, 1997b)). However, both human and rat P_{eff} values predict the quantitative amount of drug absorbed in-vivo in man very well (Fagerholm et al 1996).

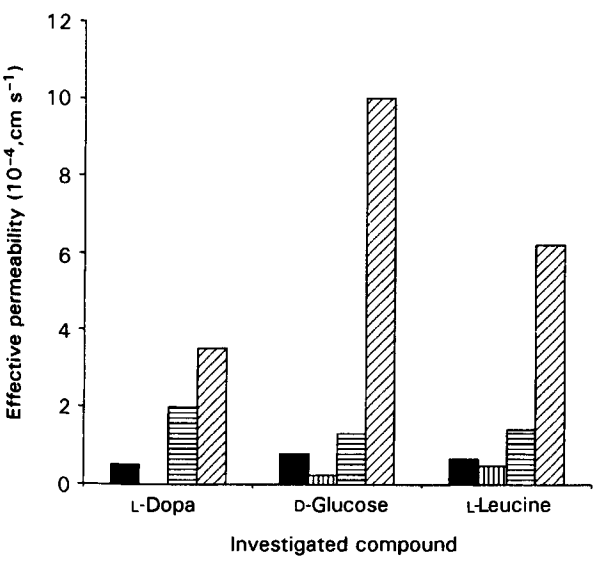

FIG. 7. The effective permeability values of L-dopa, D-glucose and L-leucine in four different permeability models. These three compounds are transported across the intestinal barrier by carrier-mediated mechanisms. ■, Rat jejunum in Ussing chambers; ▥,Caco-2 monolayers; ▤, rat jejunal perfusion; ▨, human jejunal perfusion. Permeability data were obtained from Fagerholm et al (1996) and Lennernäs et al (1996a, 1997b).

For the Caco-2 model, the permeability coefficients of the rapidly transported drugs were dependent on the hydrodynamics (Artursson & Karlsson 1991; Lennernäs et al 1996a). However, the true in-vitro permeability for antipyrine of $2–3 \times 10^{-4}$ cm s^{-1} was directly comparable with that obtained in the well-stirred in-vivo situation in the human jejunum. Similar results were obtained for the other two model drugs (naproxen and metoprolol) for passive transcellular drug absorption (Lennernäs et al 1996a) (Fig. 7). These results give further support to the hypothesis that the intestinal epithelium and not the adjacent unstirred water layer is the rate-limiting barrier to absorption of rapidly transported drugs such as antipyrine, naproxen and metoprolol (Lennernäs et al 1992, 1996a; Fagerholm & Lennernäs 1995). The permeability values of the low permeability drugs, such as atenolol and terbutaline, in the human jejunum were on average 50 times those in the Caco-2 monolayers (Fig. 7). The lower mean permeability in the Caco-2 monolayers might be due to a lower paracellular permeability in colon-derived cell line as suggested by Artursson et al (1993). Another possible explanation is that there is a larger area available in-vivo in man, as it is assumed that the absorption of hydrophilic compounds is so slow that a larger surface area of the intervillous space is exposed (Schwartz et al 1995). Thus, the permeability values of hydrophilic compounds in the Caco-2 monolayers are closer to those seen in the human colon. However, it was concluded that the passive diffusion of drugs across the human jejunal mucosa in-vivo can be predicted and classified in the Caco-2 model (Fig. 7). The effective permeability values of carrier-mediated transported compounds such as L-dopa, D-glucose and L-leucine were also much slower in Caco-2 cells than in human jejunum in-vivo at the same concentrations (Fig. 7). For instance, the carrier-mediated transport rate of L-dopa in human jejunum was approximately 340 times that in Caco-2 monolayers. However, we can not exclude the possibility that these compounds were also partly transported by passive diffusion in the Caco-2 monolayers due to saturation of the carrier. Nevertheless, the results are in agreement with previous studies in Caco-2 monolayers which show that this cell line displays a variable and generally lower expression of carrier-mediated transport than that seen in-vivo (Hu & Borchardt 1990). This is also consistent with the colonic origin of the Caco-2 cells. Prediction of carrier-mediated drug transport in man based on data generated in the Caco-2 model will, therefore, only be possible after each transport system has been characterized, and the subsequent introduction of a scaling factor to compensate for the differences between the carriers expression in Caco-2 cells and in-vivo (Lennernäs et al 1996a). It is still possible to perform studies of transport mechanisms for different drugs in the Caco-2 model with a high degree of accuracy, despite the lower expression of transport proteins by this model.

The P_{eff} value for compounds transported by both passive diffusion and carrier-mediated mechanisms across the rat jejunal segment mounted in an Ussing chamber have been compared with corresponding human data (Lennernäs et al 1997b). The P_{eff} values and their rank order were the same for passively transported compounds in the excized rat jejunal segment (in-vitro) and in the human jejunum (in-vivo). There was a high correlation between the two models when both low and high P_{eff} drugs (transported by passive diffusion) were

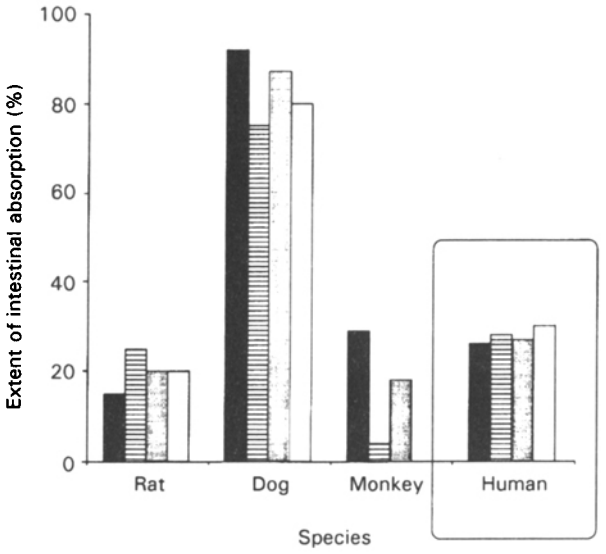

FIG. 8. The interspecies variation in the extent of intestinal absorption of four incompletely absorbed drugs. ■, Nadolol; ▤, acyclovir; ▥, methyldopa; □, pafenolol. (Permeability data was obtained from Regårdh et al 1990; Dressman & Yamada 1991).

compared (Lennernäs et al 1997b). The human in-vivo P_{eff} estimates for all drugs absorbed by passive diffusion were in general about 4–5 times those in the rat (Fig. 6). The carrier-mediated transport values for D-glucose, L-dopa and L-leucine were approximately 5–15 times higher in the in-vivo human model (Fig. 7). The higher P_{eff} values in the in-vivo model might partly be explained by the lack of blood flow or a less pronounced concentration gradient across the jejunal barrier in-vitro. In addition, the in-vitro P_{eff} values for the carrier-mediated transported compounds, might also be affected by the supply of co-factors which are crucial for an optimal function of the transport protein (Lennernäs et al 1997b).

The extent of drug absorption in different species has been reported for nadolol, acyclovir, α-methyldopa and pafenolol. Fig. 8 demonstrates the well-known fact that small, hydrophilic, and passively transported drugs are better absorbed in dogs than in other species, such as rat, man and monkey (Regårdh et al 1990; Dressman & Yamada 1991). The physiological explanation underlying this observation is still unknown, but it has been suggested that the dog villi should be higher and therefore expose a larger area for absorption than the other species (Madara & Trier 1994). For drugs that are CYP 3A4 substrates rapid transport will probably lead to a higher cytosolic drug concentration and, most likely, somewhat saturate the CYP 3A4 enzymes located in the enterocyte and consequently decrease first-pass and higher bioavailability (Wang et al 1989; Kolars et al 1991; Wu et al 1995). This is especially valid when the major part of the given oral dose is absorbed in the more proximal region of the small intestine, since these CYP 3A4 enzymes are localized to that region (De Waizers et al 1994).

Another plausible reason for the reported higher degree of absorption in dogs might be differences in the functional expression of P-glycoproteins. For instance, pafenolol, which has a bioavailability of about 20–30% in rat and man compared with 80% in dog (Regårdh et al 1990). This is mainly due to incomplete uptake, and could also be partly caused by excretion of the drug directly into the intestine in both species (Regårdh et al 1990; Lennernäs & Regårdh 1993). This phenomenon has also been observed in-vivo for other β-blocking agents such as talinolol and in-vitro been confirmed to be related to P-glycoprotein (Gramatté et al 1996). The higher bioavailibility in dog could be caused by a higher transport rate across the apical membrane as a consequence of less P-glycoproteins expressed in the dog enterocytes than that in human or rat enterocytes. A plausible reason for why the bioavailability of drugs such as pafenolol and talinolol, and not (*R*,*S*)-verapamil, should be affected by the efflux mechanisms could be that (*R*,*S*)-verapamil has a higher degree of saturability. This explanation might hold if it is assumed that (*R*,*S*)-verapamil has a more rapid passive diffusion than that of pafenolol as a consequence of more favourable physicochemical properties.

Is There a Correlation Between the Transport Rate Across the Epithelial Membrane in the Intestinal Tract and the Endothelial Membrane in the Blood–brain Barrier?

Previously it has been suggested that transcellular transport across the apical epithelial membrane in the human jejunum is the most important route for quantitative drug transport for molecules larger than 200 Da regardless of their physicochemical properties (Lennernäs 1995). This hypothesis prompts us to question whether passive diffusion and carrier-mediated transport across the intestinal apical membrane could be used to predict the permeability across the blood–brain barrier. In general, passive effective membrane permeability (transcellular route) in-vivo is determined by three factors (Stein 1986):

$$P_{eff} = KD_m/\lambda \quad (7)$$

where K is the membrane-aqueous partition coefficient of this compound, D_m is the diffusion coefficient of the compound within the membrane, and λ is the thickness of the membrane barrier. This equation describes the "solubility-diffusion" model and can explain why the membrane permeability of drugs depends on both the hydrophobicity and the molecular size. This model is general and is valid for both lipid bilayer membranes as well as other biological membranes (Stein 1986). The partition coefficient (K) describes the relative tendency of a substance to dissolve in the membrane phase (or an organic solvent that mimics the membrane), compared with its tendency to dissolve in the surrounding aqueous phase. Furthermore, the permeability decreases when the molecular size (molecular volume) increases, owing to a decreased diffusion coefficient of the molecule within the membrane (D_m). The mass dependence of diffusion within the cell membrane has been observed to be very much steeper than for diffusion in an aqueous phase (Stein 1986).

The transport rate of drugs into the brain tissue (dM/dt) and available to give a pharmacological effect, is affected by the factors described in the relationship:

$$dM/dt = P_{eff} \times A(C_{plasma} \times f_u - C_{ubrain}) \quad (8)$$

where A is the surface area, f_u is the fraction unbound in plasma, and $C_{plasma} * f_u - C_{ubrain}$ is the unbound drug con-

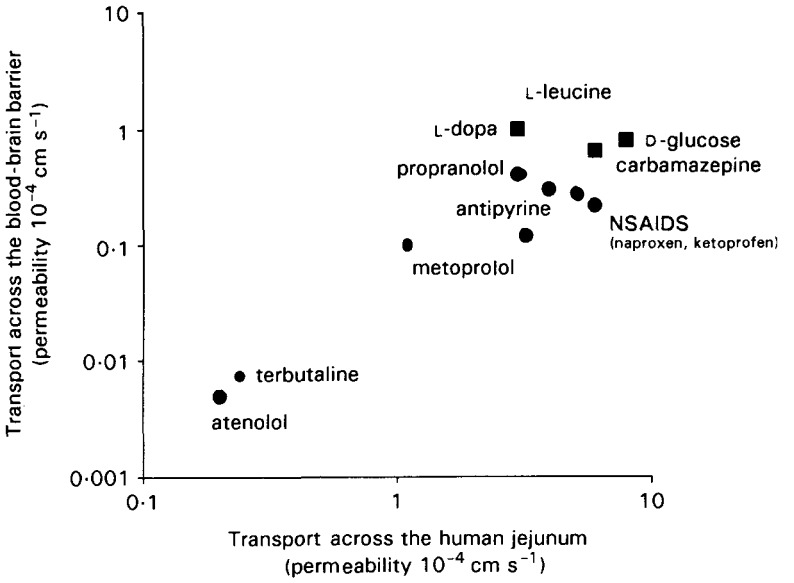

FIG. 9. The relation between the measured human jejunal permeability value and the permeability across the blood–brain barrier estimated in various models. ●, Passive diffusion; ■, carrier-mediated transport. Data was obtained from (Van Bree et al 1989; Pardridge et al 1990; Belle et al 1992; Dehouck et al 1992; Fagerholm et al 1996; Lennernäs et al 1993, 1994, 1995a,b, 1996).

centration gradient across the blood–brain barrier. The free steady-state concentration in the interstitial brain tissue is simultaneously affected by tissue binding and drug elimination from the central nervous system (both secretion and metabolism). The P_{eff} across the blood–brain barrier will be one of the major factors determining the mass of drug that will be delivered into the brain tissue. This can occur by both passive diffusion and carrier-mediated transport. The relationship between human jejunal permeability and the permeability across the blood–brain barrier in animals is described in Fig. 9, which clearly demonstrates that a drug which shows high permeability across the intestinal barrier may also be considered a highly permeable compound across the blood–brain barrier. However, the quantitative transport of drugs into the brain tissue can still be low due to extensive plasma protein binding. The potential role of efflux by P-glycoproteins in the in-vivo quantitative transport in the intestine and blood–brain barrier is still unclear. The blood–brain barrier permeability data must be considered with some caution since they are obtained from different studies using slightly different techniques (Van Bree et al 1989; Pardridge et al 1990; Dehouck et al 1992; Belle et al 1994). Even if the reported transport data for the blood–brain barrier permeability includes some uncertainties, it is still possible to obtain a rough estimation of blood–brain barrier permeability from the intestinal permeability values generated in the different intestinal models discussed previously. This is of major interest, since overcoming the blood–brain barrier is identified as the rate-limiting step in brain drug research and development in general (Pardridge 1995). Moreover, this correlation suggests that pure passive membrane diffusion is universal for membranes with different physiological functions and physicochemical properties. The absolute value of the permeability will certainly be different, but the rank order will probably agree very well between different biological membranes.

Conclusions

The regional human jejunal perfusion technique has been validated by several crucial points (Table 1). One of the most important findings is there is a good correlation between the measured human effective permeability values and the extent of absorption of drugs in man determined by pharmacokinetic studies. We have also shown that it is possible to determine the P_{eff} value for carrier-mediated transported compounds, and to classify them according to the proposed Biopharmaceutical Classification System (BCS).

Furthermore, it is possible to predict human in-vivo permeability using preclincal permeability models, such as in-situ perfusion of rat jejunum, the Caco-2 model and excized intestinal segments in the Ussing chamber. The permeability of passively transported compounds can be predicted with a particularly high degree of accuracy. However, special care must be taken for drugs with a carrier-mediated transport mechanism, and a scaling factor needs to be used. I also suggest that it is possible to roughly estimate the permeability of the blood–brain barrier using measurements of intestinal permeability, even if the role of efflux of P-glycoproteins still remains to be clarified.

The data obtained in-vivo in man emphasize the need for more clinical studies investigating the effect of physiological in-vivo factors and molecular mechanisms influencing the transport of drugs across the intestinal as well as other membrane barriers.

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