BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **33**: 316–323 (2012)
Published online 30 August 2012 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/bdd.1802

Effect of pH, mucin and bovine serum on rifampicin permeability through Caco-2 cells

José Eduardo Gonçalves[a,*], Mariane Ballerini Fernandes[a], Chang Chiann[b], Maria Nella Gai[c], Jacqueline De Souza[d], and Sílvia Storpirtis[a]

[a]*Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil*
[b]*Mathematics and Statistics Institute, University of São Paulo, São Paulo, SP, Brazil*
[c]*Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile*
[d]*Faculty of Pharmacy, Federal University of Ouro Preto, Ouro Preto, MG, Brazil*

ABSTRACT: Rifampicin, a poorly soluble drug, has great importance in therapeutics as it is the main drug used to treat tuberculosis. The characterization of its permeability and the factors that influence it represent an important tool for predicting its bioavailability. Caco-2 cell monolayers were used as models of the intestinal mucosa to assess the uptake and transport of rifampicin and the effects of various experimental conditions were investigated, in order to establish the influence of these variables on rifampicin permeability. Different pHs (5.8, 6.8 and 7.4) in the apical medium, the presence or absence of mucin (3.0% w/v) in the donor site and the presence or absence of bovine serum albumin (4.0% v/v) in the receptor chamber were the evaluated conditions. The quantification of rifampicin in the apical or basolateral chambers was performed by a validated HPLC-UV method. The change in the donor chamber pH showed that permeability values were greater at pH 6.8, although this increase does not result in an alteration of the qualitative classification of rifampicin, which has high permeability. Mucin and bovine serum showed no effects on the permeability of rifampicin at the concentration tested. Overall, the current study suggests that pH, artificial mucin and bovine serum proteins have no influence on rifampicin permeability. Copyright © 2012 John Wiley & Sons, Ltd.

Key words: rifampicin; Caco-2 cells; intestinal permeability; absorption

Introduction

Rifampicin, the main drug used to treat tuberculosis (TB), is a potent semi-synthetic antibiotic of the family of rifamycins, produced by *Nocardia strains* (*Streptomyces*) mediterranei. It has broad bactericidal spectrum, being active against all the TB bacilli, including several strains of mycobacteria, and some Gram-positive and Gram-negative bacteria. This drug acts by inhibiting the DNA-dependent RNA polymerase by forming a stable complex with the enzyme, which suppresses the initiation of the bacterial RNA synthesis in intra- and extracellular bacilli in the division process or in a latent state [1–3].

Rifampicin is a poorly soluble drug that has been known to possess a variable bioavailability. There are several hypotheses to explain this variability, some of them being inherent to the drug or dosage form, such as interaction between rifampicin and other drugs, low solubility of the drug in aqueous media, its inadequate release from the dosage form, the amount of drug in formulation, lack of adherence by the patient, among others [4–7]. The variability in bioavailability can

*Correspondence to: Av. Prof. Lineu Prestes, 580, bl. 13, Cidade Universitária, 05508-900 São Paulo, SP, Brazil.
E-mail: jgoncalves@usp.br

Received 10 May 2012
Revised 29 June 2012
Accepted 12 July 2012

also be derived from the process of degradation and inactivation caused by the gastric juice [5,8] and the adsorption of the drug by the content of the gastrointestinal tract (GIT), such as mucus.

The characterization of rifampicin permeability and the factors that influence it represent an important tool for predicting its bioavailability. A number of *in vivo, in situ* and *in vitro* experimental methods have been developed to determine the drugs intestinal permeabilities, and, apart from clarifying the mechanisms of absorption, prediction and enhancement of oral drug absorption in humans is the ultimate goal of such studies. During the past decade, cultured epithelial cell lines, especially Caco-2 cells, have received considerable attention from pharmaceutical industries as a promising *in vitro* method to assess permeability [9–11].

Caco-2 cells monolayers have been accepted widely as a potent *in vitro* model membrane for the rapid screening of intestinal drug absorption. Under normal conditions of cell culture, Caco-2 cells differentiate spontaneously to mature cells to form monolayers. Although these cells originated from a human colon carcinoma, they acquire many features of absorptive intestinal cells during culture, such as microvillus structure, hydrolysis enzymes and carrier-mediated transport systems for sugars, amino acids and several drugs [12,13]. Also, adjacent cells adhere through tight-junctions formed at the apical side of the monolayer, which can discriminate the transcellular and paracellular transport of drugs across the epithelial layer [14].

Several reports have already shown the possibility of predicting the oral absorption of drugs in humans from their permeability through Caco-2 monolayers [15–18]. However, in these reports, several inconsistencies have been identified in experimental conditions such as pH, mucus layer around the cell in the donor chamber, the composition of medium on receptor chamber and ionic composition of the medium, shaking rate and the use of some co-solvents. In *in vitro* drug transport studies, these conditions may possibly have critical effects on the calculated permeability of the tested drugs.

Studies have indicated that drug interactions with mucin may limit the bioavailability of drugs being delivered via any mucosal surface by retarding their rate of membrane transport. Braybrooks *et al*. [19] observed a 50% decrease in the apparent permeability coefficients (P_{app}) for tetracycline in the presence of mucus. Kearney and Marriott [20] found an increase in lag-time as well as a decrease in the tetracycline transport rates using reverted gut experiments in the presence of mucus. Matthes *et al*. [21] found that the disappearance rate of compounds of various polarities through a diffusion chamber was reduced when the buffer solutions in a drug–buffer mixture were replaced with mucus solution.

Besides, permeability can be affected significantly by the pH of the buffer systems. The physiological intestinal pH in humans is around 6.5 in the upper intestine (duodenum and jejunum), while it is around 7.0–7.5 in the lower intestine (ileum and colon) [22]. The pH of the basolateral solution of Caco-2 monolayers corresponds to the pH of the interstitial fluid in the small intestine villi. Under physiological conditions, this region presents the same pH as the blood (pH 7.4), since extracellular fluids can be exchanged with the serum through the wall of the micro vessels. Therefore, to reflect the *in vivo* drug transport, different pH values should be applied for apical and basolateral media [23]. In previous reports, however, the neutral pH (pH 7.4) was often used for both apical and basolateral media to measure the permeability of passively transported drugs [15,24,25]. Since an acidic pH of the apical medium will affect the passive diffusion of drugs by changing the amount of drug that will be dissociated in the solution, inconsistency in experimental conditions can cause problems in comparing drug permeabilities reported by different laboratories. Additionally, in the case of active transport, some carriers work in a proton gradient-dependent manner, so, the employment of two different pH buffers in the apical and basolateral sides should be used [26]. It becomes important, therefore, to optimize the permeability assay conditions for each studied drug.

In this study, the effects of pH, the presence of mucin in the apical compartment and the presence of bovine serum albumin in the basolateral compartment were evaluated in the Caco-2 study in order to establish the influence of these variables on the rifampicin permeability.

DOI: 10.1002/bdd

Material and Methods

Chemicals

Rifampicin was kindly provided by Fundação para o Remédio Popular (FURP) (Guarulhos, SP, Brazil). Dulbecco's modified Eagle medium (DMEM), penicillin, streptomycin, trypsin–EDTA (0.2%), fetal calf serum (FCS) and Hanks balanced salt solution (HBSS) were obtained from Cultilab (Campinas, SP, Brazil). Glutamine, sodium bicarbonate ($NaHCO_3$), D-glucose, non-essential amino acids (NEAA), bovine serum albumin (BSA) and mucin from porcine stomach type III were purchased from Sigma–Aldrich (St Louis, MO, USA).

Cell line and culture conditions

The Caco-2 human colon adenocarcinoma cell line (ATCC #HTB-37) was purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Caco-2 cells were cultured in DMEM supplemented with 4.5 g/l of D-glucose, 2.2 g/l of $NaHCO_3$, 10% of FCS, 1% NEAA, 100 IU/ml of penicillin and 100 μg/ml of streptomycin in an atmosphere of 5% CO_2 and 90% relative humidity at 37 °C. All cells used in this study were between passages 40 and 50.

Permeability experiments

Briefly, Caco-2 cells were cultured in 12-well Transwell® insert filters for 21 days to reach confluence and differentiation at a seeding density of 5×10^4 cells/cm^2. The integrity of the monolayer was examined by measuring the transepithelial electrical resistance (TEER) with an epithelial voltammeter (Millicell-ERS®, Millipore Corporation, Bedford, USA) and running standard assays using fluorescein and metoprolol as paracellular and transcellular flux markers, respectively. Only cell monolayers with a TEER above 300 Ω × cm^2 were used for the transport assays.

The transport experiments were initiated with the addition in the apical compartments of the Transwell® plates of 10 mM rifampicin in HBSS buffer at pHs 5.8, 6.8 and 7.4, while the basolateral buffer was set at pH 7.4. In order to simulate the influence of mucus on the rifampicin permeability, mucin was added to the buffer, reaching 3.0% (w/v). The influence of BSA on the rifampicin permeability was evaluated adding this supplement to the basolateral compartment to obtain 4.0% (w/v) BSA in HBSS buffer.

The plates were kept under agitation in an orbital shaker at 37 °C (25 rpm). Samples were collected from the basolateral side at 30, 60, 90, 120 and 180 min and all aliquots were mixed with 20 μl of ascorbic acid solution 0.1% (w/v) in water to stabilize rifampicin.

Quantitative determinations of rifampicin were performed by HPLC on a Shimadzu LC-10A chromatographer. Chromatographic separations were obtained using a Synergil C18 column (150 mm 4.6 mm, 4 μm, Phenomenex®) at 40 °C. The mobile phase (1 ml/min) was composed of phosphate buffer 20 mM (pH 4.0) and acetonitrile (65:35 v/v). The analytical wavelength was set at 254 nm and samples of 50 μl were injected into the HPLC system.

The apparent permeability coefficients (P_{app}, cm/s) were calculated according to the following equation:

$$P_{app} = (VR \times \Delta Q/\Delta t)/(A \times C_0)$$

where VR is the volume of the receiver compartment (basolateral or apical), $\Delta Q/\Delta t$ is the linear appearance rate of the compound on the receiver chamber (in ng/s), A is the membrane surface area (cm^2) and C_0 is the initial concentration in the donor compartment (ng/cm^3). This calculation requires that the sink conditions are fulfilled, therefore, only receiver concentrations below 10% of the donor concentration were employed in the calculations. Mass balance was calculated as the amount of drug recovery in receiver samples after each interval and in the donor chamber at the end of the experiment divided by the amount of drug in the donor chamber at the beginning of the experiment.

Statistics

The results are expressed as mean ± standard deviation (SD) of three independent experiments. The differences between mean values were analysed with unpaired or paired two-tailed Student's t-test as appropriate. Comparisons between more than three groups were performed by using one-way and two-way ANOVA with IC 95%. Statistical analyses were carried out using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA). The significance level was set at $p < 0.05$.

DOI: 10.1002/bdd

Results

Effects of pH on rifampicin permeability

The P_{app} values of rifampicin measured under three apical pH conditions are summarized in Figure 1. The results suggest that the P_{app} value increased at pH 6.8 compared with the other pHs.

Effects of mucin on rifampicin permeability

Figure 2 summarizes the effects of mucin at three different apical pHs on rifampicin P_{app} through the Caco-2 monolayer. The addition of mucin (3% w/v) to the apical medium caused no significant effect on the rifampicin permeability or to the TEER values.

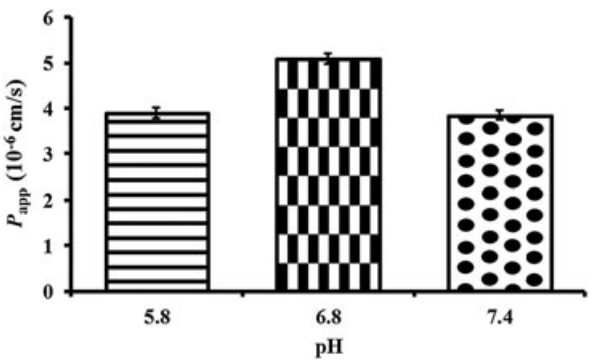

Figure 1. Evaluation of the apical pH effect on the rifampicin permeability through Caco-2 cells. Only pH 6.8 had p values < 0.05 ($n = 6$)

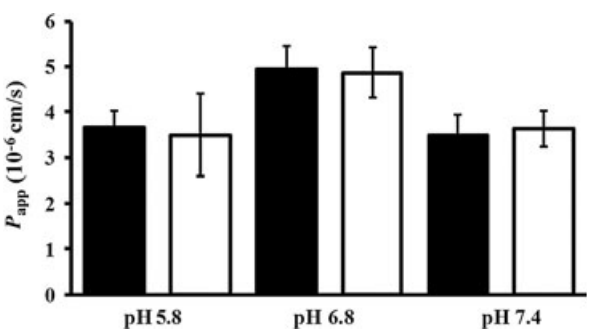

Figure 2. Evaluation of the mucin 3% (w/v) effect at three different apical pHs on the rifampicin permeability through Caco-2 cells. Black columns refer to experiments without mucin and white blocks refer to experiments with mucin. All p values are >0.05 ($n = 6$)

Effects of bovine serum albumin on permeability of rifampicin

In Figure 3, the effects of BSA at three different apical pHs on the rifampicin P_{app} through the Caco-2 monolayer are summarized. The addition of BSA (4% w/v) to the basolateral medium caused neither a significant effect on the rifampicin permeability nor to the TEER values.

Influence of simultaneous BSA and mucin in the permeability of rifampicin

The influence of the simultaneous addition of 3% (w/v) porcine mucin in the apical compartment and the addition of 4% (w/v) BSA in the permeability experiments medium was evaluated by comparison of the data obtained with and without the use of these adjuvants at three different apical pHs. The results are presented in Figure 4.

The analysis of variance (ANOVA) considering two factors (medium composition and pH), followed by the analysis of the factors that resulted in differences for the determination of confidence intervals (95%) are presented in Tables 1 and 2.

The results suggest that the medium composition and the pH together showed no significant changes in the results on rifampicin permeability.

Discussion

The pH in the GIT, which varies from 6.5 in the upper intestine to around 7.0–7.5 in the lower intestine, is one of the most important factors

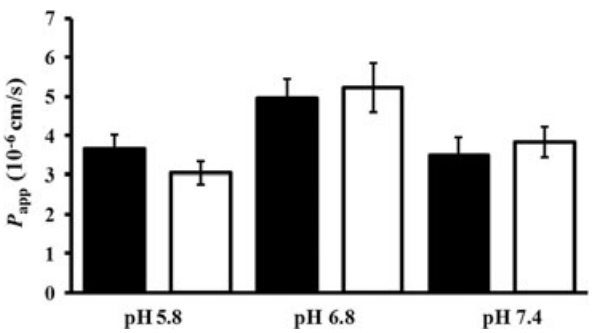

Figure 3. Evaluation of the BSA 4% (w/v) effect at three different apical pHs on the rifampicin permeability through Caco-2 cells. Black columns refer to experiments without BSA and white columns refer to experiments with BSA. All p values are >0.05 ($n = 6$)

DOI: 10.1002/bdd

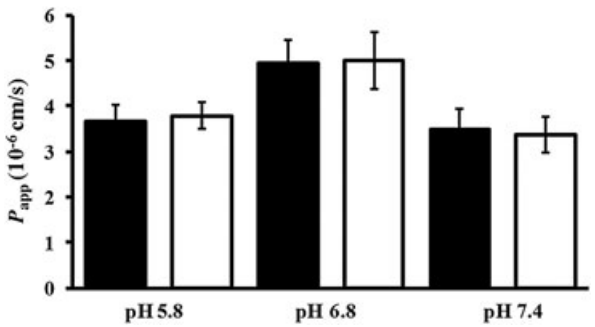

Figure 4. Evaluation of the simultaneous mucin 3% (w/v) and BSA 4% (w/v) effect at three different apical pHs on the rifampicin permeability through Caco-2 cells. Black columns refer to experiments without mucin and BSA and white columns refer to experiments with mucin and BSA. All p values are >0.05 ($n = 6$)

Table 1. Two factor ANOVA for the values of rifampicin permeability for both the pH and composition of the medium conditions

	DF	SS	MS	F-test	p
Factor 1 (f1) medium composition	3	0.0496	0.0165	0.05	0.984
Factor 2 (f2) pH	2	20.5406	10.2703	32.03	0.000[a]
f1 × f2	6	1.3137	0.2190	0.68	0.665
Error	33	10.5823	0.3207		
Total	44				

DF, degrees of freedom; SS, sum of squares; MS, mean square.
[a]$p < 0.05$.

Table 2. Confidence interval (CI) 95% of the rifampicin permeability values considering the parameter pH

pH	Difference (%)	CI
6.8–5.8	+ 26.1[a]	(0.9923)–(1.9397)
7.4–5.8	−4.0	(−0.4930)–(0.4544)
7.4–6.8	−29.1[a]	(−1.9590)–(−1.0116)

[a]Significant difference $p < 0.05$.

affecting drug absorption [22]. Under physiological conditions, an acid microclimate is found just above the epithelial cell layer in the upper part of the small intestine, acting as a driving force of the active drug transport. This acid microclimate is maintained by secretion of hydrogen ions from epithelial cells and is restricted by the mucus layer [24]. Caco-2 cells, in turn, do not produce this mucus layer, and, in contrast to what occurs *in vivo*, the apical solution is in direct contact with the cell surface. The pH value of the apical medium, thus, could have a critical effect on the membrane transport of drugs and consequently in the prediction of their permeabilities.

This study attempted to investigate the effect of pH on the transport rate of rifampicin. The pHs 5.8, 6.8 and 7.4 were selected for the apical side and pH 7.4 was fixed for the basolateral side. The results suggest that the P_{app} value increased when a pH of 6.8 was used in the apical side. Rifampicin has amphoteric characteristics with a pKa of 1.7 related to the 4-hydroxyl group and a pKa of 7.9 related to the nitrogen of the piperazine group. In aqueous solution, its isoelectric point is equal to 4.8 [27]. It is slightly soluble in water and solubility and stability vary with the pH because of its amphoteric nature. At pH 2, the solubility is equal to 100 mg/ml, at pH 5.3 it is reduced to 4.0 mg/ml and at pH 7.5 it is equal to 2.8 mg/ml [28]. The distribution coefficient (log D) of rifampicin, that is, the ratio of the sum of the concentrations of all forms of the compound (ionized and unionized) in a mixture of two immiscible solvents at equilibrium, normally water and octanol, is higher at pH 6.8, while it is lower at pH 5.8 or 7.4. Therefore, these values are in accordance with the P_{app} results observed.

However, all the results obtained indicate that rifampicin is a high permeable drug, according to Biganzoli *et al*. (1999) [29] classification, which states that P_{app} values $> 2.0 \times 10^{-6}$ cm/s are related to high absorption antibiotics (bioavailability 90%). These results are consistent with the classification made by WHO (2006), in which rifampicin is indicated as a highly permeable drug. The Biganzoli classification was employed in our laboratory after the performance of Caco-2 assays with some antibiotics such as nitrofurantoin, which has high permeability, and amoxicillin, which has low *in vitro* permeability, in which the cut-off values were similar to theirs.

Although a statistical difference in the P_{app} value at pH 6.8 was observed, it could be considered not significant in terms of the qualitative classification of the rifampicin permeability. On the other hand, the information regarding the increase of rifampicin at pH 6.8 can be used as a foundation for the development of a modified release drug product, favouring its release from the dosage form in the GIT portion that allows a greater absorption and a concomitantly lower risk of acid degradation of the drug.

DOI: 10.1002/bdd

The use of simulated mucus (mucin) and BSA in the apical and basolateral compartments, respectively, are strategies used to optimize the permeability determination of lipophilic drugs, because they can avoid the adsorption of the drug to the plastic material that constitutes the plates, the adsorption to the filters of the growth support and the drug accumulation inside the cells [23,30,31]. Mucin, however, can reduce the amount of free drug available for absorption.

The results revealed no significant differences between the P_{app} values of rifampicin in experiments with and without the addition of mucin in the apical compartment, as in the experiments with and without the addition of BSA in the receiver chamber, despite the fact that rifampicin is considered a drug with high lipophilicity. Furthermore, the ANOVA results demonstrated that the medium composition did not lead to significant differences in the values of the apparent permeability of rifampicin. Additionally, the results obtained in the two factor ANOVA (medium composition and pH) showed that these two experimental conditons together may not cause changes in the rifampicin permeability results. As already reported, the effect of albumin on the basolateral side in Caco-2 transport experiments could be different depending on the unbound fraction in the plasma, the drug permeability and the transport mechanism [32]. For drugs highly bound to plasma proteins, such as rifampicin (approximately 80% of binding) [33], the presence of BSA increases the transport rate in the absorptive direction and decreases it in the secretory direction. However, if the drug is apically effluxed, the lowering of the initial donor concentration by protein binding will lead to an increase in secretory transport and a decrease in absorptive transport to an extent related to the permeability-concentration dependency for the compound. So, the lack of difference between the P_{app} values from the assays with and without the BSA for rifampicin at the concentration evaluated could be due to a compensation factor of the P-gp efflux transport, which could neutralize the BSA effect of increasing the transport rate in the absorptive direction.

The outcomes of the evaluation of the presence or absence of simulated mucus and serum in the apical and basolateral portions, respectively, show that the use of these adjuvants is not justified when conducting permeability experiments for rifampicin at the concentration evaluated. Furthermore, they represent a source of interference in the quantification method, requiring the prior treatment of the samples, which would increase the assay's costs and would require more sensible analytical methods for quantification.

Conclusion

In this report, apart from the cell culture conditions which might affect the permeability of cellular monolayer itself, the study indicated the importance of experimental conditions for rifampicin transport study through Caco-2 cells to obtain a satisfactory prediction of *in vivo* oral absorption. This concept indicates that the experimental conditions for *in vitro* studies should reflect physiological conditions *in vivo*, however, it is also true that complex conditions can be difficult and time consuming, and, consequently, it is important to choose appropriate conditions according to the purpose of the study as well as to the physicochemical properties of the drugs to be tested. In the rifampicin case, the employment of different pH values for apical and basolateral media, mucin in the apical compartment and serum protein in the basolateral chamber in these assays brings no advantages and increases the complexicity of the assays, their use being dispensable.

Acknowledgements

This work was supported by CNPq 402422/2005-8 - Ed 542005.

Conflict of Interest

There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

1. Zhang Y. The magic bullets and tuberculosis drug targets. *Annu Rev Pharmacol Toxicol* 2005; **45**: 529–564.
2. Bacchi A, Pelizzi G, Nebuloni M, Ferrari P. Comprehensive study on structure-activity relationships

of rifamycins: Discussion of molecular and crystal structure and spectroscopic and spectroscopic and thermochemical properties of rifamycin O. *J Med Chem* 2008; **41**: 2319–2332.
3. Gumbo T. Chemotherapy of tuberculosis, mycobacterium avium complex disease, and leprosy. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 12th edn, Brunton L, Chabner B, Knollman B (eds). McGraw-Hill: New York, 2011; 1549–1570.
4. Shishoo CJ, Shah SA, Rathod IS, Savale SS, Vora MJ. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. *Int J Pharm* 2001; **228**: 53–67.
5. Singh S, Mariappan TT, Sankar R, Sarda N, Singh B. A critical review of probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. *Int J Pharm* 2001; **228**: 5–17.
6. Agrawal S, Ashokraj Y, Bharatam PV, Pillai O, Panchagnula R. Solid state characterization of rifampicin samples and its biopharmaceutic relevance. *Eur J Pharm Sci* 2004; **22**: 127–144.
7. Panchagnula R, Bedi S, Yasvanth A. Rifampicin: polymorphism or crystal defects. *Clin Res Regul Aff* 2006; **23**: 71–83.
8. Shishoo CJ, Shah SA, Rathod IS, Savale SS, Kotecha JS, Shah PB. Stability of rifampicin in the dissolution medium in presence of isoniazid. *Int J Pharmacol* 1999; **190**: 109–123.
9. Souza J, Freitas ZMF, Storpirtis S. Modelos *in vitro* para determinação da absorção de fármacos e previsão da relação dissolução/absorção. *Braz J Pharm Sci* 2007; **43**: 515–527.
10. Souza J, Benet LZ, Huang Y, Storpirtis S. Comparison of bidirectional lamivudine and zidovudine transport using MDCK, MDCK–MDR1, and Caco-2 Cell monolayers. *J Pharm Sci* 2009; **98**: 4413–4419.
11. Gonçalves JE, Souza J, Storpirtis S. Avaliação da permeabilidade de fármacos empregando culturas celulares. In *Biofarmacotécnica - Série Ciências Farmacêuticas*. Storpirtis S, *et al*. (eds). Guanabara Koogan: Rio de Janeiro, 2009.
12. Hidalgo IH. Cultured intestinal epithelial cell models. In *Models or Assessing Drug Absorption and Metabolism*, Borchardt T, Smith PL, Wilson G (eds). Plenum Press: New York, 1996; 35–50.
13. Hilgers AR, Conradi RA, Burton PS. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm Res* 1990; **7**: 902–910.
14. Tanaka Y, Taki Y, Sakane T, *et al*. Characterization of drug transport through tight-junctional pathway in Caco-2 monolayer: comparison with isolated rat jejunum and colon. *Pharm Res* 1995; **12**: 523–528. doi:10.1023/A:1016245711557.
15. Artursson P, Karlsson J. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem Biophys Res Commun* 1991; **175**: 880–885.
16. Rubas W, Jezyk N, Grass GM. Comparison of the permeability characteristics of a human colonic epithelial (Caco-2) cell line to colon of rabbit, monkey, and dog intestine and human drug absorption. *Pharm Res* 1993; **10**: 113–118.
17. Stewart BH, Chan OH, Lu RH, *et al*. Comparison of intestinal permeabilities determined in multipole *in vitro* and *in situ* models: Relationship to absorption in humans. *Pharm Res* 1995; **12**: 693–699.
18. Chong S, Dando SA, Soucek KM, Morrison RA. *In vitro* permeability through Caco-2 cells is not quantitatively predictive of *in vivo* absorption for peptide-like drugs absorbed via the dipeptide transporter system. *Pharm Res* 1996; **13**: 120–123.
19. Braybrooks MP, Barry BW, Abbs E. The effect of mucin on bioavailability of tetracycline from the gastro-intestinal tract: *in vitro in vivo* correlations. *J Pharm Pharmacol* 1974; **27**: 508–515.
20. Kearney P, Marriott C. The effect of mucus glycoproteins on the bioavailability of tetracycline: II Binding. *Int J Pharm* 1987; **35**: 211–217.
21. Matthes I, Nimmerfall F, Sucker H. Mucusmodelle zur untersunchung von intestinalen absorptions mechanismen. *Pharmazie* 1992; **47**: 609–613.
22. Horter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv Drug Deliv Rev* 2001; **46**: 75–83.
23. Yamashita S, Furubayashi T, Kataoka M, Sakane T, Sezaki H, Tokuda H. Optimized conditions for prediction of intestinal drug permeability using Caco-2 cell. *Eur J Pharm Sci* 2000; **10**: 195–204.
24. Shiah YF, Fernandez P, Jackson MJ, Mcmonagle S. Mechanism maintaining a low-pH microclimate in the intestine. *Physiology* 1985; **248**: 608–617.
25. Rubas W, Jezyk N, Grass GM. Comparison of the permeability characteristic of a human colonic epithelial (Caco-2) cell line to colon of rabbit, monkey, and dog intestine and human drug absorption. *Pharm Res* 1993; **10**: 113–118.
26. Matsumoto S, Saito H, Inui K. Transcellular transport of oral cephalosporins in human intestinal epithelial cells, Caco-2: Interaction with dipeptide transport systems in apical and basolateral membranes. *J Pharmacol Exp Ther* 1994; **270**: 498–504.
27. Maggi N, Pasqualucci CR, Ballotta R, Sensi P. Rifampicin: a new orally active rifamycin. *Chemotherapy* 1966; **11**: 285–292.
28. Gallo GG, Radaelli P. Rifampicin. In *Analytical Profiles of Drug Substances*, Florey K (ed.). Academic Press: London, 1976.
29. Biganzoli E, Cavenaghi LA, Rossi R, Brunati MC, Nolli ML. Use of a Caco-2 cell culture model for

the characterization of intestinal absorption of antibiotics. *Farmaco* 1999; **5**: 594–599.
30. Krishna G, Chen KJ, Lin CC, Nomeir A. Permeability of lipophilic compounds in drug discovery using *in-vitro* human absorption model, Caco-2. *Int J Pharm* 2001; **222**: 77–89.
31. Fossati L, Dechaume R, Hardillier E, *et al*. Use of simulated intestinal fluid for Caco-2 permeability assay of lipophilic drugs. *Int J Pharm* 2008; **360**: 148–155.
32. Neuhoff S, Artursson P, Zamora I, Ungell A-L. Impact of extracellular protein binding on passive and active drug transport across Caco-2 cells. *Pharm Res* 2006; **23**: 350–359.
33. Acocella G. Clinical pharmacokinetics of rifampicin. *Clin Pharmacokinet* 1978; **3**: 108–127.

DOI: 10.1002/bdd