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Transepithelial transport of bepridil in the human intestinal cell line, Caco-2, using two media, DMEMc and HBSS

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Abstract

The purpose of this work was to study transepithelial transport of bepridil, an anticalcic agent, through monolayer cells Caco-2, using two experimental media with different chemical components. For experimentation, the measure of the transepithelial electrical resistance (TEER) allowed us to evaluate the state of cells; and the quantities of bepridil have been quantified using a gas chromatography/mass spectrometry system. First, when using the medium alone, without bepridil, Caco-2 cell integrity is, at least, maintained for 8 h using both media. However, for 24-h studies, only the DMEMc medium, rich in essential nutriments, allowed cell integrity to be maintained. Then, with bepridil in HBSS medium, the TEER measurement showed a dose-dependent toxic effect of bepridil, whereas in the DMEMc medium, the toxic effect was only found for the highest dose (12 μg). This difference is probably related to the high binding of bepridil to proteins of the DMEMc medium, therefore minimising the concentration of the free compound. The kinetics of bepridil result from two phenomena: first, an immediate passage of a slight part of bepridil through the cell barrier and second, a high retention of most of the bepridil dose in the cell level. The transfer of bepridil from the apical to the basolateral compartment appears quantitatively and kinetically different using DMEMc or HBSS medium. The retention of the compound in the 'filter with Caco-2 cells' compartment is higher in DMEMc medium (60% at 3 μg) than in HBSS medium (46% at 3 μg), and bepridil entering the basolateral compartment is delayed in the DMEMc medium. This study exhibits the importance of the selected medium on results and interpretation of data and the predominance of DMEMc to study the transport of lipophilic compounds highly retained in cells. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Caco-2; Media; Bepridil; Transepithelial electrical resistance; Transport; Uptake

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1. Introduction

Epithelial cells of the gastrointestinal tract are the predominant barrier to the absorption of drugs administered by oral route (Artursson and Magnusson, 1990; Artursson, 1993). Drugs can cross this barrier by two parallel routes: the paracellular route (across the intercellular spaces) and the transcellular route (across the cell membrane) (Wilson, 1990). The transcellular route is generally considered to be the most important pathway for drug absorption across the intestine. Indeed, the cell membrane occupies a surface area that is > 1000 times larger than those of the paracellular spaces (Pappenheimer, 1990). Only sufficiently lipophilic compounds are able to pass the cell membrane and are generally completely absorbed (Artursson and Magnusson, 1990).

The Caco-2 human colon carcinoma cell line is considered to be an appropriate in vitro model system for intestinal epithelium and is frequently used to investigate intestinal transport of drugs ingested by oral route (Boulenc, 1997; Stammati et al., 1997). Caco-2 cells are able to spontaneously differentiate in standard culture conditions, with structural and functional characteristics similar to those of the small intestine (Pinto et al., 1983; Hidalgo et al., 1989; Neutra and Louvard, 1989). In addition, they express phase I and II enzymatic activities (Hillgren et al., 1995; Boulenc, 1997). For transport studies, several categories of media have been used, including a simple salt solution composed of inorganic components and glucose, or the culture medium itself, closer to the in vivo conditions and constituted, besides the salt solution, of vitamins, amino acids and several proteins (Karlsson and Artursson, 1991; Chandler et al., 1993; Lehr and Lee, 1993; Noach et al., 1993; Thwaites et al., 1993; Hamilton et al., 1994; Wils et al., 1994; Tomita et al., 1995). Generally, no explanation is clearly given to support the selection of a particular medium in transport studies.

In this paper, the transepithelial transport of a compound across the Caco-2 cell monolayers was studied in two of the most commonly used experimental media with different chemical compositions: a salt solution (HBSS) and the culture medium (DMEMc). During the experimentation, cell monolayer integrity was checked using the transepithelial electrical resistance (TEER) measurement. A decrease in the TEER is known to be an early indicator of cell monolayer damage, being more sensitive than changes in cell morphology, yielding even information on the state of the tight junctions between adjacent epithelial cells (Welsh et al., 1985; Atisook and Madara, 1991; Sakai et al., 1994; Twiss et al., 1994; Pasternak and Miller, 1996; Werner et al., 1996). The studied compound was bepridil, a highly hydrophobic compound with anticalcic properties, in vivo known to easily bind to proteins (Albengres et al., 1982; Tillement et al., 1982). As the filter of the culture chamber used was recovered with collagen on the apical side, the bepridil transport, and particularly, the uptake, was for comparison also evaluated using filter without cells.

2. Materials and methods

2.1. Chemicals

Bepridil and the internal standard used for the assays were a kind gift from Riom Laboratories Cerm (Riom, France).

Methanol, sodium phosphate and *n*-hexane were purchased from Merck (Nogent sur Marne, France).

2.2. Culture materials

Dulbecco's modified Eagle medium (DMEM), Hank's buffer solution salt (HBSS) and non-essential amino acids (NEAA) were obtained from Gibco Laboratories (Cergy Pontoise, France). Foetal calf serum (FCS) was from Biochrom (Berlin, Germany), Penicillin (50 000 U/ml) and streptomycin (25 mg/ml) were obtained as a mixture from Biomerieux (Marcy l'Etoile, France). Sodium pyruvate, trypsin (1 × solution), EDTA (0.02% solution) were from Sigma Cell Culture (St Quentin Fallavier, France). Collagen-coated Transwell Col inserts with polyethyleneterephthalate membrane (surface area, 0.33 cm^2; pore size, 3 μm), T-75 cm^2 flasks and plates were obtained from Costar Corporation (Brumath, France).

The Millicell-ERS voltohmmeter and electrodes were purchased from Poly Labo (Strasbourg, France).

The Caco-2 cell line was a kind gift from Dr M. Smit, Organon (Oss, The Netherlands).

2.3. Cell culture

Caco-2 cells were routinely plated and grown in T-75 cm^2 tissue culture flasks, using DMEM supplemented with 11.5% heat-inactivated FCS, 1% NEAA, 1% sodium pyruvate and 1% penicillin–streptomycin mixture (complete medium, DMEMc). Cells were cultured in an atmosphere of 95% air and 5% CO_2 at 37°C. The medium was changed every 2 days.

For experiments, cells of passage 30–40 have been used. Cells were harvested with trypsin-EDTA and seeded at a density of 64 000 cells/cm^2 on collagen-coated Transwell cell culture inserts introduced in the 24 wells of plates. The culture medium (DMEMc) was added to the apical (200 µl) and to the basolateral (600 µl) chambers. The cells were fed every second day and allowed to grow and differentiate for up to 22 days before being used in drug transport experiments.

2.4. Bepridil transport studies

Stock Bepridil solutions (100 µg/ml) were prepared in DMEMc or HBSS and then diluted to give solutions of 50 and 25 µg/ml.

The integrity of cell monolayers was checked by measurement of the TEER. When the TEER was less than 200 Ω cm^2, filters were discarded. The medium was replaced on both sides of the monolayers by the experiment medium (HBSS or DMEMc). Filters (with and without cells) were incubated in 95% air, 5% CO_2 at 37°C during 15 min. The TEER of the monolayers was measured at that time and this measure was considered as the start of the study ($t = 0$). Then, 120 µl of the apical medium were replaced by 120 µl of the adequate bepridil solutions in order to give an initial quantity of bepridil of 3, 6 or 12 µg in the apical chambers. Filters were incubated in 95% air, 5% CO_2 at 37°C and experiments were carried out without stirring.

TEER was measured from 0.5 to 24 h. At protocol times 0.07, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8 and 24 h, the whole of apical and basolateral media were collected for further analysis. The filters, with or without cells, were transferred into glass tubes and 200 µl of methanol and 200 µl of distilled water were added. Samples were kept frozen at −20°C until analysis. For each collecting time, three monolayers and three filters without cells have been checked.

2.5. Analytical procedure

The quantitative analysis of bepridil was carried out by a gas chromatography/mass spectrometry (GC/MS) method, which was validated (linearity, precision and accuracy), and the limit of quantitation was of 0.01 µg Calibration curves have systematically been realised at various quantities of bepridil from 0.05 to 6 µg.

2.5.1. Extraction

Aliquots of the apical (100 µl) and basolateral (150 µl) media, as well as the MeOH/H_2O solution containing filters, were supplemented with the internal standard (0.5 µg) and with 100 µl of saturated aqueous disodium hydrogen phosphate. The mixture was extracted twice with 3 ml of *n*-hexane. The 10 ml glass tubes were mechanically shaken for 10 min and centrifuged at 1600 × *g*. The organic phases were taken up, transferred to a 10 ml glass tube and evaporated to dryness at room temperature using a Speed Vac Concentrator (Savant, France). The dry residue was dissolved in methanol before analysis by GC/MS.

2.5.2. Analysis by GC/MS

The gas chromatograph was a Hewlett-Packard HP 5890 instrument coupled to a mass detector (HP 5970 A) and equipped with a 15 m × 0.25 mm ID fused silica capillary column (RTX-5 Amine, Chrompack) (film thickness, 0.5 µm). The temperatures were set at 250°C for the injector, 280°C for the transfer line and 180°C for the MS source. The oven temperature was set at 120°C for 1 min and then programmed to 280°C at 25°C/min. Helium with a flow rate of 2 ml/min was used as the carrier gas. Ions used for quanti-

tation by selected ion monitoring (SIM) were at $m/z=170$ for bepridil and at $m/z=128$ for the internal standard..

2.6. Statistical analysis

Statistical comparisons were made using the Mann–Witney non parametric U-test, the student t test or analysis of variance (ANOVA) test. Values of $P<0.05$ were considered significant.

3. Results and discussion

All results are reported as the mean ± SD of the mean.

3.1. Media and bepridil effects on TEER time course

TEER is expressed as a percent of the TEER measured at zero time.

3.1.1. Media influence

The medium influence on TEER values was evaluated by using reference monolayer results (without bepridil) and is shown in Fig. 1. During the first 8 h, profiles are relatively similar in both media. The TEER of the reference monolayers tends to increase during the first hour and then slightly to decrease until 8 h. The first increase is probably due to a recovery of cells after the medium change and equilibration. The mean values of the TEER between 0.5 and 8 h in DMEMc and HBSS are, respectively, 93.2 ± 7.9% and 99.0 ± 11.9% (no significant difference (NS)). Yet, at 24 h, a difference between the TEER values for the two media become evident ($P<0.05$). In DMEMc, the mean TEER ($n=3$) is 88.8 ± 5.1%, whereas in HBSS, the TEER value is 17.5 ± 2.0%. In DMEMc, the 24-h value is close to the values obtained at 8 h (87.0 ± 6.0%, NS). On the contrary, using HBSS medium, the 24 h (17.5 ± 2.0%) and 8 h values (83.5 ± 15.0%) are quite different. To confirm these differences, an additional study was carried out using 38 monolayers for each medium and TEER was measured at 24 h. In DMEMc medium, values are in the range 74.3–119.8% and the mean value is 87.3 ± 7.4% (CV = 8.5%). Using HBSS medium, values are in the range 15.5–21.8% and the mean value is 18.3 ± 1.5% (CV = 8.2%). Differences between the two media are highly significant ($P<0.001$).

Therefore, cell integrity is at least maintained for 8 h following application of both media and

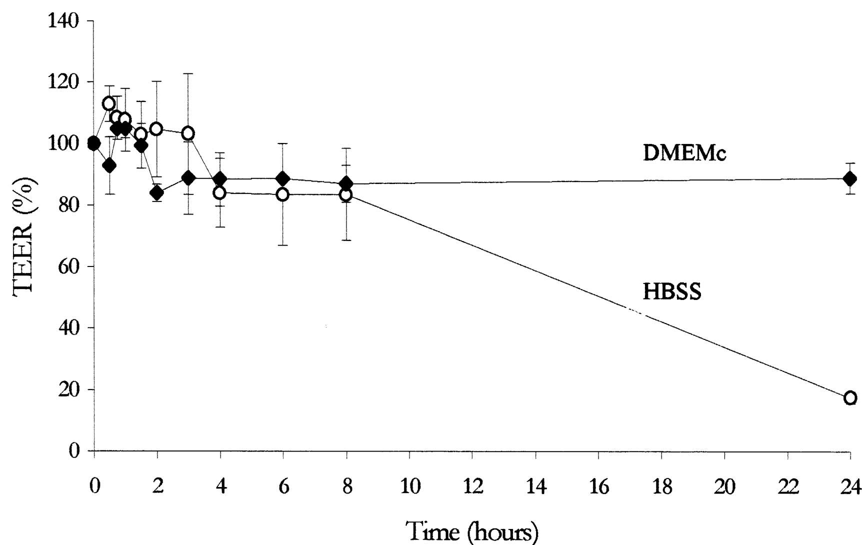

Fig. 1. Influence of both media (○, HBSS; ◆, DMEMc) on the TEER of reference monolayers.

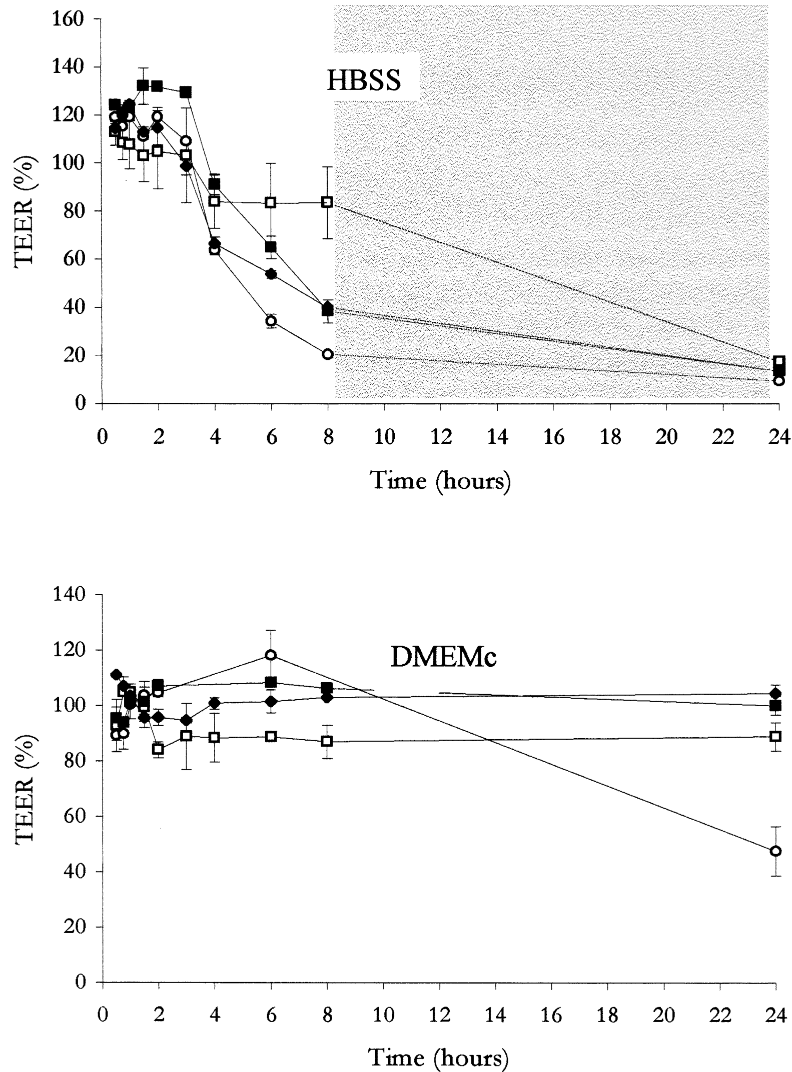

Fig. 2. Effect of different doses of bepridil on TEER using HBSS and DMEMc media: □, 0 μg (reference); ◆, 3 μg; ■, 6 μg; ○, 12 μg; ░, Alteration of cells in HBSS.

only DMEMc medium, rich in essential nutriments, could be used for 24 h.

3.1.2. Bepridil effect

During the first 3 h, in HBSS medium (Fig. 2a), the TEER slightly increases and remains above the TEER of reference monolayers for the three bepridil quantities used. After 3 h, TEER decreases more when the introduced quantity of bepridil is higher and the bepridil effect on the TEER appears to be dose-dependent. This corresponds to a toxic effect of the compound itself, the medium having no negative effect until 8 h. By using DMEMc medium (Fig. 2b), TEER profiles are similar for reference and treated monolayers and TEER values of treated monolayers remain above reference values. No toxic effect of the compound was found, except at 24 h for the highest 12 μg dose (50% of decrease). Therefore, between 3 and 8 h, compared with

Table 1
Kinetics of bepridil in the three compartments of the model, without Caco-2 cells using the HBSS medium

Time (h)	Quantity of bepridil recovered (μg) in the three compartments at different doses								
	12 μg			6 μg			3 μg		
	Apical	Basolateral	Membrane without cells	Apical	Basolateral	Membrane without cells	Apical	Basolateral	Membrane without cells
0.07	8.27	0.95	0.79	2.83	1.12	0.69	1.60	0.18	0.36
0.5	4.82	6.62	0.82	2.05	2.41	0.56	0.93	1.13	0.41
0.75	4.30	6.40	0.79	1.84	2.60	0.55	0.82	1.25	0.37
1	4.54	6.90	0.74	1.66	2.98	0.55	0.76	1.75	0.36
1.5	3.77	7.70	0.73	1.39	3.22	0.55	0.66	1.84	0.38
2	3.12	8.64	0.79	0.84	3.22	0.58	0.54	1.87	0.36
3	2.14	9.96	0.72	0.73	3.91	0.51	0.44	2.25	0.33
4	1.79	9.61	0.68	0.70	3.73	0.48	0.32	2.20	0.30
6	1.46	9.98	0.67	0.47	4.09	0.50	0.27	2.46	0.31
8	1.40	9.97	0.66	0.46	4.31	0.53	0.29	1.99	0.33
24	1.57	9.32	0.73	0.31	3.41	0.52	0.36	2.00	0.31

Table 2
Kinetics of bepridil in the three compartments of the model, without Caco-2 cells using the DMEMc medium[a]

Time (h)	Quantity of bepridil recovered (μg) in the three compartments at different doses								
	12 μg			6 μg			3 μg		
	Apical	Basolateral	Membrane without cells	Apical	Basolateral	Membrane without cells	Apical	Basolateral	Membrane without cells
0.07	8.09	1.25	0.72	4.79	0.04	0.25	2.03	0.05	0.26
0.5	5.08	5.40	1.19	2.98	1.42	0.56	1.42	0.59	0.42
0.75	4.10	6.61	1.26	2.41	2.21	0.79	1.32	0.70	0.41
1	4.13	8.14	1.30	2.31	2.69	0.82	1.38	0.83	0.35
1.5	4.15	6.25	1.49	2.69	2.30	0.59	1.14	1.01	0.41
2	3.24	6.72	1.33	2.59	2.48	0.61	0.99	1.21	0.39
3	ND	ND	ND	ND	ND	ND	0.91	1.33	0.38
4	ND	ND	ND	ND	ND	ND	0.74	1.57	0.39
6	2.05	8.65	1.51	1.70	4.37	0.56	0.65	1.71	0.30
8	ND	ND	ND	1.04	3.92	0.50	0.49	1.88	0.28
24	1.09	9.34	1.48	0.49	4.47	0.52	0.41	2.05	0.26

[a] ND, not determined.

Table 3
Kinetics of bepridil in the three compartments of the model, with Caco-2 cells using the HBSS medium

Time (h)	Quantity of bepridil recovered (μg) in the three compartments at different doses								
	12 μg			6 μg			3 μg		
	Apical	Basolateral	Membrane with cells	Apical	Basolateral	Membrane with cells	Apical	Basolateral	Membrane with cells
0.07	10.31	0.08	0.48	4.73	0.07	0.36	2.26	0.02	0.23
0.5	7.64	0.28	2.70	3.76	0.13	1.46	1.76	0.05	0.85
0.75	7.25	0.34	2.70	3.37	0.18	1.64	1.71	0.08	1.13
1	7.31	0.59	3.60	2.95	0.23	2.01	1.47	0.09	1.18
1.5	6.88	0.94	3.37	2.81	0.38	2.30	1.45	0.18	1.19
2	6.34	1.56	3.52	2.47	0.49	2.39	1.04	0.35	1.30
3	5.42	2.28	3.58	1.91	0.74	2.74	0.98	0.52	1.40
4	4.62	3.12	3.79	1.60	1.20	2.71	0.80	0.80	1.34
6	3.25	4.52	3.89	1.24	1.89	2.39	0.57	1.06	1.25
8	2.45	5.40	3.85	1.06	2.12	2.30	0.51	1.35	1.24
24	1.26	6.58	4.14	0.82	2.57	2.03	0.45	1.69	0.92

Table 4
Kinetics of bepridil in the three compartments of the model, with Caco-2 cells using the DMEMc medium[a]

Time (h)	Quantity of bepridil recovered (μg) in the three compartments at different doses								
	12 μg			6 μg			3 μg		
	Apical	Basolateral	Membrane with cells	Apical	Basolateral	Membrane with cells	Apical	Basolateral	Membrane with cells
0.07	9.53	0.05	0.34	5.22	0.02	0.31	2.35	<LOQ	0.12
0.5	7.21	0.10	2.42	3.68	0.05	1.43	2.04	0.01	0.58
0.75	7.02	0.13	2.66	3.72	0.06	1.54	1.72	0.02	0.91
1	6.18	0.16	3.05	3.54	0.08	1.45	1.52	0.04	1.18
1.5	6.17	0.26	3.24	3.25	0.14	2.07	1.37	0.09	1.46
2	6.60	0.29	3.25	3.19	0.18	2.48	1.09	0.14	1.49
3	ND	ND	ND	ND	ND	ND	0.89	0.24	1.61
4	ND	ND	ND	ND	ND	ND	0.77	0.31	1.81
6	3.56	1.50	5.21	1.51	0.84	3.64	0.61	0.48	1.67
8	ND	ND	ND	1.13	1.28	3.35	0.53	0.60	1.66
24	0.96	5.26	5.17	0.57	2.35	2.78	0.27	0.97	1.45

[a] ND, not determined (altered monolayers); LOQ, limit of quantitation.

HBSS, the DMEMc medium seems to protect cells from the toxicity of bepridil.

3.2. Distribution of bepridil between the three compartments of the model

Results, expressed in quantities (μg) of bepridil recovered in the three compartments of the transport model (i.e. apical, filter with or without cells, and basolateral), are reported in Tables 1 and 2 (without cells) and Tables 3 and 4 (with cells), and exhibit the importance of medium on results and interpretation of data.

3.2.1. Without Caco-2 cells

3.2.1.1. Filter compartment without cells. In the absence of cells, some binding of bepridil to filters was found. Nevertheless, if the phenomenon appears quite saturable in the HBSS medium (percent of binding at 3 μg, 11.6 ± 1.2%; at 6 μg, 9.1 ± 1.0%; at 12 μg, 6.2 ± 0.6%; and statistical

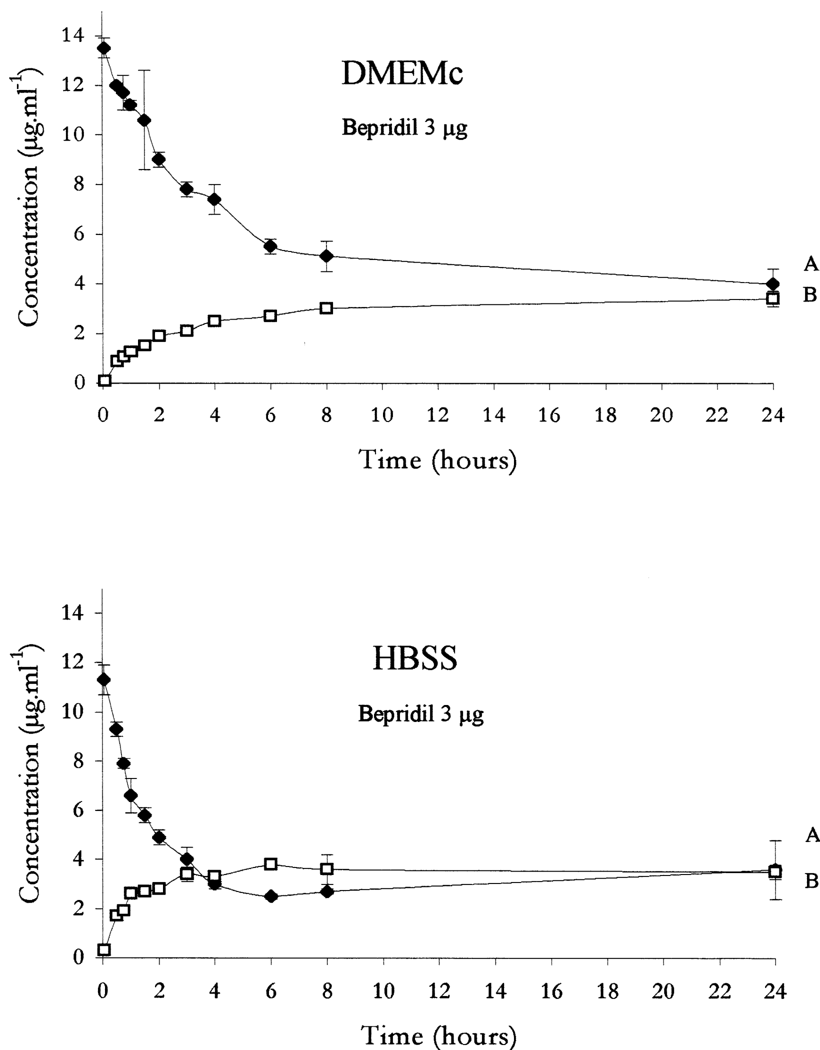

Fig. 3. Bepridil concentrations recovered in apical and basolateral compartments (filters without cells), using the DMEMc medium (a) and the HBSS medium (b) (bepridil test dose, 3 μg) (◆, apical level (A); □, basolateral level (B)).

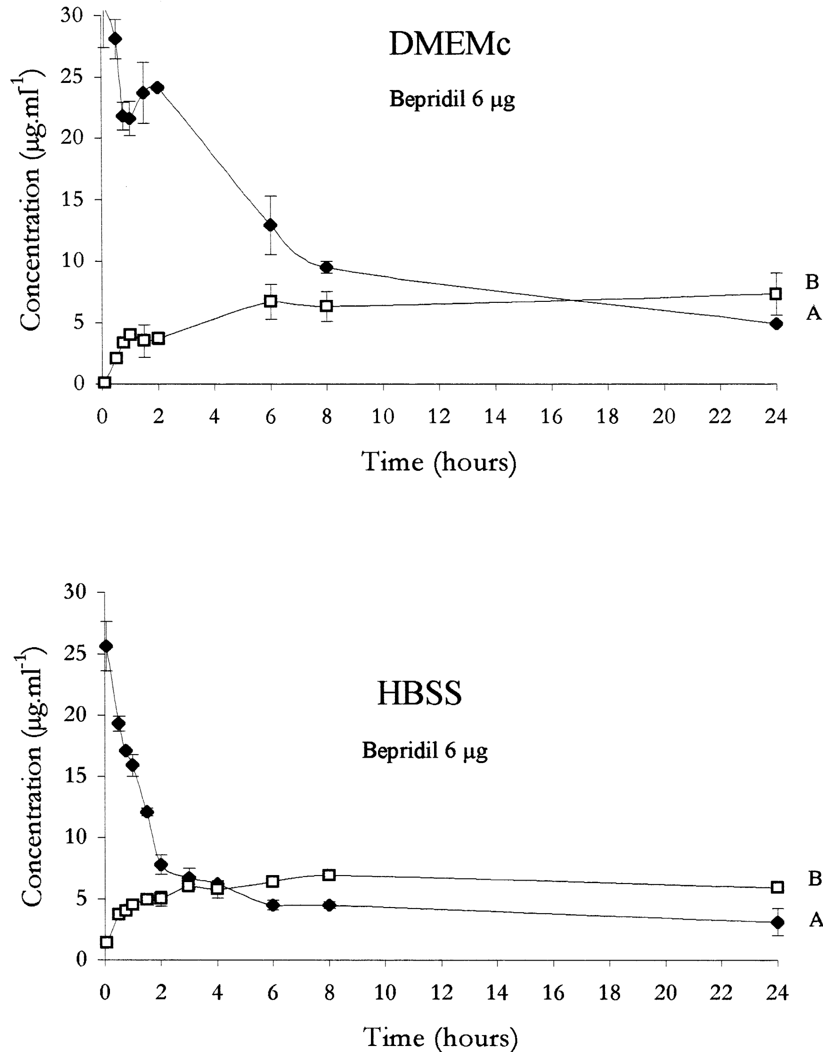

Fig. 4. Bepridil concentrations recovered in apical and basolateral compartments (filters without cells), using the DMEMc medium (a) and the HBSS medium (b) (bepridil test dose, 6 μg) (◆, apical level (A); □, basolateral level (B)).

comparison for 6–12 μg, $P < 0.001$), the binding to the filter remains constant in DMEMc, according to the level of the compound (3 μg, 11.7 ± 2.3%; 6 μg, 9.6 ± 2.8%; 12 μg, 10.3 ± 2.6%). As serum proteins have a high affinity for bepridil (Albengres et al., 1982), the foetal calf serum of the DMEMc medium may mask therefore the saturable effect observed in HBSS.

3.2.1.2. 'Apical' and 'basolateral' compartments. Results, expressed in concentrations (μg/ml) (Figs. 3 and 4), exhibit the medium effect on the bepridil distribution between compartments with time. The transport of bepridil across the filter barrier is more rapid in the HBSS medium than in DMEMc and the expected equilibrium between compartments is observed later in DMEMc (i.e. at 24 h (Fig. 3a and Fig. 4a) versus 6 h in HBSS (Fig. 3b and Fig. 4b)). Remarkably, the equilibrium appears to be a pseudo-equilibrium. Concentrations are higher in the basolateral compartment (B) than in the apical compartment (A) and this is

particularly evident in HBSS after 6 h (6 μg level, comparison A–B, $P < 0.01$; Fig. 4b) and delayed, in DMEMc at 24 h (6 μg level; Fig. 4a).

3.2.2. With Caco-2 cells

Profiles of the kinetics of bepridil in the three compartments with both media are shown in Fig. 5.

3.2.2.1. Filter compartment. Results, expressed in quantities of bepridil (HBSS (Table 3) and DMEMc (Table 4)), show that the quantities of bepridil bound on the filter compartment with cells first rapidly increase with time until about 3 h, to reach a plate value, and more especially for the lowest dose of bepridil (i.e. in HBSS, 46.0% of the 3 μg dose bound at 3 h, against 30% for the 12

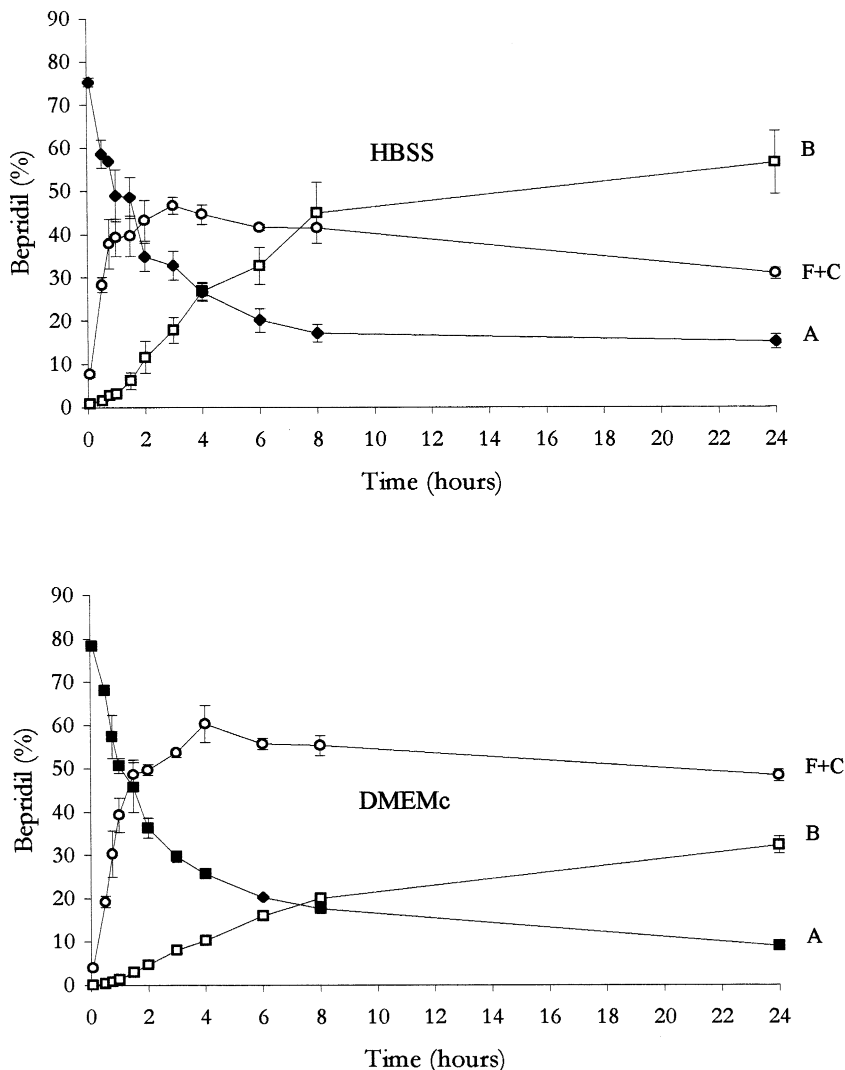

Fig. 5. Evolution of bepridil (%) in the three compartments of the model with Caco-2 cells, at the test dose of 3 μg (% of bepridil recovered,: ◆, in the apical compartiment (A); □, in the basolateral compartment (B); ○, in the filter with cells compartment (F + C)).

Table 5
Linear regression (percent of bepridil recovered in basolateral compartment versus time) from 0.5 to 8 h at different test doses in both media[a]

Bepridil test dose (μg)	Media	
	HBSS	DMEMc
3	$y = 6.04x - 1.33$ (0.98)	$y = 2.71x - 0.86$ (1.00)
6	$y = 5.36x - 1.65$ (0.99)[b]	$y = 2.73x - 1.45$ (0.99)
12	$y = 6.01x - 0.25$ (0.99)	$y = 2.18x - 0.80$ (0.99)[b]

[a] $y = ax + b$ (correlation coefficient).
[b] From 0.5 to 6 h.

μg dose). Moreover, the increase is quantitatively more important in DMEMc than in HBSS (i.e. 60% of the 3 μg dose bound at 4 h against 45%, respectively). After 6 h, the quantities of bound bepridil tend to decrease for the lowest dose (3 μg) in both media.

3.2.2.2. 'Apical' and 'basolateral' compartments. The medium effect, previously shown without drug, is patently found with bepridil. The high retention in the 'filter with cells' compartment delays bepridil entering the basolateral compartment. This delay is more important in DMEMc than in HBSS, probably due to additive binding of bepridil to medium proteins.

3.2.3. Rates of transfer

The kinetics of bepridil was followed in the three compartments of the model, and data, expressed in percent of bepridil with time, are reported in Fig. 5 for the test dose 3 μg (as an example). Experimental results exhibit asymptotic curves, contrary to what could be expected in first-order kinetics. The rate of bepridil transport into the basolateral compartment, in the absence of binding of bepridil to cells, is evaluated using the slope of the tangent to the curve at zero time. Yet, a linear regression equation ($y = ax + b$) can be obtained by plotting the percent of bepridil in the basolateral compartment versus time, from 0.5 to 6 or 8 h, with correlation coefficients above 0.98 (for the three doses of bepridil; Table 5). As the b (ordinate to zero) value is close to zero, the equation can be assimilated to $y = ax$ where a represents the rate of transport into the basolateral compartment (in percent of the initial dose per hour). In fact, as shown in Fig. 5, the relation (percent of bepridil with time) is not strictly linear. The kinetics probably results from two kinds of transport: a very rapid first step (free bepridil) followed by a quite slow one (bound bepridil). When the rate of transport shortened, the percent of bepridil in the basolateral compartment increases and tends to an asymptote value between 8 and 24 h. An absorption arising from an important binding on cell proteins of the intestine may be assessed, and this cannot be emphasized when carrying out in vivo studies.

The determination of area under the curve (percent of bepridil in the 'filter with cells' compartment versus time), using the trapezoidal rule, allowed us to calculate the mean percent of bepridil bound in the 'filter with cell' compartment per hour (32–38% in HBSS and 40–51% in DMEMc). A difference between HBSS and DMEMc profiles is once again observed. The initial transport is faster in HBSS (a around 6%/h) than in DMEMc (a around 2.7%/h). Proteins in DMEMc probably delay the transport of bepridil and the rate is therefore shortened by a factor of 2. The hypothesis of a higher affinity of bepridil for DMEMc proteins than for cell proteins can be done. Finally, the higher percentage of bepridil recovered in the 'filter with cells' compartment, using the DMEMc medium, might be related to an additive binding of medium proteins (which bind bepridil) to cells.

In conclusion, this study exhibits the importance of the selected medium on results and interpretation of data. Although both media allowed to maintain cell integrity for 8 h, the predominance of DMEMc was shown for 24-h transport studies of lipophilic compounds such as bepridil, highly retained in cells. The transfer of bepridil from the apical to the basolateral compartment appears quantitatively and kinetically different using HBSS and DMEMc, and this was related to the presence of proteins in the DMEMc medium.

With regard to the high retention of bepridil in Caco-2 cells, the unbinding of the compound, if it occurs, cannot be shown with this static model. A model including a periodic renewal of the basolateral medium, acting as pseudo elimination, is now under investigation.

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