Jejunal Permeability: A Comparison Between the Ussing Chamber Technique and the Single-Pass Perfusion in Humans

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INTRODUCTION

Investigation of the transport of systemically acting drugs across the intestinal membranes is fundamental in order to develop strategies such as; (a) improvements of the mass of drug absorbed from the oral product, and (b) biopharmaceutical criterias for selection of the most appropriate candidate drug regarding intestinal absorption in humans. Crucial to rational drug design and development is the access to different intestinal permeability models, but direct comparisons with human data are lacking. Recently technical progress in the development and validation of a new regional human perfusion approach has been made, which is used to study membrane transport of drugs *in vivo* (1). Therefore, it is now possible to directly compare permeability values obtained in the different *in vitro/in situ* models with these *in vivo* human effective permeabilities. Recently, such correlations have been reported for Caco-2 cells and *in situ* perfusion preparations of the rat jejunum (2,3). However, a direct evaluation of the ability of the Ussing chamber technique to predict permeability values for both passively and carrier-mediated transported drugs in humans has not yet been published. As a consequence, we will directly compare the effective permeability coefficients (P*eff*) determined in human jejunum (*in vivo*) and excised jejunal segment from rat (*in vitro*).

The P*eff*-value of a drug is a biopharmaceutical variable that is possible to use regardless of the transport mechanism across the mucosal barrier (4). A majority of drugs are transported across the intestinal mucosa by passive diffusion, and is determined by the membrane/lumen partitioning (K) and the membrane diffusion coefficient (D_m) (P*eff* = $K \cdot D_m/\lambda$: "Overton's rule") (5,6). λ represents the transport distance for a molecule across the intestinal cell lining. The general view is that lipophilic compounds are transported by the transcellular pathway, whereas hydrophilic and charged molecules, which have lower P_{eff} due to low K and/or D_m-values, are transported through the water filled space between the epithelial cells, the so called paracellular route (6–9). However, the quantitative importance of drug transport by the paracellular route *in vivo* has been questioned recently (6,8,10). Instead, the passive transcellular diffusion was suggested to be the dominating transport route in quantitative aspects for compounds larger than 200 Da, regardless of the physicochemical properties of the drug (6).

The aim of this study was to determine the effective permeability for 12 compounds with different transport mechanisms (passive and carrier-mediated) in excised jejunal rat segments *in vitro* using the Ussing chamber technique, and then compare to corresponding permeabilities in humans *in vivo*.

MATERIAL AND METHODS

Animal Experiments (*in Vitro*)

Female Spraque-Dawley rats weighing 250–300 g were used in the experiments. All animals had free access to food and water prior to sacrifice. For preparation of the tissue segment, the rat was anaesthetised with Isofluran (FORENE® Abbott, USA) and the proximal jejunum was excised, washed thoroughly using cold (10°C) Krebs-Bicarbonate Ringer solution (KBR) as described earlier (7,11). Thereafter, the intestinal tissue was put into a cold and bubbled (95/5% O_2/CO_2) KBR solution for further preparation.

After the serosa of the proximal jejunum was stripped off using blunt dissection with the tissues submerged in cold and bubbled KBR, the tissues were mounted in modified Ussing chambers with rotors for effective stirring conditions. The effective area of the tissue segment was 1.14 cm^2 and the viability as PD (potential difference), SCC (short-circuit current), and TEER (transepithelial electrical resistance) was continuously monitored as described previously (11). All the experiments were carried out unidirectionally at 37°C with the tissues unclamped, and any tissue with electrical values less than 4 mV and 30 $Ohm \cdot cm^2$ for PD and TEER, respectively, was excluded before the experiments were started.

Each permeability experiment was started by changing the solution on both sides of the membrane to fresh KBR and the drug to be tested was added to the mucosal solution. Samples were withdrawn from both compartments at regular time intervals for up to 180 min and the P*eff* was calculated according to equation 2.

Human Experiments (*in Vivo*)

A regional single-pass perfusion approach (2.0–3.0 ml/min) was used in healthy volunteers, which is based on a double balloon technique with a 10 cm long jejunal segment, which are described in more details elsewhere (1). The jejunal effective permeabilities (P*eff*) have previously been published (1–3,6,8).

Drugs

In this report we used 12 compounds which are transported across the jejunal mucosa by different mechanisms (passive and carrier-mediated), and exhibit various physico-chemical properties (Table 1). The wide range of lipophilicity was demonstrated by log D values (octanol/water, pH 7.4) ranging −5.2–1.3. However, the molecular weights represent a more narrow range, 113–426.

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Table 1. Physicochemical Characteristics Substrateconcentrations (Conc.), and Number of Experiments (n) of 12 Different Compounds Studied in Rat Jejunum Using the Ussing Chamber Technique

	Physicochemical properties			Experimental information	
	MW [g/mole]	pKa	log D [oct/water, pH 7.4]	Conc. [mM]	n [rat/man]
Passive absorption					
Antipyrine	188	1.5 (base)	0.4[b]	traces	5/75[a,d,e]
Atenolol	266	9.6 (base)	−1.8	0.25	5/8[d]
Inogatran	439	1.3, 7.6, >12 (base)	0.0	0.25	5/8[b]
Enalaprilat	348	2.8, 3.5, 7.6 (acid)	−5.2	2.87	2/8[d]
Propranolol	259	9.5 (base)	1.3	traces	10/8[f]
Metoprolol	267	9.7 (base)	0.0	0.25	4/8[c]
Naproxen	230	4.4 (acid)	0.2	1.78	2/8[c]
Creatinine	113	4.9 (base)	−2.2	traces	3/75[f]
Terbutaline	225	8.8, 10.1, 11.2[c] (base)	−1.4	0.25	5/15[c]
Carrier-mediated absorption					
D-glucose	180	− (−)	−3.0[d]	11	3/53[a,d,e]
L-dopa	197	2.3, 8.7, 9.7, 13.4 (acid)	n.i.	traces	7/22[c]
L-leucine	131	2.3 (acid), 9.6 (base)	n.i.	traces	5/22[g]

Note: n.i.; no information available. ; traces = only radiolabeled substance added (corresponding to 10–30 μM for the different compounds). Base = basic compounds; log D = partition between octanol/water at pH 7.4. The pH and osmolality in the solution were 6.5 and 290 mOsm/L and 7.4 and 290 mOsm/l, for *in vivo* human jejunum perfusions and rat experiments, respectively.
[a] Lennernäs, H. *et al.*, 1992.
[b] Astra Hässle internal report, 1996.
[c] Fagerholm. U. *et al.*, 1996.
[d] Lennernäs. H. *et al.*, 1994.
[e] Lennernäs. H. *et al.*, 1996.
[f] Lennernäs. H. *et al.*, 1996 unpublished data.
[g] Lennernäs. H. *et al.*, 1996.

Chemical Assays

All chemicals used were of analytical grade. The drugs were analysed by different HPLC methods, which are described elsewhere (1–3,6,8). D-glucose and L-leucine were measured using routine clinical chemistry (1,3). ^{14}C-D-glucose, ^{14}C-L-lopa and ^{14}C-L-leucine were analysed in a liquid β-scintillation counter (LKB Rackbeta, SWEDEN)

Data Analysis

The P*eff* and other variables were calculated from the steady-state level in the perfusate leaving the human jejunal segment by using the well-stirred model according to eq. 1 (1):

$$Peff = \frac{Qin \cdot (Cin - Cout)}{Cout \cdot 2\,\pi RL} \quad (1)$$

where Q_{in} is the inlet perfusate rate, C_{in} and C_{out} are the inlet and outlet perfusate concentration (corrected for fluid flux), respectively, R is the radius (R = 1.75 cm) and L is the length of the jejunal segment (10 cm) (1).

The effective (apparent) permeability (P*eff*) across the excised rat jejunal segment (rat) in the Ussing chamber was calculated according to eq. 2:

$$Peff = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_0} \quad (2)$$

where dQ/dt is the steady-state appearance rate on the serosal side of the membrane, A is the exposed surface area and C_0 the starting concentration on the mucosal side (2). The data are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

In the present study we have determined the effective permeability (P*eff*) for compounds transported by passive diffusion and carrier-mediated mechanisms across the rat jejunal segments, using the Ussing chamber technique *in vitro* (Table 2 and Figures 1–2). The permeability values for the passively transported compounds were ranked similarly in humans and rats, but the P*eff* for passive transport were about 5–6 times higher than in the rat segment (Fig. 1). There was a high correlation between these two models when both low and high P*eff*-values for passively transported drugs were compared (R^2 = 0.95) (Fig. 2). It is also interesting to note the parallel shift to the left in relation to the human *in vivo* data for the present *in vitro* model, which also agreed with the correlation reported for the Caco-2 model (Fig 1) (2).The similarities regarding rank order support the use of the Ussing chamber technique for predictions of the intestinal absorption of drugs in humans during discovery and development of new oral drug candidates.

The P*eff* for the hydrophilic, low permeable and incompletely absorbed compounds enalaprilat, creatinine, atenolol, inogatran (pINN) and terbutaline were on average 3.4 times higher in the human jejunum (*in vivo*) than in the excised jejunal

Table 2. Absorption Variables of 12 Compounds Obtained in Rat Jejunum Using the Ussing Chamber

Compound	Effective permeability, Peff [$\cdot 10^{-4}$ cm/s]			Fraction absorbed, fa [%]
	Rat (Ussing) (*in vitro*)	Man[a]# (perfusion) (*in vivo*)	Rank Order[a] rat/human	fraction abs.# *in vivo* in humans
Passive absorption				
Antipyrine	0.40 ± 0.07	5.6 ± 1.6	2/2	100
Atenolol	0.06 ± 0.003	0.15 ± 0.2	7/7	50
Enalaprilate	0.06 (0.03 – 0.08)	0.1 ± 0.3	8/8	60
Propranolol	0.29 ± 0.02	2.8 ± 1.3	4/3	100
Metoprolol	0.35 ± 0.07	1.5 ± 0.9	3/4	≥95
Naproxen	0.45 (0.39 – 0.51)	10 ± 3.6	1/1	100
Creatinine	0.08 ± 0.01	0.29 ± 0.16	5/6	0
Terbutaline	0.07 ± 0.080	0.3 ± 0.3	6/5	60
Inogatran	0.03 ± 0.01	0.03 ± 0.03	9/9	—
Carrier-mediated absorption				
D-glucose	0.57 ± 0.21	11 ± 8.2	—/—	100
L-dopa	0.36 ± 0.20	3.4 ± 1.7	—/—	≈100
L-leu	0.71 ± 0.27	6.2 ± 2.9	—/—	100

Note: The human data are historical data which are published elsewhere (refs. 6, 8, 14, 16–19). The P_{eff} estimates are presented as the mean ± SD.

[a] Compounds utilizing carrier-mediated transport are not ranked. # These human permeability data and values of fraction absorbed are taken from refs. 6, 8, 14, 16–19. The human P_{eff} for propranolol is obtained from Lennernäs et al, unpublished data. For propranolol, enalaprilate and naproxen mean and range are given.

segment of the rat (Tables 1–2). The P*eff* for inogatran—which has a dipeptide like structure—was almost identical in rat and man, i.e., 0.04 and 0.03 $*10^{-4}$ cm/s, respectively. The mean ratio between the human and rat models regarding P*eff*-estimates for the four high permeability drugs, metoprolol, propranolol, naproxen and antipyrine was 11 (Table 2).

In general, model differences regarding the quantitative permeability values, assuming the transcellular route to predominate regardless of permeability class (low or high), might be partly explained by species differences due to the available intestinal surface area for absorption, lipid composition of the intestinal mucosa and methodological differences such as oxygenation and viability of the tissue, thickness of the diffusional pathways, blood flow and stirring conditions. However, the more pronounced difference between the *in vivo* and *in vitro* models obtained for drugs with a high P*eff*-value may be due to a complex interplay between different factors. For instance, the sink conditions, usually created by the capillary network in the villi, can be absent or less functioning in the *in vitro* model, which might affect the permeability across the intestinal mucosa for drugs with high permeability coefficients (12). The lower supply of cofactors might also influence the organisation of the membrane lipids and proteins, which thereafter can affect the solubility of the drug in the membrane as well as the membrane diffusion coefficient ($Peff = K \cdot D_m/\lambda$) (5,6). In addition, the effective surface area on the top of the villus might be less available in the *in vitro* model, which should influence the P*eff* of rapidly transported compounds, which are known to be absorbed in that region of the villus (13). Another possible factor is the unstirred water layer (UWL), which may be the rate-limiting diffusion barrier for drugs with a high transport rate across the membrane *in vitro*, and effect both transport towards the membrane and the effective surface area available for absorption. In the human *in vivo* model we have earlier reported that the membrane is the rate-limiting step, since the P*eff*-values for both D-glucose and antipyrine were unchanged despite increased luminal stirring caused by changing the perfusion rate between 1.5–6.0 ml/min (14). This is most likely due to gastrointestinal motility in vivo producing a highly efficient luminal stirring, which is sufficient for a reduction of the unstirred water layer *in vivo* (14).

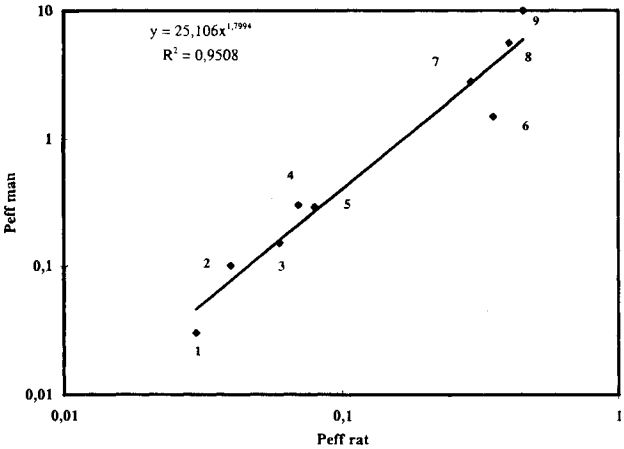

Fig. 1. Correlation between extent of absorption in humans and the effective permeabilities in human jejunum (*in vivo*) and rat jejunum in the Ussing chamber model (*in vitro*). The numbers represent the following compounds: 1. Inogatran; 2. Enalaprilat; 3. Atenolol; 4. Terbutaline; 5. Creatinine; 6. Metoprolol; 7.Propranolol; 8. Antipyrine; 9. Naproxen.

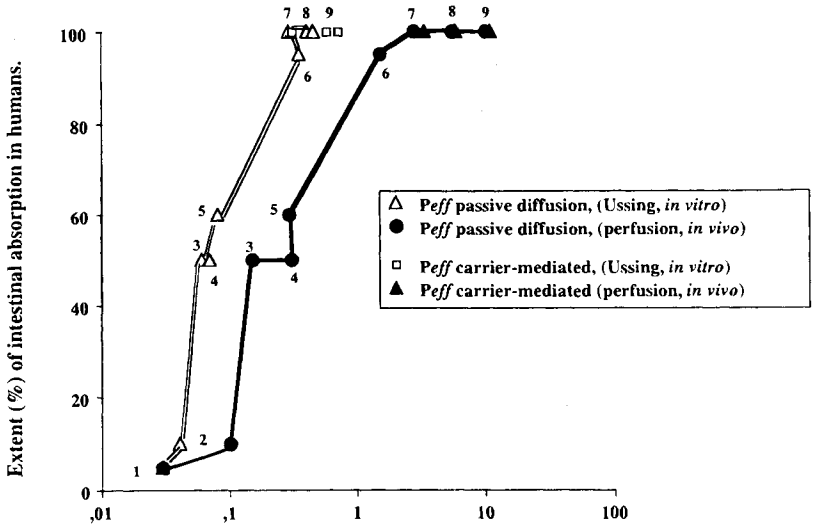

Fig. 2. Correlation between the effective permeabilities obtained in human jejunum (*in vivo*) and rat jejunum in the Ussing chamber model (*in vitro*). The numbers represent the following compounds: 1. Inogatran; 2. Enalaprilat; 3. Atenolol; 4. Terbutaline; 5. Creatinine; 6. Metoprolol; 7.Propranolol; 8. Antipyrine; 9. Naproxen.

Naproxen (a NSAID) had approximately a 20 times higher P*eff*-value in the human perfusion model compared to the *in vitro* model (Table 2). This might be due to a pharmacological induced enhancement effect on the membrane by an inhibition of cyclooxygenase during the absorption phase (15). A significant effect on the epithelial membrane (decreased potential difference, PD) was seen in the in vitro rat experiments using naproxen at a high concentration (>1 mg/ml). The lower concentration of naproxen (0.41 mg/ml) did, however, not cause any electrical changes, giving a P*eff*-value of $0.45 \cdot 10^{-4}$ cm/s (Table 2). Therefore the P*eff* of this class of drugs, NSAIDS, might be dependent of its own pharmacological effect *in vitro*, and as well the concentration exposing the investigated tissue.

The carrier-mediated transport for D-glucose, L-dopa and L-leucine were 19, 11 and 8 times higher in the human *in vivo* model (Figure 2 and Table 2). The higher P*eff*-values for these compounds *in vivo* might partly be explained by a lower supply of co-factors to the transport proteins, diminished concentration gradient across different compartments in the mucosa, different exposed area of the intestinal villi, and a more pronounced UWL as discussed for high passive permeable drugs. Furthermore, it might also be different functional expression of the number of transport proteins between the two species. An example illustrating the effect of blood flow and supply of cofactors is the observation that the P*eff*-estimates (at the same concentrations) for D-glucose and L-dopa are 8.5 and 1.7 times, respectively, lower in the perfused rat jejunum (*in situ*) compared to human perfusion data *in vivo* (1,3). The effect of low expression of carriers is demonstrated by P*eff* of D-glucose, L-dopa and L-leucine in the Caco-2 cell model, which were 44, 340 and 1215 times lower compared to our human *in vivo* data at the same concentrations (2). Based on these two other permeability models it seems like the Ussing chamber technique will be ranked between the Caco-2 model and the single-pass perfusion model regarding functional expression of carrier-mediated transport. Consequently, prediction(s) of drug transport by carrier-mediated processes in humans based on data obtained in excised rat segments, will also in general require a characterisation of each transport system and subsequent introduction of a scaling procedure.

CONCLUSIONS

This is the first report of a direct comparison of the effective intestinal permeability of passively transported drugs in the Ussing chamber with corresponding *in vivo* data from humans. The comparison clearly demonstrates the usefulness of the present *in vitro* model as a tool for screening and prediction of drug absorption in humans. Special concerns are drugs with permeability values which may be influenced by local pharmacological effects on the tissue, and/or variability in the functional transport routes of any carrier-mediated protein involved. Finally, it is crucial to understand the basic mechanisms of the transport process, especially for drugs that might be transported by carrier-mediated mechanisms.

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