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Caco-2 permeability, P-glycoprotein transport ratios and brain penetration of heterocyclic drugs

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Abstract

In this study the gastrointestinal absorption and P-glycoprotein (Pgp) efflux transport of heterocyclic drugs was investigated with the Caco-2 cell model.

Based on the calculation of the physico-chemical properties a good oral absorption was predicted for all the drugs tested in this study which corresponded well with the measured Caco-2 permeabilities (P_{app}). Generally a high permeability of the tested heterocyclic drugs was measured being in agreement with earlier published human in vivo absorption data.

Based on the transport data of domperidone and verapamil it was found that the Pgp efflux transporter was expressed in the Caco-2 cells. Many of the drugs tested were indicated to be potential Pgp efflux substrates. Since Pgp is expressed at the Blood Brain Barrier (BBB) as well, it was expected that CNS penetration will be impaired if a drug is a Pgp substrate. However, no correlation could be found between brain penetration in rats and the Pgp efflux ratio as measured with the Caco-2 cells.

From the data it is concluded that Pgp efflux ratio's as determined in in vitro High Throughput Screening (HTS) tests, where the transport conditions are fixed (pH gradient, concentration, etc.), cannot routinely be used to predict a possible limited brain penetration.

Keywords: Caco-2; P-glycoprotein transport; Brain penetration

1. Introduction

In the discovery and development of New Chemical Entities (NCEs) the absorption of the drug and possible interactions with transporter systems are important variables (Testa et al., 2001). Transporters are of special interest since they can be of influence on the absorption and distribution of drugs. Drugs intended to treat depression and psychotic disorders need to cross firstly the enterocytes in the gastrointestinal (gi) tract and secondly the Blood Brain Barrier (BBB) to enter the Central Nervous System (CNS) (van der Sandt, 2001; Pratt and Taylor, 1990; Williams, 1995).

In the gi-tract and in the BBB transporter systems are present, from which the P-glycoprotein (Pgp) transporter is the most frequently studied (Cordon-Cardo et al., 1989; Thiebaut et al., 1987; Rodrigues, 2002). Pgp serves as an ATP-dependent efflux pump that exports a large number of structurally unrelated substrates out of the cell. In general it is thought that Pgp limits intestinal absorption as well as penetration of the CNS, since this transporter

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is widely expressed in the gi-tract and the BBB (Rodrigues, 2002).

Since in Caco-2 cells the Pgp transporter is strongly expressed, due to their tumourous nature (Romsicki and Sharom, 1999; Rodrigues, 2002), this cell system is widely used in in vitro screening technologies to predict gastrointestinal absorption and possible Pgp transport of NCEs (Stoner et al., 2000; van der Sandt, 2001; Testa et al., 2001). However, little information is available which links Pgp transport to possible in vivo effects (Trouman and Thakker, 2001; Chiou et al., 2001; Ahmed et al., 2000).

The objective of this study is to evaluate the Caco-2 permeability and possible (Pgp) efflux transport of a series of heterocyclic drugs. Furthermore, using the (Pgp) efflux transport data of the Caco-2 experiments it is examined whether (Pgp) efflux influences brain penetration in rats in vivo.

2. Materials and methods

2.1. Compounds tested

The chemicals were supplied by the following manufacturers. Sigma–Aldrich: salicylic acid, acetylsalicylic acid, carbamazepine, imipramine hydrochloride, caffeine, verapamil, amitriptyline hydrochloride, ranitidine hydrochloride, clonidine hydrochloride, desipramine hydrochloride, PEG4000, pyrilamine maleate, antipyrine, haloperidol, acetaminophen and indomethacin. Janssen Research Foundation: risperidone and domperidone. NV Organon: quinidine, ibuprofen, morphine, Org 25907 (3-OH-4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedionemonohydrochloride), Org 9935 (4,5-dihydro-6-(5,6-dimethoxybenzo[*b*]thien-2-yl)-5-methyl-3(2*H*)-pyridazinone), Org 12962 (1-[6-chloro-5-(trifluoromethyl)-2-pyridinyl]piperazine hydrochloride), Org 23430 (4-[(4-fluorophenyl)-4-chlorothienyl-2-methylene]-methylpiperidinebutane-1,4-dioate), Org 5222 (*trans*-DL-5-chloro-2,3,3*a*,12*b*-tetrahydro-2-methyl-1*H*-dibenz[2,3:6,7]oxepino[4,5-*c*]pyrrole (*Z*)-2-butenedioate), Org 13011 (1-[4-[4-[4-(trifluoromethyl)-2-pyridinyl]-1-piperazinyl]butyl]-2-pyrrolidinone(*E*)-2-butenedioate), Org 33062 (4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedionemonohydrochloride), Org 23366 (1-[4-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]-1-oxobutyl]pyrrolidine methanesulfonate), Org 34037 (*R*(−)-6-(4-chlorophenyl)-2,3,5,6-tetrahydroimidazo [2,1-a]isoquinoline(*E*)-2-butenenedioate), Org 32782 (methyl 2,6-di-deoxy-2,6-di-amino-alpha-D-mannopyranosyl-(1-2)-*O*-alpha-D-mannopyranoside diacetate), Org 34167 ((−)-2-(1,2-benzisoxazol-3-yl)-alpha-(2-propenyl)benzenemethanamine hydrochloride) and mannitol. The purity of all the used compounds was higher than 98%.

The radio-labelled compounds were supplied by the following manufacturers. ICN: [^{3}H]mannitol. Amersham: [^{14}C]PEG 4000. Perkin-Elmer Life Science: [^{3}H]verapamil and [^{14}C]caffeine. Janssen Research Foundation: [^{3}H]domperidone and [^{3}H]risperidone. NV Organon: [^{3}H]Org 12962, [^{14}C]Org 23430, [^{3}H]Org 5222, [^{3}H]Org 13011, [^{14}C]Org 23366, [^{3}H]Org 34037 and [^{3}H]Org 32782.

2.2. Calculation of the physico-chemical properties

The chemical structures of the drugs were retrieved from the Organon compound database. The Clog *P*, MW and the static polar surface area (PSA, Å^{2}) were calculated using the methods described in (Kelder et al., 1999). The static PSA does not take into account the different conformations of the chemical structures, but gives essentially the same results as the dynamic PSA. It has the advantage of a much shorter time of calculation (Kelder et al., 1999).

2.3. Cell culture

The Caco-2 cells (ATCC, code HTB 37, human colon adenocarcinoma, passage numbers **29**–**33**) were grown in culture medium consisting of Dulbecco's Modified Eagle Medium (DMEM), supplemented with heat-inactivated foetal calf serum (10%, v/v), non-essential amino acids (1%, v/v), L-glutamine (2 mM) and penicilline/streptomycine (100 IU μg/ml and 0.1 mg/ml, respectively). The Caco-2 cells were cultured by seeding about 2,000,000 cells in 80 cm^{2} tissue culture flasks containing culture medium. Near confluent Caco-2 cell cultures were harvested by trypsinisation and resuspended in culture medium. The cells were routinely cultured in a humidified incubator at 37 °C in air containing 5% CO_2.

Caco-2 cells were seeded on semi-permeable filter inserts (Costar 24-well Transwell plates) at ca. 21,000 cells per filter growth area 0.33 cm^2 (containing 0.1 ml culture medium). The cells on the insert are cultured for 22–24 days at 37 °C in a humidified incubator containing 5% CO_2 in air.

To check the differentiation status of the formed monolayer the transepithelial electrical resistance (TER) was measured (Millicell-ERS epithelial volt-ohmmeter, Millipore Co., Bedford, USA). The TER of the cell monolayers was calculated according to the following equation:

$$\mathrm{TER} = (R_{\mathrm{monolayer}} - R_{\mathrm{empty\ filter}}) \times A(\Omega\,\mathrm{cm}^2)$$

where $R_{\mathrm{monolayer}}$ is the resistance measured, $R_{\mathrm{empty\ filter}}$ is the resistance of control filters without cells (approximately 140 $\Omega\,cm^2$) and A is the surface area of the filter insert (0.33 cm^2). After 2–3 weeks in cell culture, the monolayers developed a TER of approximately 600 $\Omega\,cm^2$.

2.4. Drug transport experiments

All test substances were tested at a high (1 mM) and low (0.1 mM) concentration. Antipyrine and PEG4000 were tested at 100 and 10 μM, whereas mannitol, verapamil, caffeine, Org 12962, Org 5222 and Org 13011 at 10 and 1 mM, respectively. Three filter inserts were used per concentration. Transport of the drugs was assessed after apical exposure and after basolateral exposure. Per drug tested all Caco-2 tests were performed with cells of the same passage number.

It was decided not to use BSA in the transport medium of the Caco-2 cells, since most drugs do not bind equally to proteins (e.g. SHBG, HSA, BSA) (Kragh-Hansen, 1981; Watanabe and Sato, 1996; Baker, 1998). In our study protein binding of the drugs varied from 0–99% (Hardman and Limbird, 1996). Furthermore, the Caco-2 permeability might be influenced by the presence of BSA in the receptor compartment as is the case for midazolam and dexamethasone (Fischer et al., 1999; Yamashita et al., 2000), i.e. in a diffusion experiment the permeability is mainly determined by the free fraction.

In the current study a physiologically relevant pH gradient (pH = 6.5/7.4) was applied since the apparent permeability values in the apical to basolateral direction are reportedly more predictive of human intestinal absorption than using pH 7.4 on both sides (Boisset et al., 2000; Yamashita et al., 2000).

For apical exposure, culture medium was removed from the filter insert prior to moving them to a new 24-well plates containing 0.6 ml fresh transport medium (Hanks Balanced Salt Solution, pH = 7.4, 25 mM D-glucose, 50 mM HEPES, 1.25 mM $CaCl_2$, 0.5 mM $MgCl_2$). The transport study starts by filling the apical chambers with 100 μl of the test solution (in Hanks Balanced Salt Solution, pH = 6.5, 25 mM D-glucose, 50 mM HEPES, 1.25 mM $CaCl_2$, 0.5 mM $MgCl_2$). After 1, 2 and 4 h the inserts were transferred to new 24-well plates containing fresh transport medium. Samples were withdrawn of the receptor compartments. The applied pH gradient was present during the complete course of the experiment.

For basolateral exposure, culture medium at the apical side was replaced by 100 μl fresh transport medium (pH = 6.5) and the transport study started by transferring the filter inserts to new 24-well plates containing 0.6 ml test solution (pH = 7.4). All cultures were incubated on a rotating platform in a humidified incubator containing 5% CO_2 in air at 37 °C. Samples of the receptor compartments were collected at 1, 2 and 4 h after application of the test substances and directly after sampling the original volume was restored by adding fresh transport medium. The applied pH gradient was present during the complete course of the experiment.

2.5. Calculations

The apparent permeability coefficient (P_{app}, cm/s) was calculated using the following equation: $P_{\mathrm{app}} = (\mathrm{d}Q/\mathrm{d}t)/(1000 \times A \times C_0)$, where $\mathrm{d}Q/\mathrm{d}t$ is the initial permeability rate (mol/s), A is the surface area of the filter insert (0.33 cm^2) and C_0 is initial concentration (mol/l).

The permeability ratio was calculated according to:

$$P_{\mathrm{ratio}} = \frac{P_{\mathrm{app,ba}}}{P_{\mathrm{app,ab}}}$$

where $P_{\mathrm{app,ba}}$ is the permeability from the basolateral to the apical side (blood to intestine) (cm/s) and $P_{\mathrm{app,ab}}$ is the permeability from the apical to the basolateral side (intestine to blood) (cm/s).

2.6. Concentration measurements

All the concentrations of the non-radio-labelled compounds were determined by HPLC (HP11000 with DAD detection and temperature controlled column compartment). The following columns were used: Luna C8 (Phenomenex), Luna C18 (Phenomenex), Phenyl–Hexyl (Phenomenex) and Supelcosil LC-NH2 (Supelco). The temperature of the column was held at 30 °C. Detection was performed at 210, 250, 280, 300 and 310 nm. The injection volume was 5–40 μl depending on the peak area of the lowest concentration used. The runtimes were typically between 5 and 6 min.

The concentrations of the ^{14}C- and ^{3}H-labelled compounds were measured by using a LKB/Wallac S1409 scintillation Counter and Packard Ultima Gold scintillation liquid.

3. Results and discussion

3.1. Caco-2 permeability data

Calculations of the physico-chemical properties show that most compounds used in this study are predicted to have a good oral absorption, except for mannitol and PEG4000 (Table 1). Both compounds are highly polar and are known for their bad oral absorption. Similar results are obtained for the prediction of drugs into the CNS.

The wide majority of the tested drugs is well transported over the Caco-2 cell monolayers which is consistent with the polar surface area data (Tables 1 and 2) (Kelder et al., 1999; Palm et al., 1997), i.e. the permeability is approximately 10^{-5} cm/s or higher, corresponding to a high absorption (antipyrine (97%), caffeine (100%), desipramine (95–100%), imipramine (100%), ranitidine (50%), clonidine (100%), acetominophen (60–70%), acetyl salicylic acid (68–100%), quinidine (100%), indomethacin (100%), salicylic acid (100%) and ibuprofen (100%)) (Yazdanian et al., 1998; Norinder et al., 1997; Gres et al., 1998). Even the low-permeability compounds (morphine (bioavailability 80% (Yee, 1997)) and Org 9935) are transported at a much higher rate than the reference compounds with a known low permeability (mannitol and PEG4000 having a bioavailability of 13 and 1%, respectively) (Gres et al., 1998). The Caco-2 permeability data from this study are comparable to data reported in literature indicating that the cell system used in this study worked adequate (Yee, 1997; Yazdanian et al., 1998; Camenisch et al., 1998). For the drugs which did not show polarized transport (see Section 3.2) the apical to basolateral permeability was not influenced by the concentration in the donor compartment ($\log P_{ab,low} = 0.97 \times \log P_{ab,high}$; $r^2 = 0.82$).

Although several reports are available in which the metabolic capability of Caco-2 cells is described, no metabolism was detected in this study (Schmiedlin-Ren et al., 1997; Raeissi et al., 1999; Lampen et al., 1998; Prueksaritanont et al., 1996), i.e. metabolite formation would lead to differences in the drug retention time and extra peaks in the HPLC chromatograms, due to changes in the chemical structure. Since the retention time did not change, during transport over the cells, it is concluded that metabolism was absent.

3.2. P-glycoprotein transport

To investigate possible efflux transport of the CNS drugs by the Pgp transporter the permeability ratio was calculated. The calculation of the permeability ratio is explained in detail in Section 2.5. In general it is assumed that a permeability ratio of 2 and higher is indicative for Pgp transport, corresponding to a net efflux transport of the drug (Karlsson et al., 1993), i.e. drugs are preferentially transported towards the gastrointestinal (apical) side of the Caco-2 cells. The Caco-2 cells used in this study have a high expression of Pgp (Versantvoort et al., 2002). The calculated permeability ratios are given in Table 2.

For domperidone, a well known Pgp transported dopamine antagonist (Schinkel, 1999), permeability ratios are found of 15.1 and 36.3. This indicates that Pgp was expressed in the Caco-2 cells. Also the verapamil data show that Pgp was expressed (Hendrikse, 1999; Sandstrom et al., 1998). At a low concentration in the donor compartment a ratio of approximately 6 is found. At higher concentrations, however, the transporter becomes saturated resulting in a permeability ratio of approximately 0.6 (Sandstrom et al., 1998).

Since Pgp is widely expressed in the BBB one would expect a low-brain/CNS penetration when

Table 1
Calculated physico-chemical parameters of the drugs tested

Compound number	Compound	Clog P ($0 < X < 6$)[a] ($-1 < X < 6$)[b]	PSA ($0 < X < 70$)[a] ($0 < X < 120$)[b]	MW ($0 < X < 450$)[a] ($0 < X < 600$)[b]	Prediction of oral absorption	Prediction of CNS absorption	pK_a
1	Mannitol	−2.05	97.9	182.2	Moderate	Bad	
2	PEG 4000	0.14	181.5	1061.3	Bad	Bad	
3	Verapamil	4.47	54.5	454.6	Good	Bad	8.8[e]
4	Antipyrine	0.2	20.2	188.2	Good	Good	1.4[c]
5	Caffeine	−0.04	45.8	194.2	Good	Moderate	0.2[c]
6	Desipramine HCl[f]	4.47	14	266.4	Good	Good	10.1[c]
7	Imipramine HCl[f]	5.04	8	280.4	Good	Good	9.5[c]
8	Amitriptyline HCl[f]	4.85	4	277.4	Good	Good	9.4[c]
9	Carbamazepine[f]	1.98	34.9	236.3	Good	Good	3
10	Ranitidine HCl	0.63	70.8	314.4	Good	Moderate	8.2
11	Domperidone[f]	4.27	56.5	425.9	Good	Good	7.6/11.1/11.8[d]
12	Clonidine HCl	1.43	29.8	230.1	Good	Good	8.3[c]
13	Pyrilamine Maleate	3.23	26.8	285.4	Good	Good	4.0/8.9[c]
14	Haloperidol[f]	3.85	32.6	375.9	Good	Good	8.3[c]
15	Acetaminophen	0.49	38.6	151.2	Good	Good	9.5[c]
16	Acetylsalicylic acid	1.02	49.6	180.2	Good	Good	3.5[d]
17	Quinidine HCl	2.79	39.1	324.4	Good	Good	4.2/8.3[c]
18	Morphine	0.57	45.4	285.3	Good	Good	9.9[c]
19	Indomethacin	4.18	53	357.8	Good	Good	4.5[c]
20	Salicylic acid	2.19	44.9	138.1	Good	Good	3.0[c]
21	Ibuprofen	3.68	28.6	206.3	Good	Good	5.2[c]
22	Risperidone[f]	2.58	49.8	410.5	Good	Good	3.1/8.2[d]
23	Org 12962[f]	1.71	24	265.7	Good	Good	8.4[d]
24	Org 23430[f]	5	4	321.8	Good	Good	9[d]
25	Org 5222[f]	4.58	12.8	285.8	Good	Good	8.6[d]
26	Org 33062[f]	0.83	56.5	359.5	Good	Good	7.3[d]
27	Org 25907[f]	0.4	72.8	375.5	Good	Moderate	
28	Org 13011[f]	1.41	34.2	370.4	Good	Good	7.3[d]
29	Org 34037[f]	4.63	13.8	282.8	Good	Good	9.8[d]
30	Org 23366[f]	4.72	20.2	424.5	Good	Good	8.6[d]
31	Org 9935	3.21	49.7	304.4	Good	Good	
32	Org 32782	4.5	27.4	268.1	Good	Good	9[d]
33	Org 34167[f]	3.14	38.5	264.3	Good	Good	8.4[d]

Clog P: calculated log P, PSA: polar surface area, MW: Molecular weight.
[a] Criteria for CNS penetration.
[b] Criteria for oral absorption. The criteria for oral and CNS penetration are based on (Lipinski et al., 1997) and (Kelder et al., 1999).
[c] Newton and Kluza (1978).
[d] Own measurement.
[e] Hasegawa et al. (1984).
[f] CNS drug.

a high-permeability ratio is measured. Therefore it is interesting to compare the permeability ratios from this study with brain penetration data in rats (Table 3) (Kelder et al., 1999). Brain penetration is expressed as the ratio between the maximum concentration in the brain and the maximum concentration in the blood (C_{brain}/C_{blood}). The following criteria are generally accepted for CNS penetration and Pgp transport:

- if the C_{brain}/C_{blood} ratio is higher than 1, CNS penetration is considered to be good;
- if the permeability ratio is higher than 2, a drug is considered to be transported by Pgp.

Table 2
Caco-2 permeability and transport ratio of the drugs tested

Compound number	Compound	High concentration			Low concentration		
		$P_{app,ab}$	S.D.[a]	P_{ratio}	$P_{app,ab}$	S.D.[a]	P_{ratio}
1	Mannitol	6.30E-07	0.51	0.71	7.06E-07	0.99	1.40
2	PEG 4000	3.22E-07	0.39	0.99	2.70E-07	0.29	1.20
3	Verapamil	6.24E-05	0.45	0.59	9.17E-06	15.4	6.47
4	Antipyrine	5.56E-05	0.45	1.63	9.68E-05	0.60	1.04
5	Caffeine	6.05E-05	0.47	1.18	4.23E-05	0.42	1.63
6	Desipramine HCl	2.12E-05	0.62	3.98	–	–	–
7	Imipramine HCl	1.89E-05	0.22	4.82	1.37E-05	0.48	6.04
8	Amitriptyline HCl	2.10E-05	0.04	3.86	1.73E-05	0.43	4.51
9	Carbamazepine	2.70E-05	0.27	1.27	5.01E-05	0.00	1.27
10	Ranitidine HCl	4.59E-06	0.47	0.67	2.43E-05	0.00	0.47
11	Domperidone	3.18E-06	1.17	15.11	1.48E-06	0.63	36.26
12	Clonidine HCl	2.18E-05	0.18	2.99	3.40E-05	0.00	2.95
13	Pyrilamine Maleate	1.87E-05	0.13	0.27	2.51E-05	0.00	–
14	Haloperidol	3.93E-06	0.49	–	1.68E-05	0.00	–
15	Acetaminophen	3.16E-05	0.05	1.41	6.16E-05	0.46	1.81
16	Acetylsalicylic acid	6.67E-05	0.39	0.43	3.35E-05	0.00	0.45
17	Quinidine HCl	1.28E-05	0.13	3.15	4.17E-06	–	9.87
18	Morphine	2.54E-06	0.00	3.51	–	–	–
19	Indomethacin	6.17E-05	0.85	0.62	3.93E-05	0.49	0.94
20	Salicylic acid	4.35E-05	0.46	0.50	5.80E-05	1.00	0.48
21	Ibuprofen	5.64E-05	0.32	0.38	5.83E-05	0.00	0.52
22	Risperidone	1.42E-05	0.11	5.04	1.12E-05	0.08	5.73
23	Org 12962	2.62E-05	0.08	1.32	2.40E-05	0.22	3.23
24	Org 23430	6.10E-06	–	6.56	6.10E-06	1.00	6.44
25	Org 5222	2.85E-05	0.20	1.02	9.11E-06	0.44	5.89
26	Org 33062	1.49E-05	0.27	4.25	2.53E-05	0.00	4.00
27	Org 25907	1.74E-05	0.30	3.33	8.42E-06	0.00	12.27
28	Org 13011	1.39E-05	0.15	1.08	2.22E-05	0.10	2.31
29	Org 34037	2.12E-05	0.03	3.81	1.66E-05	0.20	5.59
30	Org 23366	1.38E-05	0.17	3.54	3.54E-06	0.76	13.71
31	Org 9935	1.96E-06	1.28	0.46	8.42E-06	0.00	0.67
32	Org 32782	2.58E-05	0.17	2.99	1.25E-05	0.07	5.67
33	Org 34167	1.42E-05	–	1.04	1.25E-05	–	0.89

P_{app}: apparent Caco-2 permeability (cm/s), ab: apical to basolateral transport, ba: basolateral to apical transport, high concentration: high concentration in the donor compartment, low concentration: low concentration in the donor compartment. P_{ratio}: permeability ratio = $P_{app,ba}/P_{app,ab}$, –: no data available.

[a] Standard deviation must be multiplied by the exponent of the P_{app} value.

When the brain penetration (C_{brain}/C_{blood}) is plotted as function of the permeability ratio it can be seen if CNS penetration is in agreement with the supposed Pgp transport of the drug (this is indicated with the shaded areas in Fig. 1).

From the presented data it becomes clear that brain penetration data are not 'always' consistant with the measured efflux ratios (Yamazaki et al., 2001; Adachi et al., 2001; Fricker et al., 1996). Several drugs are supposed to be transported by Pgp (efflux ratio higher than 2) and still show a good brain penetration ($C_{brain}/C_{blood} > 1$). In a HTS experiment a drug with an efflux ratio higher than 2 would be typically considered to be a potential Pgp substrate. Recently it has been reported that multiple efflux systems of the ABC superfamily are expressed in differentiated Caco-2 cell monolayers (Taipalensuu et al., 2001), i.e. other transporters than Pgp may contribute to the measured efflux ratio. Also a possible species difference in the Pgp substrate susceptibility might be present, as was shown by Yamazaki et al. (2001) in Pgp-mediated transport studies using Pgp of mouse and human

Table 3
Brain penetration data in rats (Kelder et al., 1999)

Compound number	Compound	C_{brain}/C_{blood} [a]
3	Verapamil	10
6	Desipramine HCl	10
7	Imipramine HCl	11.2
8	Amitriptyline HCl	9.5
9	Carbamazepine	1
10	Ranitidine HCl	0.059
11	Domperidone	0.17
12	Clonidine HCl	1.29
22	Risperidone	0.96
23	Org 12962	43.7
25	Org 5222	10.7
27	Org 25907	1.78
28	Org 13011	1.44
33	Org 34167	1.00

[a] Radio-labelled drugs were orally administered to male Wistar rats. Blood and brain samples were taken at fixed time points. The brains were perfused via the aorta with saline until free of blood (within 1 min). Parent compound peak values were used to calculate the C_{brain}/C_{blood} ratio.

origin. In the current study Pgp of rat (brain penetration data) and human (Caco-2 data) origin are compared.

Also experimental variables may contribute to possible variations in the measured (Pgp) efflux ratio. Recently it has been reported that the basolateral to apical permeability of basic drugs may be influenced by the pH gradient over the Caco-2 cells (Mizuuchi et al., 2000; Baker et al., 2002), i.e. the permeability of the basolateral to apical transport is overestimated for drugs with high-pK_a values (pK_a > 8–9). Since many of the drugs in Table 3 are basic from nature (Table 1), this may have been a factor of influence. However, the data of verapamil and domperidone, both being bases, are in line with earlier published results (Sandstrom et al., 1998; Hendrikse, 1999; Schinkel, 1999). Also the applied concentration in the donor compartment could be of influence on the measured permeability ratio, since the Pgp transporter can become saturated (Sandstrom et al., 1998). Furthermore, if the P_{app} is high, as reported in this study for most of the tested drugs (Table 2), the influence of Pgp may become less pronounced (Lentz et al., 2000).

Comparing the data of this study with Maher Doan et al. (2002) and Baker et al. (2002) shows that antipyrine, carbamazepine, domperidone, indomethacin, risperidone and verapamil are correctly predicted. The CNS drugs desipramine, imipramine, amitriptyline and clonidine give opposite results, whereas the imipramine results possibly can be explained by the presence of the pH gradient over the Caco-2 cell monolayer (Baker et al., 2002).

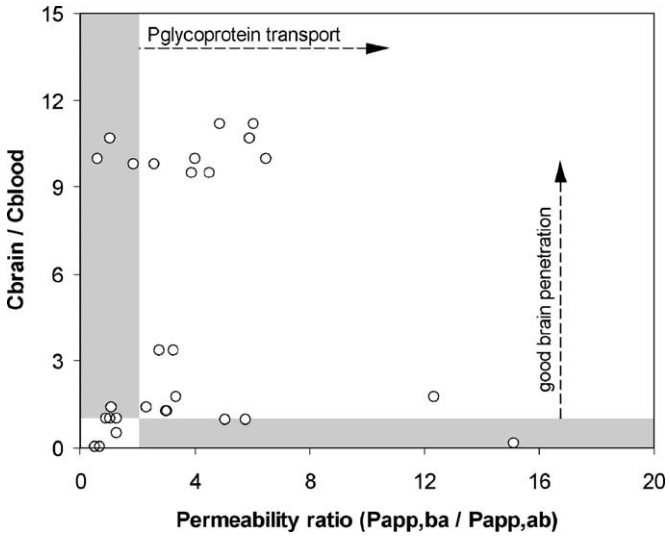

Fig. 1. Brain penetration plotted as function of the permeability ratio. The shaded areas indicate where Pgp and brain penetration data are consistent with each other. At a low-Pgp efflux ratio a high brain penetration and at a high-Pgp efflux ratio a low brain penetration is expected.

All the above discussed variables may contribute to false negative and false positive predictions when standard transport conditions are used in the Caco-2 transport experiments. Hence, the Pgp efflux ratios derived from High Throughput Screening (HTS) experiments, where the transport conditions are fixed (pH gradient, concentration, etc.), cannot be routinely used to predict a possible limited brain penetration. Positive identification of Pgp efflux transport clearly needs to be established by performing a transport experiment in the presence of a known Pgp inhibitor.

Besides Pgp efflux of CNS drugs also the physico-chemical properties are of influence on brain penetration. Comparing the molecular descriptors to differentiate between CNS and non-CNS drugs (Table 1) shows that the CNS group had fewer hydrogen bond donor sites (data not shown), greater lipophilicity and a lower polar surface area. This corresponds well with the results of Maher Doan et al. (2002). However, a relationship between molecular weight and possible Pgp transport (Maher Doan et al., 2002) could not be established as the large majority of drugs in this study had molecular weight lower than 400.

In the evaluation of the significance of Pgp efflux data from in vitro tests, it is interesting to review several recent reports on the clinical relevance of Pgp on the oral absorption in the gastrointestinal tract (Trouman and Thakker, 2001; Sakaeda et al., 2001; Chiou et al., 2001). Drugs which are known as Pgp substrates show an average bioavailability of 47%, suggesting that being a substrate for Pgp does not always result in poor bioavailability (Sakaeda et al., 2001). On average the studied Pgp transported drugs had the same pharmacokinetic parameters as other drugs (fraction absorbed, bound fraction, urinary excretion, total, renal and hepatic clearance) (Sakaeda et al., 2001). In a recent review it was shown that marketed drugs, which are known Pgp substrates, all had linear pharmacokinetics in humans (C_{max} and AUC linearly related to dose), indicating that the absorption was not impaired by Pgp (Chiou et al., 2001). In the light of these results it is clear that the role of Pgp in gastrointestinal absorption might be overestimated and the results of the current study indicate that the same may apply to CNS penetration.

4. Conclusions

Based on the calculation of the physico-chemical properties a good oral absorption was predicted for all the heterocyclic drugs tested in this study. This corresponds well with the measured Caco-2 permeabilities. For almost all the drugs tested a high permeability was measured. Even the low-permeability drugs (morphine and Org 9935) were transported at a much higher rate as the reference compounds with a known low permeability (mannitol and PEG4000). The high Caco-2 permeability was in agreement with earlier published human in vivo absorption data. Comparison of the Caco-2 permeability with data reported in literature showed that the Caco-2 cell system worked well. For the drugs which did not show polarized transport the apical to basolateral permeability was not influenced by the concentration in the donor compartment.

Based on the transport data of domperidone and verapamil it was found that the Pgp efflux transporter was expressed in the Caco-2 cells used. Approximately 50% of the drugs tested were indicated to be potential Pgp substrates (desipramine, amitriptyline, clonidine, risperidone, Org 23430, Org 33062, Org 34037, verapamil, imipramine, cimetidine, domperidone, quinidine, Org 12962, Org 5222, Org 25907, Org 23366 and Org 32782). Since Pgp is expressed at the BBB as well, CNS penetration might be impaired if a drug is a Pgp substrate. However, no correlation could be found between brain penetration and the efflux ratio.

This may be caused by physiological (species difference, presence of multiple efflux systems) and experimental (e.g. overestimation of the P_{app} in the presence of a pH gradient, concentration in the donor compartment, etc.) variables contributing to possible variations in the measured Pgp efflux ratio.

From the data it is concluded that Pgp efflux ratios as determined in in vitro HTS screening tests, where the transport conditions are fixed (pH gradient, concentration, etc.), cannot be routinely used to predict a possible limited brain penetration.

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