Validation of Cell Culture Models for the Intestine and the Blood-Brain Barrier and Comparison of Drug Permeation

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Summary
Cell culture models are useful tools to study the uptake of drugs across the barriers of the human body, like the intestine, the skin or the blood-brain barrier. Cell-based in vitro models not only help to reduce the number of animals used but are also much faster to perform, more cost effective and give more reproducible data than animal studies. Given the increasing number of new drugs and chemicals under development, there is an urgent need for the establishment of such in vitro models. However, the validity of such in vitro models is reflected by its ability to accurately predict the behaviour of a substance at the corresponding in vivo barrier. Here, we compare a well-established cell culture model for the intestine, based on Caco-2 colon carcinoma cells, with a primary cell culture model of the blood-brain barrier. We find that Caco-2 cells and cells of the blood-brain barrier have different barrier properties. Therefore, cells used for cell-based assays should be derived from the corresponding tissue to reflect the in vivo barrier characteristics.

Zusammenfassung: Validierung von Zellkultur-Modellen für den Darm und die Blut-Hirn-Schranke und Vergleich der Arzneistoffpermeation
Zellkulturmodelle sind nützliche Hilfsmittel, um die Aufnahme von Arzneistoffen über natürliche Barrieren des menschlichen Körpers, wie z.B. den Darm, die Haut oder die Blut-Hirn-Schranke, zu untersuchen. Zellbasierte in vitro Modelle helfen nicht nur den Verbrauch von Versuchstieren zu reduzieren, sondern sind auch schneller durchzuführen, kosteneffektiver und liefern besser reproduzierbare Daten als Tierstudien. Aufgrund der steigenden Anzahl neuer Arzneimittel und Chemikalien in der Entwicklung besteht ein großer Bedarf an geeigneten in vitro Modellen. Die Validität eines in vitro Modells hängt allerdings von dessen Fähigkeit ab, das Verhalten eines Wirkstoffes an der in vivo Barriere vorherzusagen. In der vorliegenden Studie wird ein gut etabliertes Zellkulturmodell für den Darm, basierend auf Caco-2 Kolon-Karzinom Zellen mit einem Primärzellmodel der Blut-Hirn-Schranke verglichen. Wir konnten zeigen, dass Caco-2 Zellen und Zellen der Blut-Hirn-Schranke unterschiedliche Barriere-Eigenschaften besitzen. Für ein geeignetes Zellkulturmodell sollten Zellen eingesetzt werden, die ursprünglich aus dem entsprechenden Gewebe stammen, auf dessen Barriere-Eigenschaften sich das Zellkulturmodell bezieht.

Keywords: Caco-2, Porcine Brain Endothelial Cells (PBEC), blood-brain barrier (BBB), Biopharmaceutical Classification System (BCS)

1 Introduction

The German Federal Institute for Drugs and Medical Devices (BfArm) recently announced a revision of the drug approval process (bioavailability and bioequivalence) as laid down in article 21 of the German Drug Law (AMG). The update was based on work carried out by an expert group at the European Agency for the Evaluation of Medicinal Products (EMEA) and published as a "Note for Guidance on the Investigation of Bio-availability and Bioequivalence – CPMP/EWP/QWP/1401/98" (referred to in the following as "Note for Guidance"). After a transitional period of three months, which ended on the 26th of June 2003, the new announcement came into force, replacing the previous 9th announcement on bioavailability and bioequivalence (CDER, 2000).

The first German conference on the subject of the Biopharmaceutical Classification System (BCS) was held in March 2003. The conference, which was attended by delegates from BfArm, from the German Pharmaceutical Industry Association (BPI), from pharmaceutical companies and from contract research organisations, provided an opportunity to summarise German experience of the BCS and to formulate common expectations of the system in Germany. The first biowaiver was issued by BfArm in 2002 for the drug compound sotalol hydrochloride. Work on this initial pilot scheme involved close collaboration between the Committee on Generic Compounds at the BPI and members of BfArm. BfArm representatives told delegates that ten requests for biowaivers had been received over the last two years and that the requests had been granted in 50% of the cases.

The BfArm decisions were based on the EMEA Note for Guidance (EMEA,

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1998), which is itself modelled on the guidance published in 2000 by the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services under the name "Waiver of In Vitro Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System". In contrast to the FDA guidance, the EMEA guidance does not specifically exempt highly permeable, highly soluble compounds (so called FDA class I compounds). In principle, the Note for Guidance allows biowaivers to be issued for all classes of compounds provided that appropriate supporting data is provided. According to the EMEA Note for Guidance, a request for a biowaiver that is based on the use of alternative *in vitro* experiments should incorporate a complete characterisation of the drug substance (e.g. *in vitro* permeability, solubility and physicochemical characterisation) and the drug product (content, release, etc.), and should take particular account of the effects of excipients used in the formulation. If the metabolic stability of the drug substance is an issue, the report submitted with the request must contain experimental data collected by the applicant or drawn from the published literature.

The conference agreed that given the time and costs associated with *in vivo* bioequivalence studies, the number of requests for biowaivers is expected to rise. It was estimated that the costs of the *in vitro* methods and solubility measurements are about one tenth or one fifteenth of the costs associated with a full *in vivo* bioequivalence study (approx. €150,000). Furthermore, the time required to acquire the data and to compile the requisite documentation would decrease from months to weeks. Further scientific work leading to a more differentiated view of drug classification in terms of solubility and permeability is desirable.

2 Aim

The US FDA and the European agency EMEA have issued guidelines under which pharmaceutical companies can request a waiver from *in vivo* bioequivalence studies based on the BCS (CDER, 2000; EMEA, 1998). The theoretical basis of the BCS system was first described in 1995 (Amidon, 1995). BCS is a scientific framework for classification of substances according to their aqueous solubility and their intestinal permeability (Yu, 2002). The BCS also takes the dissolution of the drug product into account and hence covers the three main factors which govern the rate and extent of drug absorption from immediate-release (IR) solid oral dosage forms (e.g. tablets, capsules):

-dissolution rate
-solubility
-permeability

According to the BCS, drug substances can be classified as belonging to one of four classes:

-Class 1: high solubility and high permeability
-Class 2: low solubility and high permeability
-Class 3: high solubility and low permeability
-Class 4: low solubility and low permeability

As the pH solubility profile of the test compound during passage through the gastrointestinal tract may influence drug dissolution rate and permeability, the solubility profile should be determined in the pH range of 1-7.5 at 37±1°C. In addition, the stability of the drug compound at different concentrations of physiological fluids that mimic gastrointestinal fluid and gastric juice must be taken into account. The drug substance is classified as highly soluble when the highest dose strength is soluble in 250 ml of aqueous media over the pH range 1-7.5.

Both the FDA and EMEA (CDER, 2002; EMEA, 1998) recommend the use of monolayers of suitable epithelial cells to classify the permeability of drug compounds. One epithelial cell line that has been widely used as a model system for measuring intestinal permeability is Caco-2 (Fogh et al., 1977; Hidalgo et al., 1989; Madara et al., 1987).

In the presented study, a correlation was established between the drug permeability and the fraction of drug absorbed in humans by determining the permeability using Caco-2 cells. In addition, the dependency of the permeability values on the following parameters was studied: inter-day precision, the effects of drug application on apical-to-basolateral absorption and on basolateral-to-apical absorption, number of days in culture and passage number.

Another goal of the presented study was the comparison of drug transport across Caco-2 cells with transport across Porcine Brain Endothelial Cells (PBEC), a cell culture model for the blood-brain barrier (BBB) established by Franke (1999). A special focus of the study was to measure the activity of the efflux transporter p-glycoprotein (PGP) and other active transporter proteins, using the PGP substrates rhodamine123 and digoxin in the presence and absence of the PGP inhibitor verapamil.

The results confirm that the Caco-2 and PBEC models can readily differentiate highly permeable compounds, which are known to be well absorbed, from less permeable compounds that are poorly absorbed. In addition, we found striking differences in the permeability of PGP substrates in the Caco-2 and PBEC models. Our data indicate that these differences are the result of different substrate specificities of different PGP-like transporters found in the cell membranes of Caco-2 and PBEC. Thus, the cells used to study drug uptake across a specific barrier should be derived from the corresponding tissue to reflect the pertinent barrier properties.

3 Materials and methods

3.1 Caco-2 cells

Caco-2 cells were obtained from the German Cell Culture Collection DSMZ, DSMZ-No.: ACC 169. Aliquots of passage 3 were stored in liquid nitrogen. All cell cultures tested negative for mycoplasma (PCR testing) prior to cryoconservation.

Dulbecco's Modified Eagle's Medium (DMEM), non-essential amino acids (NEA), and gentamycin sulphate were purchased from Biochrom KG (Berlin, Germany), while the trypsin-EDTA solution was from Sigma Chemicals (Deisenhofen, Germany). Fetal calf serum (FCS) was supplied by Greiner Labortechnik (Frickenhausen, Germany). The refer-

ence compounds were from local suppliers and were of the highest chemical-grade purity.

Caco-2 cells were cultured at 37°C, 90% humidity, 10% CO_2 in cell culture flasks in DMEM containing 10% FCS and antibiotics. The medium was changed three times a week, and cells were passaged once per week until confluence was about 80%. Caco-2 cells were detached by means of trypsin-EDTA and seeded with 10^4 cells/cm² on Transwell™ clear filters (Costar®, Wiesbaden, Germany) with an area of 1.13 cm² and a pore size of 0.4 µm. Monolayer growth was monitored weekly by measuring the transepithelial electrical resistance (TEER) values. TEER values of monolayers were calculated by subtracting the TEER of empty filters from the measured TEER.

3.2 PBEC

PBEC were prepared as described by Gindorf et al. (Gindorf et al., 2001). Cells were cultured at 37°C, 90% humidity, 10% CO_2 in cell culture flasks in M199 medium with 10% Ox serum (OS) and antibiotics. On the third day after preparation, the cells were subcultured. The cells were harvested by trypsination and seeded on collagen-treated polycarbonate filters. After three days on Transwell™ filters, the M199 medium was replaced by DMEM/Ham's F12 medium. A volume of 500 µl was added per apical compartment and a volume of 1500 µl was applied per basolateral compartment, respectively.

3.3 Quality control of monolayer batches

The quality of a batch of cell monolayers was monitored by measuring the TEER of a subgroup of randomly selected monolayers and by measuring their permeability with respect to three test compounds. Quality control measurements were performed in triplicate for each cell transport condition (e.g. transport direction, presence or absence of inhibitors). The following quality criteria had to be met before the Caco-2 and PBEC monolayer batches were released for permeability studies with test compounds (Tab. 1).

3.4 Test compounds

Test compounds were dissolved in DMSO and diluted with KRB (pH 7.4) or cell culture medium (DMEM/Ham's F12, for PBEC studies) to a concentration of 50 µM, respectively (< 1% DMSO final concentration). The solutions were warmed to 37°C and used as transport solution. Note that PBEC have been shown to be resistant to DMSO up to a concentration of 1%.

3.5 Caco-2 permeability studies

Immediately before the experiment, the cells were washed twice with KRB buffer. The buffer was then replaced with KRB buffer containing the test compound. After 30-35 minutes pre-incubation, samples were withdrawn from the donor and acceptor compartments. The first sampling was defined as the start of the experiment (t = 0 min). During the transport study further samples were withdrawn from the acceptor compartments at defined times. The volumes withdrawn were replaced with fresh KRB buffer or KRB buffer with the corresponding inhibitor. When not being sampled, the monolayers were incubated in a CO_2 incubator. At the end of the study a second sample was taken from each donor compartment. Finally, the transepithelial electrical resistance (TEER) was remeasured; a TEER value above 200 Ω x cm² (not including the TEER of the empty filters) indicated the monolayer was intact.

The volumes of the apical and basolateral compartments used in the transport experiments were 500 µl and 1500 µl respectively. The recovery of the test compounds was calculated by comparing pre-incubation samples with samples withdrawn at the end of the transport study.

3.6 PBEC permeability studies

A PBEC monolayer batch is defined as PBEC isolated, seeded and cultured in parallel under the conditions described above on Transwell™ filters. All transport studies were performed on PBEC monolayers in triplicate. For the transport studies, half of the cell culture medium from the donor compartments was removed and replaced with transport solution. The pre-incubation process, sampling and TEER determination were performed as described for Caco-2 cells. The acceptance criteria for PBEC are depicted in Table 1.

Tab. 1: Quality control criteria for Caco-2 and PBEC cell monolayer batches. Qualification criteria have to be met by every monolayer.

Test item	Caco-2	PBEC
Number of passages validated for experiments	10-50	Individual preparations, no passages
Age of monolayer	14–30 days	4-7 days
Low permeability	P_{app} of fluorescein (ab) < $1 \cdot 10^{-6}$ cm/s	P_{app} of fluorescein (ab) < $1 \cdot 10^{-6}$ cm/s
High permeability	P_{app} of propranolol (ab) >$5 \cdot 10^{-6}$ cm/s	P_{app} of propranolol (ab) >$5 \cdot 10^{-6}$ cm/s
Expression of P-glycoprotein	P_{app} rhodamine (ba)/$_{app}$ rhodamine (ab) > 2.0	P_{app} rhodamine (ba)/P_{app} rhodamine (ab) > 2.0
Tightness of barrier before transport	TEER of monolayers (not including TEER of empty filters) > 200 Ω·cm²	TEER of monolayers (not including TEER of empty filters) > 500 Ω·cm²
Tightness of barrier after transport	TEER (not including TEER of empty filters) > 200 Ω·cm²	TEER (not including TEER of empty filters) > 500 Ω·cm²

3.7 Analysis of samples

The fluorescent compounds were analysed by fluorescence measurement (Victor², Wallac) and radiolabelled compounds were measured by liquid scintillation counting (Wallac 1450 Microbeta, Wallac). All other compounds were analysed by LC-PDA (Waters Alliance 2790). All test methods had been validated for each compound.

3.8 Permeability calculation

The apparent coefficient of permeation (P_{app}) was calculated using the following equation:

Eq. 1 $$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{m_O} \cdot \frac{1}{A} \cdot V_{Donor}; \text{ [cm/s]}$$

dQ/dt: permeability rate (steady state transport rate) obtained from the transport-time profile of the substrate [e.g. counts/s]
A: area of the exposed cell monolayer [cm^2]
m_0: the original mass of the marker substance in the donor compartment [e.g counts]
V_{Donor}: volume of donor compartment [cm^3].

4 Results

4.1 Correlation of Caco-2 permeability coefficients and "absorbed fraction" data for model compounds

To demonstrate that Caco-2 cell monolayer permeability correlates well with *in vivo* absorption, the P_{app} values for 19 model compounds were plotted against their published fractional absorption values (Fa) in humans (Fig. 1). The sources of human absorption data are listed in references (Artursson, 1991; Cheong et al., 1999; Goodman, 1999; Rubas et al., 1993; Sweetman, 2000; Yee, 1997; Zhao et al., 2001). We found a good correlation between the absorbed fractions in humans and the apparent permeability co-efficients for most test compounds (Fig. 1).

The reference compounds fluorescein and rhodamine123, which were used to demonstrate the intactness of the monolayer and PGP activity, respectively, were not included in Figure 1, because the relevant Fa data was not available in the literature. The permeability coefficient of [3H]PEG4000 (compound 11), which has a known low Fa value (zero marker), was found to be 5.00 x E-06 cm/s in the Caco-2 system. This high value may have been due to impurities of lower molecular weight, or to the instability of the radioactive labelling (PEG Supplier, personal communication). In the case of radiolabelled digoxin (compound 14), the literature values for the

Tab. 2: Comparison of the apparent permeation coefficients of model drugs and the BCS permeability class. The P_{app} values are mean values from experiments performed in triplicate. Data were determined at cell passage 14.

No.	Compound	P_{app} ab [cm/s]	BCS permeability class	Fa [%]
1	propranolol	3.42E-05	high	99/90
2	carbamazepin	3.18E-05	high	70
3	antipyrine	2.91E-05	high	97
4	naproxen	2.58E-05	high	99
5	verapamil	2.42E-05	high	100
6	metoprolol	2.29E-05	high	95
7	ketoprofen	1.86E-05	high	92
8	citalopram	1.40E-05	high	90
9	[14C]caffeine	1.35E-05	high	100
10	[3H]clonidin	9.75E-06	high	75/95
11	[3H]PEG4000	5.00E-06	low	0
12	fluorescein	5.17E-07	low	-
13	[3H]PEG400	4.54E-07	low	10
14	[3H]digoxin	3.78E-07	low/efflux substrate	81
15	rhodamine123	3.61E-07	low/efflux substrate	-
16	[3H]atenolol	2.57E-07	low	55/50
17	[3H]PEG900	2.41E-07	low	10
18	furosemide	1.98E-07	low	61
19	[14C]mannitol	1.77E-07	low	16

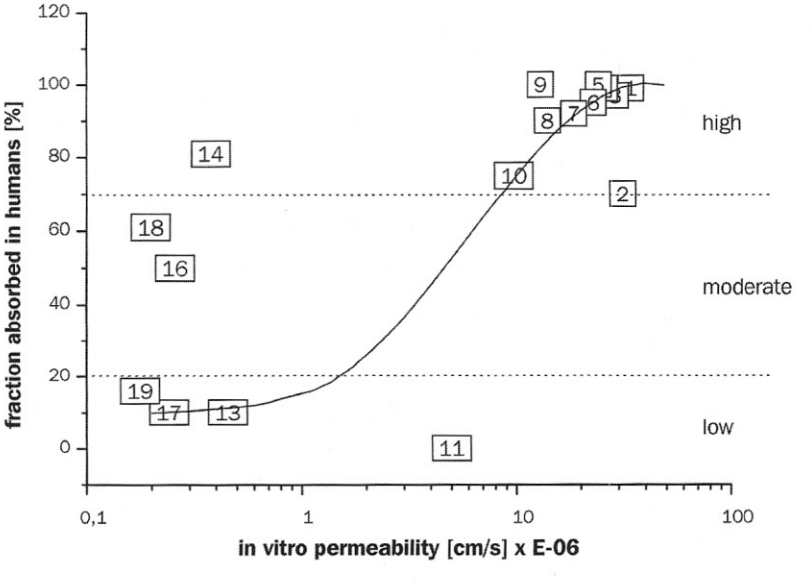

Fig. 1: Comparison of the apparent permeation coefficients of model drugs measured in the Caco-2 cell monolayer system with absorbed fractions in humans. Data were determined at cell passage 14.

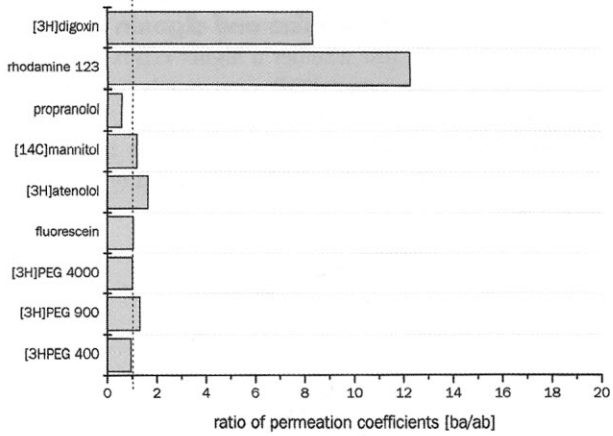

Fig. 2: Ratios (ba/ab) of the permeation coefficients of model drugs in the Caco-2 cell monolayer system. The data are mean data of experiments performed in triplicate. Data were determined at cell passage 14.

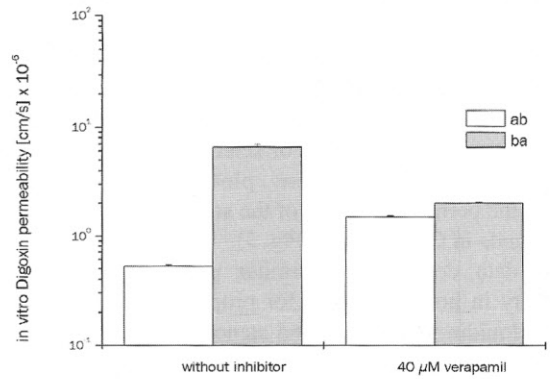

Fig. 3: Comparison of the apparent permeation coefficients of digoxin in the absence and presence of 40 µM verapamil. The data are mean data from experiments performed in triplicate. Data were determined at cell passage 11.

absorbed fraction in humans vary because of its interaction with PGP (Hidalgo et al., 1989). A weak correlation of the absorbed fraction in humans with Caco-2 permeability was also found for atenolol and furosemide. For both compounds, the excipients of the oral dosage form have been demonstrated to increase permeability greatly (Kanfer, 2002).

4.2 Caco-2 monolayers exhibit apical efflux due to p-glycoprotein

To further validate the suitability of the Caco-2 *in vitro* model, the monolayers were tested for PGP activity. Rhodamine123 and digoxin are substrates of PGP, and verapamil is a PGP inhibitor (Cheong et al., 1999). The ratio of the P_{app} values from the basolateral side (which corresponds with the blood circulation side) to the apical cell side (representing the intestinal lumen) to the transport rate in the reverse direction depends on the operating transport mechanisms. The ratios for selected compounds are summarised in Figure 2.

A clear directionality was found for digoxin and rhodamine123 only. None of the other test compounds demonstrated a clear directionality in their transport.

4.3 PGP inhibition by verapamil in Caco-2 cells

At a specific Caco-2 cell monolayer passage, the apparent permeation coefficients of digoxin were measured with and without 40 µM verapamil in the assay buffer. The data are summarised in Figure 3.

Without inhibitor, digoxin exhibits a substantial P_{app} (ba) to P_{app} (ab) transport polarity ratio of 12.5. In the presence of the PGP inhibitor verapamil, the transport polarity of digoxin is reduced to a value of 1.3. These results confirm that PGP enhances apical efflux activity.

4.4 Caco-2 cell passage number and its impact on the permeability of selected compounds

Figure 4 shows the permeability of fluorescein and propranolol as a function of various cell passage numbers in relation to the quality criteria listed in Table 1. The permeability results do not appear to be dependent on passage

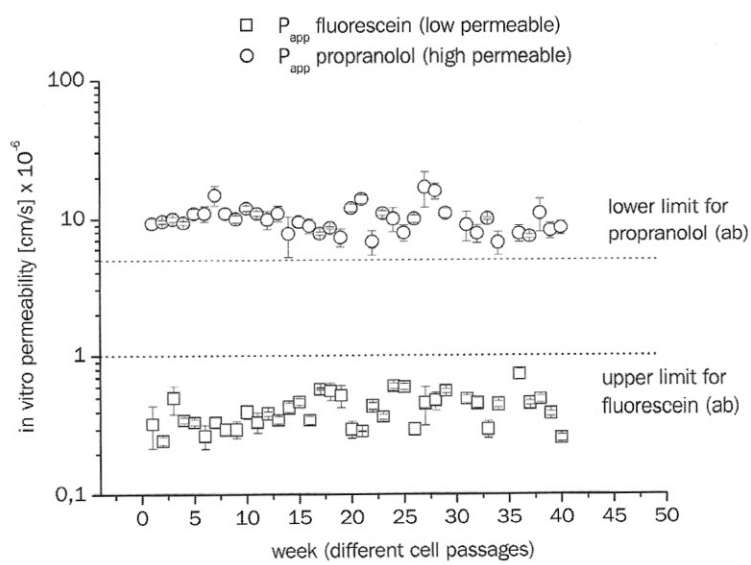

Fig. 4: Comparison of the apparent permeation coefficients of the model drugs propranolol and fluorescein for different passages. The values are mean values from experiments performed in triplicate.

number. The Caco-2 cells gave reproducible data over 40 passages.

4.5 Comparison of Caco-2 and PBEC permeability

To compare Caco-2 and PBEC permeability, the permeability data of selected compounds in PBEC were plotted against the permeability data of the same compounds in Caco-2 cells (Fig. 5).

Our data demonstrates similar permeability in both cell types for propranolol, clonidin, fluorescein and atenolol. However, the permeability found for rhodamine and digoxin were significantly different in the two cell types. While the permeability found for rhodamine was higher in PBEC, the digoxin permeability was higher in Caco-2.

4.6 Apical efflux of rhodamine in PBEC and its inhibition by verapamil

The difference in rhodamine permeability in Caco-2 and PBEC may be the result of different PGP activities in the two cell types. To test this hypothesis, the PBEC monolayers were tested for PGP activity using rhodamine. Differences in rhodamine permeability between transport from the apical to the basolateral (ab) side and transport from the basolateral to apical side (ba) are a result of polarised active transporters involved in the transport process. The rhodamine permeability for three independent PBEC preparations is summarised in Figure 6.

Rhodamine permeability was found to be within the same range for all three preparations. The permeability for the ab transport ranged between 0.5-0.7 x E-6, while the ba permeability was found to be in the range of 1.5-1.8 x E-6. Similar rhodamine permeability was found for other preparations (data not shown) when the qualification criteria were met (Table 1). Therefore, the preparation method used for PBEC cells reproducibly allows the preparation of cells with the same barrier properties. In addition, the ba transport was reproducibly found to be 2-3-fold faster then the ab transport, demonstrating that transporter activity doesn't change with different preparations. The effect was inhibited by 40 µM of the PGP inhibitor verapamil, demonstrating that PGP-like transporters are involved. Given the largely reduced apical efflux of rhodamine in PBEC in comparison to Caco-2 cells, the higher rhodamine permeability in PBECs (Fig. 5) may be the result of the significantly higher PGP activity found in Caco-2 cells.

4.7 Comparison of apical efflux of rhodamine and digoxin in PBEC

To test whether a higher efflux is found for other PGP compounds in Caco-2 cells in comparison to PBEC, we also studied the efflux of digoxin in PBEC. The ratio of the permeability of rho-

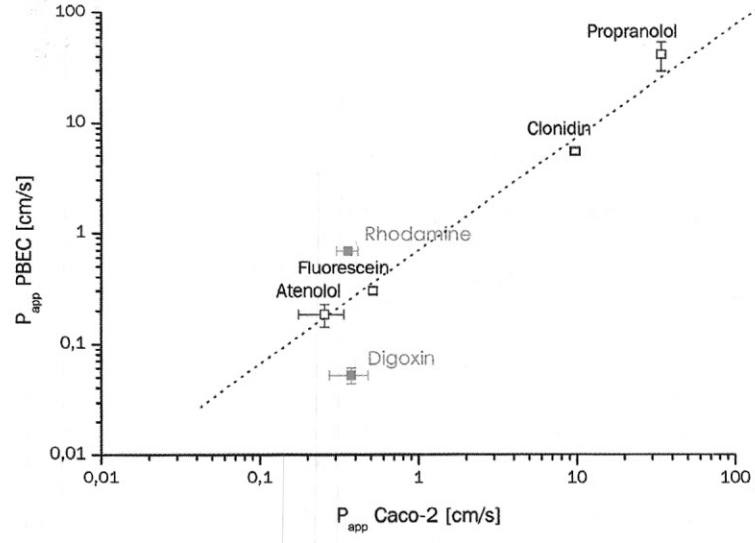

Fig. 5: Comparison of permeation coefficients of model drugs in the PBEC and Caco-2 monolayer system. The data are mean data of experiments performed in triplicate (error bars indicate SD).

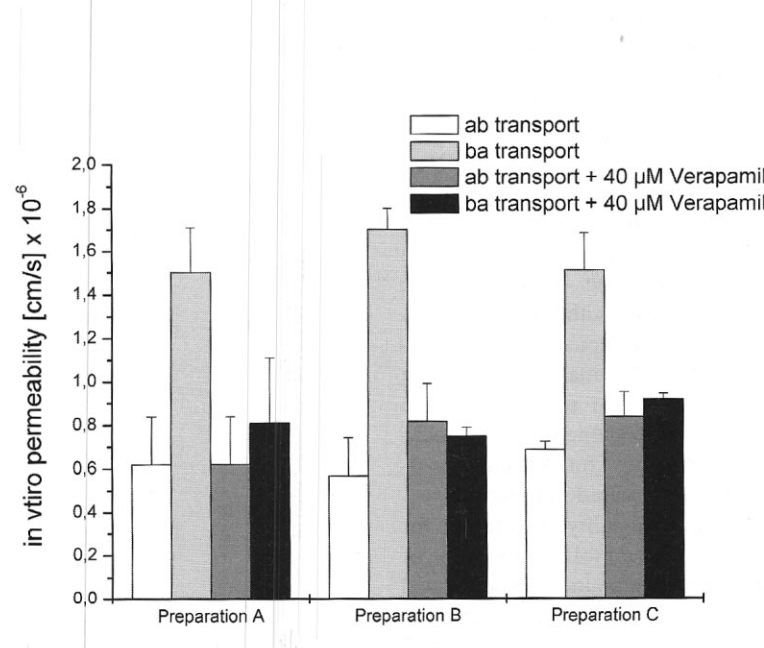

Fig. 6: Comparison of the apparent permeation coefficients of rhodamine123 in the PBEC monolayer system in the absence or presence of PGP inhibitor verapamil (40 µM). The data are mean data of experiments performed in triplicate. Data were determined on three different PBEC preparations (A, B, C) to demonstrate reproducibility of results.

damine and of digoxin in Caco-2 and PBEC is shown in Figure 7.

Interestingly, the ratio of ab to ba transport for digoxin was found to be within the same range for both cell types (Caco-2, 8-fold; PBEC, 7.2-fold). In contrast to this finding, the rhodamine efflux differed greatly for both cell types (Caco-2, 12-fold; PBEC, 2-fold). In conclusion, the differences found in the apical efflux of rhodamine in the two cell models cannot be the result of different PGP expression alone, but must also be the result of different substrate specificities of active transporters. Note that the permeability of digoxin and rhodamine was measured in the same preparation of PBEC and the same passage of Caco-2 cells respectively.

5 Discussion

According to the recommendation of the FDA and EMEA, the Caco-2 cell culture model was validated for the classification of compounds in terms of intestinal permeability. Several parameters of the Caco-2 cell permeability assay were evaluated to assess the robustness of the system. Caco-2 cell monolayers can be used to study compounds with permeability ranging from 1.77 x E-07 cm/s (e.g. mannitol) to 3.42 x E-05 cm/s (e.g. propranolol), enabling the Caco-2 system to discriminate between high and low permeability compounds as classified in the BCS. Caco-2 cell monolayers show directional transport for the PGP substrates digoxin and rhodamine123, with a significantly higher basolateral-to-apical transport than in the reverse direction. Directional transport can be inhibited by the PGP inhibitor verapamil at a concentration of 40 µM. The permeability determined for many of the model compounds correlated well with reported absorption in humans. A weak correlation was found only for the PGP substrates digoxin and furosemide. The Caco-2 cell monolayers demonstrated a very low inter-day variability for monolayers in culture. The inter-day variability was always lower than the measured inter-compound differences.

Caco-2 cell monolayers could be used for more than 40 passages without noticeable changes in the permeability of the model compounds (Fig. 4). The present Caco-2 system is therefore a valid tool to determine the gastrointestinal permeability of a test compound in accordance with the FDA's BCS. Therefore, in accordance with the 3R principle (Russell and Burch, 1959), the Caco-2 cell assay not only reduces the complexity of uptake studies, but also refines their reproducibility and can therefore be used to replace animal studies in the drug approval process.

Comparing the *in vitro* permeability of different test compounds in Caco-2 cells and PBEC reveals similarities between these cell culture models. As demonstrated for Caco-2 cells, PBEC can be used to study compounds with a wide range of permeability ranging from 5.2 x E-08 to 3.4 x E-05. Furthermore, the permeability data obtained for non-PGP substrates was within the same range for both cell culture models (Fig. 5). In addition, as demonstrated for Caco-2 cells, a clear directionality was found for the PGP substrate rhodamine123 (Fig. 7), although the PBECs showed only a two-fold higher basal-to-apical transport than in the reverse direction (Caco-2 cells, 12-fold). Permeability and directionality were shown to be within the same range for individual preparations. Our data therefore demonstrates the good reproducibility of the preparation method. Furthermore, directionality was inhibited by adding verapamil, proving the involvement of PGP-like transporters.

However, comparing the permeability data obtained from Caco-2 and PBEC also revealed some striking differences between Caco-2 and PBEC (Fig. 5). Interestingly, these differences were found exclusively for the PGP substrates rhodamine and digoxin. While the permeability of rhodamine123 was found to be about twice as high in PBEC, the permeability of digoxin was found to be ten-fold lower in Caco-2 cells. Comparing the PGP activity in Caco-2 and PBEC (Fig. 7) indicated that differing rhodamine permeability might be the result of different activity of active transporters. The different directionality found for rhodamine in Caco-2 and PBEC is unlikely to be the result of the total amount of receptor found in Caco-2 cells, since a similar degree of directionality was found for digoxin in Caco-2 and PBEC. Therefore, the differences found for rhodamine permeability are likely to be the result of different substrate specifics of different PGP-like transporters in Caco-2 and PBEC. However, digoxin also had different permeability in Caco-2 and PBEC (Fig. 5), and similar PGP activities were found in both cell types (Fig. 7). Therefore PGP activity alone cannot explain the difference in digoxin permeability found for both cell types. The reason for the differing digoxin permeability is unclear, especially since both cell types demonstrate similar permeability for non-PGP compounds. A different degree of metabolisation may be involved. Further experiments are needed to explain this observation.

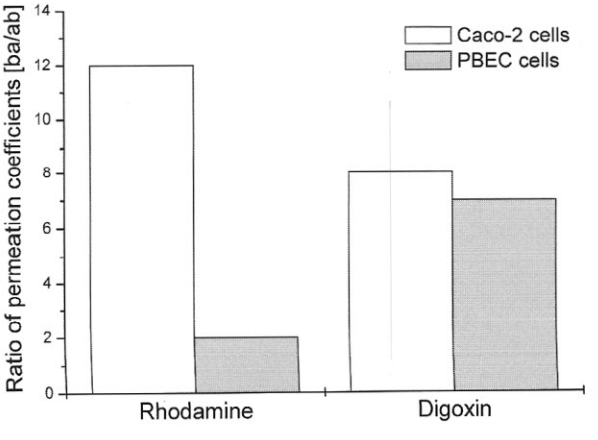

Fig. 7: Ratio of permeation coefficients of rhodamine and digoxin in Caco-2 and PBEC cells.

In conclusion, our data demonstrate that the Caco-2 cell model can be used to study drug uptake via the intestine in the drug approval process. The PBEC culture model gives similar results for non-PGP drugs, but striking differences were found for the uptake of drugs that are substrates of active transporters. Our data therefore demonstrate the necessity of specific test systems for different epithelial barriers.

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