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Correlation of in vitro and in vivo models for the oral absorption of peptide drugs

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Summary. The aim of this study was to evaluate two in vitro models, Caco-2 monolayer and rat intestinal mucosa, regarding their linear correlation with in vivo bioavailability data of therapeutic peptide drugs after oral administration in rat and human. Furthermore the impact of molecular mass (Mm) of the according peptides on their permeability was evaluated.

Transport experiments with commercially available water soluble peptide drugs were conducted using Caco-2 cell monolayer grown on transwell filter membranes and with freshly excised rat intestinal mucosa mounted in Using type chambers. Apparent permeability coefficients (P_{app}) were calculated and compared with in vivo data derived from the literature.

It was shown that, besides a few exceptions, the Mm of peptides linearly correlates with permeability across rat intestinal mucosa ($R^2 = 0.86$; $y = -196.22x + 1354.24$), with rat oral bioavailability ($R^2 = 0.64$; $y = -401.90x + 1268.86$) as well as with human oral bioavailability ($R^2 = 0.91$; $y = -359.43x + 1103.83$). Furthermore it was shown that P_{app} values of investigated hydrophilic peptides across Caco-2 monolayer displayed lower permeability than across rat intestinal mucosa. A correlation between P_{app} values across rat intestinal mucosa and in vivo oral bioavailability in human ($R^2 = 0.98$; $y = 2.11x + 0.34$) attests the rat in vitro model to be a very useful prediction model for human oral bioavailability of hydrophilic peptide drugs.

Presented correlations encourage the use of the rat in vitro model for the prediction of human oral bioavailabilities of hydrophilic peptide drugs.

Keywords: Peptides – Oral drug delivery – In vitro in vivo correlation – Caco-2 – Rat intestinal mucosa

1. Introduction

Due to recent advances in recombinant biotechnology, many therapeutic peptides and proteins have become readily available (Sayani and Chien, 1996). But non-invasive delivery of these important therapeutic compounds is still limited due to their low bioavailability. Therefore most therapeutic proteins have to be delivered via invasive routes of administration leading to pain and discomfort for the patients. Due to the progress in large-scale manufacturing of peptide drugs, convenient application routes gained more and more interest. Among them, oral administration is one of the most accepted ways of dosing for the patients. However, administration of most peptides via the oral route is limited because of their low permeability across intestinal mucosa (Bernkop-Schnürch and Göckel, 1997). Besides degradation in the gastrointestinal tract, factors such as high molecular mass, hydrophilicity, and the tendency to undergo aggregation account for the low oral bioavailability (Shah et al., 2002) of peptide drugs.

For the development of efficacious oral drug delivery systems (Walter et al., 1996) the estimation of oral drug absorption in human based on in vitro and in vivo animal studies is of considerable importance. Therefore, Caco-2 cell monolayers, a human intestinal cell line, and freshly excised rat intestinal mucosa mounted in Ussing type chambers are generally accepted as primary in vitro absorption screening models. There have been several trials to predict human in vivo absorption with the use of Caco-2 and rat intestinal permeation models (Levet-Trafit et al., 1996; Walter et al., 1996; Yee, 1997; Lau et al., 2004). Besides primary in vitro studies, animal in vivo pharmacokinetics play a major role in predicting oral in vivo bioavailability in human (Lau et al., 2004), during the process of drug development. However animal studies alone may not always be sufficient for predicting drug absorption in human, especially in the case of degradable peptides, by reason of different intestinal enzymes in animal and human. Because of this, a comparison of different absorption models like the in vitro

model Caco-2, derived from human colorectal carcinoma, with animal in vitro and in vivo studies might be useful. The selection of 21 all perfectly water soluble peptides with therapeutic potential were based on the availability of in vivo data of oral bioavailability in rat or human. The purpose of the present study was to evaluate correlations between Caco-2 cell monolayer, freshly excised rat intestinal mucosa mounted in Ussing type chambers and in vivo bioavailabilities of peptides in rat and human. Additionally we attempted to correlate the molecular mass of the evaluated therapeutic proteins with P_{app} and in vivo data.

2. Materials and methods

2.1 Data set

The in vivo oral bioavailabilities in rat or human as well as in vitro P_{app} coefficients across Caco-2 monolayer and rat intestinal mucosa mounted in Ussing-type chambers for 21 water soluble peptide drugs (Table 1) were taken from 40 references. The used peptides cover a relatively wide range of molecular mass, ranging from 335 Da to 22 kDa. From Table 1, it can be seen that for some peptide drugs, Caco-2 P_{app} values from different sources, displaying obvious variations, exist. However, Artursson et al. already showed that results obtained with Caco-2 monolayers vary due to factors such as culture time, passage number and used culture medium (Artursson and Borchardt, 1997). Additionally, in the references indicated TEER values of the Caco-2 monolayers strongly varied, displaying TEER values from 300 to 1200 Ω/cm^2. In the case of octreotide, and desmopressin where we have found references for Caco-2 P_{app} coefficients, displaying a huge variation, we decided to perform the permeation again in our laboratory. Furthermore we performed permeation studies across freshly excised rat intestinal mucosa and/or Caco-2 monolayer if the according peptide drug is commercially available and unless data are found in the literature.

2.2 Materials

The peptides (Arg)-vasopressin (AVP), buserelin, D-ala-leu-enkephalin, desmopressin, dynorphin E-2078, IRI-695, leuprolide, melanotan II, octreotide, oxytocin, PheAlaVal, PheAlaValAla and thyreotropin-releasing-hormone (TRH) were obtained from Bachem AG (Bubendorf, Switzerland). Parathyroid-hormone 1–34 (PTH 1–34) was custom synthesised by piCHEM R&D (Austria). Human PTH (1–34) specific ELISA kit was obtained from Immunotopics International (San Clemente, CA). Phosphate buffered saline (PBS) contained 8 g of NaCl, 0.2 g of KCl, 1.536 g of Na_2HPO_4, and 0.2 g of KH_2PO_4 per liter (pH 6.8). Modified Eagle Medium (MEM) contained 9.66 g/L MEM powder (Sigma), 2.2 g/L sodium-bicarbonate, 2 mmol/L glutamine (Sigma), 100 U/L penicillin (Sigma), 100 U/L streptomycin (Sigma) and 20% of fetal calf serum (FCS) (pH 6.8). N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) and all other compounds and reagents were obtained from Sigma (Austria). All chemicals were of analytical grade.

2.3 In vitro permeation studies

2.3.1 Permeation studies on freshly excised rat intestinal mucosa

For permeability studies non fasting male Sprague Dawley rats weighting between 250 and 300 g were used. After sacrificing the rats, the first 20 cm of the proximal jejunum were immediately removed. The excised intestine was cut into strips of 1.5 cm, rinsed free of luminal contents and mounted in Ussing type chambers (0.64 cm^2 surface area) without stripping off the underlying muscle layer. The preheated transport medium, containing 250 mM NaCl, 2.6 mM $MgSO_4$, 10 mM KCl, 40 mM glucose and 50 mM $NaHCO_3$ buffered with 50 mM HEPES pH 6.8 was added to the apical and basolateral side. In order to ensure oxygenation and agitation, a mixture of 95% O_2 and 5% CO_2 was bubbled through each compartment. The Ussing chambers were then placed in a water bath at 37 °C. After a 20 min equilibration period, test compounds dissolved in transport medium in a final concentration of 0.05% (w/v) were added to the donor chamber. After 1, 2 and 3 h, 100 μL samples were taken out from the acceptor chamber. The amount of permeated peptides was analyzed by high performance liquid chromatography (HPLC) (Merck HITACHI) equipped with an L-2200 autosampler. Separation of AVP, D-ala-leu-enkephalin, desmopressin, dynorphin E-2078, IRI-695, leuprolide, melanotan II, oxytocin, PheAlaVal, PheAlaValAla and TRH was carried out by using a 25 cm reversed phase Nucleosil 5C18 column (Seibersdorf GmbH). The mobile phase used for separation of these peptides consisted of A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile (90% A to 20% A in 18 min). Separation of octreotide was carried out by using a 15 cm Supelcosil® LC-8-DB column (Sigma-Aldrich) with a mobile phase consisted of 77% A (0.5% (m/v) tetramethylammoniumhydroxid pH 2.7) and 23% B (acetonitrile). A flow rate of 1 mL/min was performed for all analyzed peptides. The peptides AVP, D-ala-leu-enkephalin, desmopressin, dynorphin E-2078, IRI-695, leuprolide, melanotan II, octreotide, oxytocin, PheAlaVal, PheAlaValAla and TRH were monitored by using a diode array detector (L-2450 Merck HITACHI) at 210, 254, 210, 210, 210, 220, 210, 225, 214, 210, 210 and 215 nm, respectively. PTH 1-34 was analysed by using a specific ELISA kit.

Calculations were done by interpolations from standard curves obtained with increasing amounts of the according peptide drugs.

2.3.2 Permeation studies on Caco-2 cell monolayer

Caco-2 cells (passage number 70) were seeded onto 12 well Transwell polyester membranes (Transwell®, COSTAR, 0.4 μm pore size, 12 mm diameter). The cells were cultured in MEM supplemented with 20% fetal calf serum (FCS). The culture medium was exchanged every other day and the cells were stored in a 5% CO_2-incubator at 37 °C. Permeation studies were performed with monolayers cultured for 24 days. Caco-2 cell monolayers with trans-epithelial electrical resistance (TEER) values in the range of 400–500 Ω/cm^2 were used for the permeation studies. The transport medium contained 250 mM NaCl, 2.6 mM $MgSO_4$, 10 mM KCl, 40 mM glucose and 50 mM $NaHCO_3$ buffered with 50 mM HEPES pH 6.8. Prior to all experiments, each monolayer was washed with phosphate buffer saline (PBS). Then 1 mL of transport medium was added to the apical and 1.5 mL to the basolateral compartment. After a 20 min equilibration period in the 5% CO_2 incubator TEER was measured again to assure integrity of the monolayers. An appropriate volume of buffer from the donor chamber was replaced by a solution of the according peptide in a final concentration of 0.05% (m/v). During the experiment the Transwell plates were stored in an incubator. After 1, 2 and 3 h, 100 μL samples were taken out from the acceptor chambers and replaced by preheated buffer. The amount of permeated peptides was analyzed by HPLC as already described above.

2.3.3 Determination of the TEER

The integrity of the monolayer was evaluated by measuring the trans-epithelial electrical resistance (TEER) using an EVOM® (World Precision Instruments, Sarasota, USA) connected with a pair of electrodes. Caco-2 cell monolayers with trans epithelial electrical resistance (TEER) values in the range of 400–500 Ωcm^2 were used for the permeation studies. The TEER was measured before and after the transport studies to ensure integrity of the monolayer during the experiment.

Table 1. Summary of in vitro permeability coefficients across rat intestinal mucosa or Caco-2 monolayer and in vivo oral bioavailability in rat or human. Displayed data were observed from the literature as well as from transport experiments performed in our laboratory (indicated by [x])

Compound	Mm [Da]	P_{app} rat intestinal mucosa ($\times 10^{-6}$)	P_{app} Caco-2 monolayer ($\times 10^{-6}$)	F_{abs} Rat in vivo [%]	F_{abs} human in vivo [%]
PheAlaVal	335.4	5.8 ± 1.8[x]	2.0 ± 0.5[x]	2.8 (He, 1996)	–
TRH	362.4	4.7 ± 0.9[x]	2.05 ± 0.44 (Urayama et al., 2003) 1.9 ± 0.3[x]	1.5 (Yokohama et al., 1984)	2.0 (Yokohama et al., 1984)
Azetirelin	384.4	4.6 ± 0.46 (Yamamoto, 2001)	2.32 ± 0.76 (Urayama et al., 2003)	1.6 (Sasaki et al., 1997)	–
PheAlaValAla	406.5	4.5 ± 0.4[x]	1.5 ± 0.5[x]	1.1 (He et al., 1996)	–
IRI-695	483	4.9 ± 0.4[x]	0.1 ± 0.3[x]	2.0 (Adusumalli et al., 1996)	–
DMP 728	561	1.25 ± 0.08 to 3.21 ± 0.39 (Aungst and Saitoh, 1996)	0.35 ± 0.19 (Ribadeneira et al., 1996)	2–4 (Burcham et al., 1995)	–
D-ala-leu-enkephalin	569.7	3.01 ± 0.39 (Uchiyama et al., 1998)	0.7 ± 0.2[x]	0.4 (Lee and Amidon, 2002)	–
Metkephamid	601	–	0.049 ± 0.008 (Lang et al., 1997)	0.22 (Lipka et al., 1996)	–
Hexarelin	887.0	1.3 ± 0.9 (Fagerholm et al., 1998) 1.89 ± 0.3[22]	<0.15 (Westberg et al., 2001) (below detection limit)	–	0.3 (Deghenghi and Camanni, 1994)
Oxytocin	1007.2	2.09 ± 0.19 (Lundin et al., 1991)	1.6 ± 0.3[x]	–	–
Octreotide	1019.2	1.5 ± 0.3[x]	1.7 ± 0.5 (Michael et al., 2000) 1.3 ± 0.7[x] 0.008 ± 0.004 (Fricker et al., 1996)	0.28 (Michael et al., 2000)	0.6 (Drewe et al., 1993)
Melanotan II	1024	2.5 ± 0.3[x]	2.4 ± 0.3[x]	4.6 (Lan et al., 1994)	–
Dynorphin E-2078	1035	1.7 ± 0.7[x]	1.3 ± 0.2[x]	0.7 (Murahashi et al., 1989)	–
Desmopressin	1069.2	0.53 ± 0.29 to 1.13 ± 0.48 (Pantzar et al., 1994)	0.13 (Artursson and Karlsson, 1991) 4.8 ± 1.6 (Michael et al., 2000) 2.7 ± 0.4[x]	0.92 (Michael et al., 2000)	0.1 (Fjellestad et al., 1993)
AVP	1084.3	2.2 ± 0.8[x]	1.4 ± 0.7[x]	0.68–0.93 (Miyazaki et al., 2000)	–
Buserelin	1239.5	1.0 ± 0.3[x]	0.04 ± 0.01 (Thanou et al., 2000)	0.8 (Hou et al., 2004), 0.1 (Luessen et al., 1996)	–
Leuprolide	1269.5	1.6 ± 0.4[x]	0.52 ± 0.02 (Guo et al., 2004)	0.17–0.58 (Zheng et al., 1999)	–
Calcitonin salmon	3431.9	1.91 ± 0.45 (Sinko, 1999)	0.17 ± 0.03 (Song, 2002)	0.022 (Sinko, 1995)	0.5–1.4 (Buclin, 2002)
PTH 1–34	4117.7	0.0002[x]	0.002[x]	–	–
Insulin	5700	0.49 ± 0.1 (Yamamoto, 2001)	0.007 (Ichikawa, 2003)	0.021 (Yamamoto, 2001) 0.25 (Eaimtrakarn et al., 2002)	<1 (Shah, 2002)
hGH	22 124	3.45 ± 0.34 (Stoll et al., 2000)	1.34 ± 0.19 (Wu and Robinson, 1999)	1.0 (Moore et al., 1986)	–

2.3.4 Data analyses

The apical to basolateral (AP to BL) permeability coefficients (P_{app}) of the according peptides were calculated according to the following equation $P_{app} = Q/A * c * t$, where P_{app} is the apparent permeability coefficient (cm/sec), Q is the total amount of peptide permeated after 3 h (μg), A is the diffusion area of the Ussing chamber (0.64 cm^2) or of the Caco-2 monolayer (1.13 cm^2), c is the initial concentration of according peptide in the donor chamber (μg/cm^3), and t is the total time of the experiment (sec). The results are reported as means ± SD of at least three trials.

2.4 Statistics of the correlation models

The statistics of the bivariate correlations were tested by their linear correlation coefficient (R^2).

3. Results and discussion

3.1 Impact of molecular mass

The high molecular mass of peptide drugs is considered as one of the main obstacles associated with absorption after oral administration. As for hydrophilic peptides a mainly paracellular diffusion through the tight junctions across the gastrointestinal barrier is estimated, and as permeation through these size restricted aqueous pores depends on the molecular mass (Mm) of drugs (Hou et al., 2004), a correlation of P_{app} and Mm of the according peptides should be expected. Therefore we attempted to correlate the molecular mass of the evaluated peptides with P_{app} and in vivo data.

3.1.1 Correlation between Mm with rat intestinal P_{app}

When all rat in vitro permeability coefficients of the peptide drugs from Table 1 are plotted as a function of Mm, no linear correlation can be found. However, if peptide drugs with a Mm > 3000 (calcitonin, PTH, insulin and hGH) were excluded, a strong correlation with $R^2 = 0.86$; $y = -196.22x + 1354.24$ was observed (Fig. 1). These observations indicate that salmon calcitonin (Mm of 3432), insulin (Mm of 5700) and hGH (Mm of 22124) demonstrate higher permeability through rat intestinal mucosa, in relation to their molecular mass, as it might be anticipated. On the one hand, specific peptide transporters could account for such high P_{app} values, and on the other hand high molecular flexibility of polypeptides are suggested to strongly influence paracellular transport (Salamat-Miller and Johnston, 2005). Paracellular transport for a charged polypeptide even with a Mm of 26.6 kDa, in absence of tight junction modulators, has been reported (Salamat-Miller and Johnston, 2005). This report challenges the overall assumption that high molecular mass compounds are not able to passively cross intestinal membranes via the tight junctions. So far no specific peptide transporter in the gastrointestinal tract for insulin, calcitonin or hGH has been reported. However, it was reported that besides paracellular transport other routes may be involved in insulin absorption (Lane and Corrigan, 2006). Derossi et al. reported transcellular receptor-independent absorption of several peptides (Derossi et al., 1998).

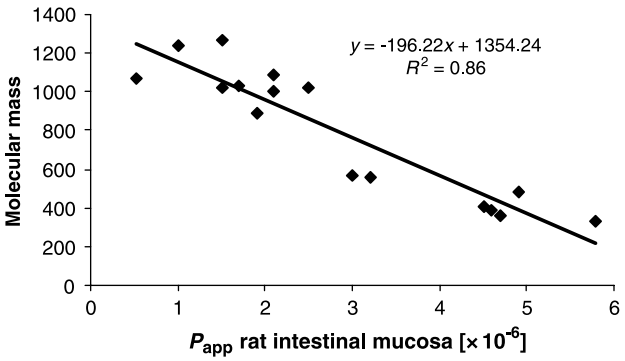

Fig. 1. Correlation between permeability coefficients across rat intestinal mucosa mounted in Ussing-type chambers and molecular mass of the according peptide drugs. The best fit line is based on a linear regression analysis indicated with R^2

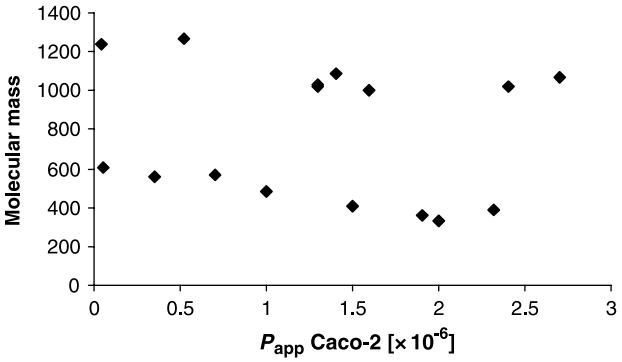

Fig. 2. Correlation between permeability coefficients across Caco-2 monolayer and molecular mass of the according peptide drugs. No linear correlation indicated by R^2 was observed

3.1.2 Correlation of Mm and Caco-2 P_{app}

No linear correlation was observed by plotting all 21 peptide drugs. Even when peptides with Mm > 3000 were excluded, no linear correlation could be found (Fig. 2). Also if just the Caco-2 P_{app} values from our laboratory ($n = 12$) were correlated versus Mm, no linear correlation was found. Several studies have shown good correlations by using this human colorectal carcinoma cell line, however, large interlaboratory differences in the cultivation of Caco-2 monolayer and other reasons such as poor paracellular permeability across Caco-2 monolayer might explain why no linear correlations were found between Mm and Caco-2 P_{app}.

3.1.3 Correlation of Mm and rat oral bioavailability

When rat in vivo bioavailability of all peptide drugs with Mm < 3000 from Table 1 were plotted as a function of Mm, no correlation was observed. However, when the P-glycoprotein (P-gp) substrates D-ala-leu-enkephalin, metkephamid (white squares in Fig. 3) and the cyclic peptide melanotan II were excluded, a moderate to strong correlation with $R^2 = 0.64$; $y = -401.90x + 1268.86$ was found. P-gp, an apically polarized efflux pump, which is located

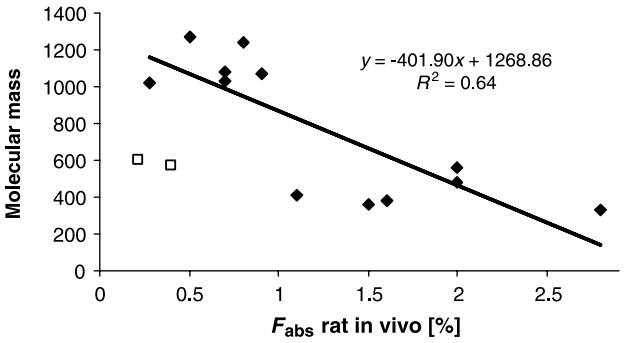

Fig. 3. Correlation between in vivo oral bioavailability in the rat and molecular mass of the according peptide drugs. The best fit line is based on a linear regression analysis indicated with R^2. The P-gp substrates metkephamid and D-ala-leu-enkephalin (white square) were excluded from the plot demonstrating a significantly lower oral bioavailability

in the membrane of enterocytes (Troutman and Thakker, 2003) limits the oral bioavailability of a lot of structurally diverse compounds such as enkephalin-analogues and several other substances (Kim, 2002) by translocating substrates from the inner side of the membrane to the outer side. In Fig. 3 it can be seen that the P-gp substrates D-ala-leu-enkephalin and metkephamid demonstrate a significantly lower oral bioavailability in the rat as compared with non-P-gp substrates. In contrast, the cyclic heptapeptide melanotan II displayed significantly higher oral bioavailability ($F_{abs} = 4.6\%$). The high apparent partition coefficient of 2.82 (n-octanol/water at pH 7.35) might explain the high oral bioavailability of melanotan II (Lan et al., 1994).

3.1.4 Correlation of Mm and human oral bioavailability

A strong linear correlation with $R^2 = 0.91$; $y = -359.43 + 1103.83$ between the Mm of peptide drugs and oral bioavailability in human is demonstrated in Fig. 4. However, the poor available human in vivo data should be considered. So, based on increasing human in vivo data, this prediction model will need to be continuously refined.

3.2 Prediction models based on Caco-2 monolayer

Transport studies across Caco-2 monolayer represent one of the most accepted in vitro permeation models. Several studies showed good correlations between Caco-2 permeability and the fraction of drug absorbed after oral administration (Lau et al., 2004). However limitations such as the lack of intestinal phase 1 metabolic enzymes like CYP3A4 and furthermore the absence of villi and mucus-layer have to be noted. Additionally, Caco-2 cells represent tighter junctions in comparison with human or animal small intestine, which is explained by the colonic origin of these cells (Matsson et al., 2005). Due to huge inter-laboratory discrepancies, a direct comparison of permeability of the same drugs might often fail. As shown in Table 1, big variations for the peptide drugs desmopressin and octreotide have been found. However, after repeated permeation studies across Caco-2 monolayer performed in our laboratory, it was possible to exclude an obvious outlier of octreotide (given with a permeability coefficient of 0.008×10^{-6}). In the case of octreotide we used the P_{app} value evaluated in our laboratory for the correlations.

3.2.1 Correlation of Caco-2 and rat intestinal P_{app}

Just a poor linear correlation with $R^2 = 0.48$; $y = 0.35x + 0.19$ between Caco-2 and rat intestinal mucosa P_{app} values can be seen in Fig. 5. However, this correlation clearly shows that hydrophilic peptides exhibit significantly higher permeability across rat intestinal mucosa than across the cultured Caco-2 monolayers. As reported earlier, permeability of hydrophilic peptide drugs across Caco-2 monolayer is lower compared to permeability

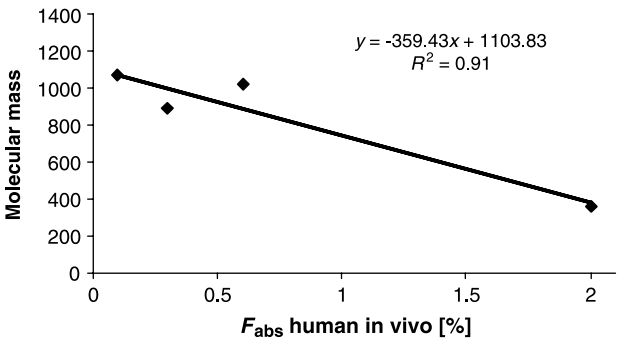

Fig. 4. Correlation between in vivo oral bioavailability in human and molecular mass of the according peptide drugs. The best fit line is based on a linear regression analysis indicated with R^2

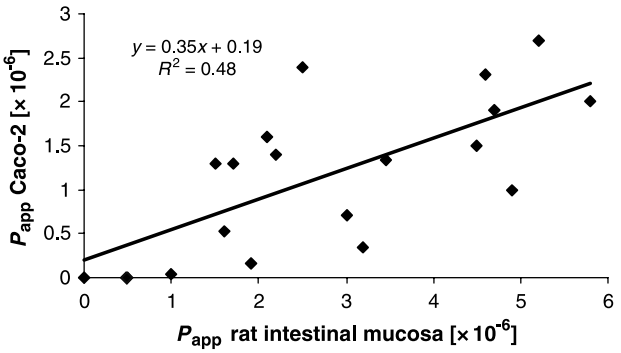

Fig. 5. Correlation between permeability coefficients across rat intestinal mucosa mounted in Ussing-type chambers and Caco-2 monolayer. The best fit line is based on a linear regression analysis indicated with R^2

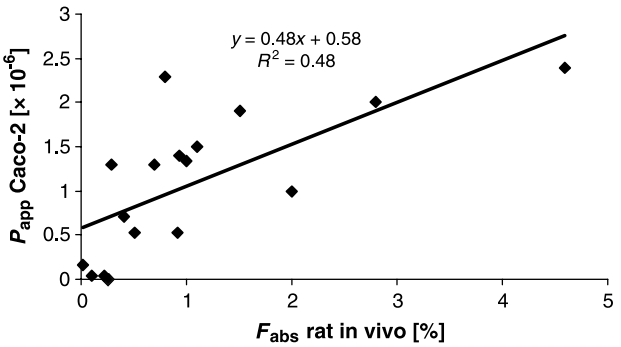

Fig. 6. Correlation between permeability coefficients across Caco-2 monolayer and in vivo oral bioavailability in the rat. The best fit line is based on a linear regression analysis indicated with R^2

through intestinal mucosa (Walter et al., 1996). This was explained on the one hand by a reduced absorption area due to the lack of villi and on the other hand by lower paracellular permeability through the tighter junctions in Caco-2 monolayer. Besides the Caco-2 model, Madin-Darby canine kidney (MDCK) cells have been reported as model for the permeation of passively absorbed drugs (Tang et al., 2002), however, in regard to oral drug delivery, Caco-2 cells are used more frequently.

3.2.2 Correlation of Caco-2 P_{app} and rat oral bioavailability

A correlation with $R^2 = 0.48$; $y = 0.48x + 0.58$ was observed after plotting Caco-2 P_{app} values versus rat in vivo bioavailability (Fig. 6). As mentioned above, different sources of Caco-2 P_{app} values might account for this moderate correlation. Furthermore, it has already been reported that permeability of several drugs across Caco-2 monolayer is lower than in comparison with intestinal mucosa (Walter et al., 1996). Due to their extraction from human colon carcinomas, Caco-2 permeation models are more related to colon permeability.

3.2.3 Correlation of Caco-2 P_{app} and human oral bioavailability

Due to the poor available data, no attempts to correlate Caco-2 P_{app} with human oral bioavailability of hydrophilic peptide drugs were made. Furthermore, there have been reports that Caco-2 monolayer can only be used to predict passive transcellular transport and not paracellular transport, by reason of smaller pore size resembling to the colonic epithelia (Yee, 1997).

3.3 Prediction models based on rat intestinal P_{app}

Permeation studies across freshly excised rat intestinal mucosa mounted in Ussing type chambers are generally accepted as primary in vitro absorption screening models. Several drug transporters such as P-gp and MRP efflux pumps are expressed in both, in rat and in human intestinal mucosa. Furthermore Mouly et al. reported that the quantitative expression of the efflux pump P-gp in the different human intestinal segments is comparable to the rat intestinal parts (Mouly and Paine, 2003). In addition, mRNAs for peptide transporter 1 (PepT1), peptide transporter 3 (PTR3), peptide/histidine transporter 1 (PHT1) and the human peptide transporter 1 (HPT-1) were widely expressed in the rat gastro-intestinal-tract (Herrera-Ruiz et al., 2001). Therefore the rat model seems to be suitable for several compounds in predicting drug absorption in human.

3.3.1 Correlation of rat intestinal P_{app} and rat oral bioavailability

A plot of rat intestinal P_{app} versus rat in vivo oral bioavailability showed a strong correlation with $R^2 = 0.81$; $y = 1.89x + 1.27$ (Fig. 7). The outliers desmopressin, DMP and melanotan II were excluded from this correlation because they obviously showed higher in vivo bioavailabilities as it should be estimated from their in vitro data. The cyclic peptide DMP was reported to be a substrate of P-gp (Saitoh and Aungst, 1997) however, P-gp should be expressed in both, in the rat in vitro as well as in the rat in vivo model. One explanation for the high oral in vivo bioavailability of DMP and melanotan II could be their cyclization, because conformational changes can influence membrane permeability (Saitoh and Aungst, 1997). Otherwise the reported high stability of DMP in

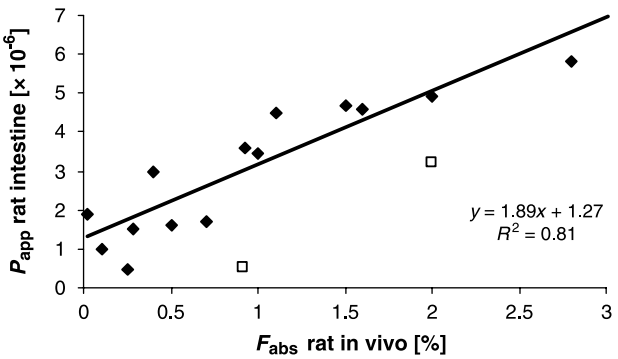

Fig. 7. Correlation between permeability coefficients across rat intestinal mucosa mounted in Ussing-type chambers and in vivo oral bioavailability in the rat. The best fit line is based on a linear regression analysis indicated with R^2. The outliers desmopressin, the cyclic P-gp substrate DMP (white squares) and the cyclic heptapeptide melanotan II were excluded from this linear correlation because they represent noticeable higher in vivo bioavailability in relation to their in vitro P_{app} values

gastric and intestinal fluid as well as in plasma (Aungst and Saitoh, 1996), and the high apparent partition coefficient of melanotan II could explain their proportional high oral bioavailability. In the case of the second outlier desmopressin, the existence of an active transport mechanism was reported to be unlikely, however Pantzar et al. confirmed the existence of regional absorption differences in vitro as well as in vivo in rats (Pantzar et al., 1995).

3.3.2 Correlation of rat intestinal P_{app} and human oral bioavailability

A very strong linear correlation with $R^2 = 0.98$; $y = 2.11x + 0.34$ between the rat in vitro permeation model and human oral in vivo bioavailabilities was found (Fig. 8). However it should be noted that the data set is limited because of poor available human in vivo data. Nevertheless such a strong linear relationship encourages the use of the rat model for the prediction of human oral bioavailabilities of hydrophilic peptide drugs.

3.4 Correlation of rat and human oral bioavailabilities

Several animal models are used to predict intestinal drug absorption in human. Frequently used models are rats, mice, guinea pigs, pigs and dogs. In a comparison concerning the paracellular transport of hydrophilic macromolecules, rats appeared to be better predictors than dogs for human drug absorption (He et al., 1998). In an investigation of different species of animals (monkeys, guinea pigs, dogs and rats) concerning their intestinal enzymatic activity, enterokinase activity in the rat duodenal mucosa was found to resemble closest the activity in the human duodenum (Malis et al., 1977). Contrarywise, Komura et al. (2002) implied that monkeys would be better predictors then rats, guinea pigs or dogs for human small intestinal metabolism of CYP3A4 substrates. However, the usefulness of the rat model for predictions of oral bioavailability of peptide drugs in human was demonstrated in Fig. 7. A direct linear correlation of rat and human in vivo data, however, is yet not meaningful, due to limited available data.

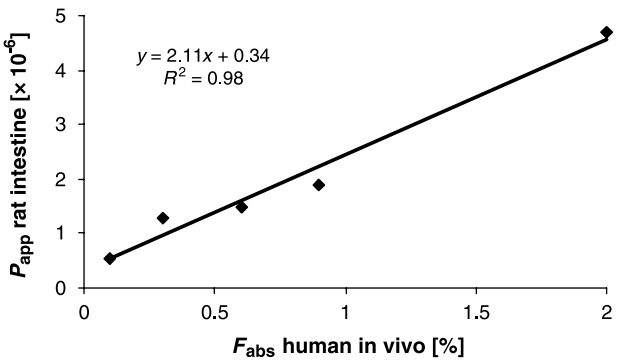

Fig. 8. Correlation between permeability coefficients across rat intestinal mucosa mounted in Ussing-type chambers and in vivo oral bioavailability in human. The best fit line is based on a linear regression analysis indicated with R^2

4. Conclusion

Although, recent studies challenged the impact of Mm on paracellular transport, our study demonstrates that besides a few exceptions, the Mm of peptides linearly correlates with their permeability. For P_{app} values of hydrophilic peptides across Caco-2 monolayer higher permeability of the same compound across rat intestinal mucosa or in vivo in the rat might be expected. Finally, the presented correlations attest the rat in vitro model to be a better prediction model for human oral bioavailabilities of hydrophilic peptide drugs in comparison with the Caco-2 monolayer.

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