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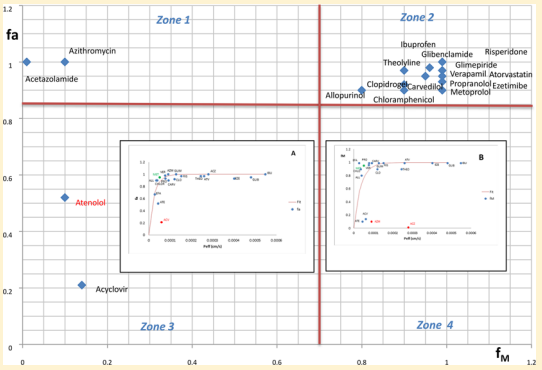
Comparative Oral Drug Classification Systems: Acetazolamide, Azithromycin, Clopidogrel, and Efavirenz Case Studies

Maria Julia Mora, R. Onnainty, and Gladys Ester Granero*

Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA), CONICET and Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, X5000-HUA Córdoba, Argentina

ABSTRACT: Biopharmaceutics classification systems based on the properties of solubility and permeability or the extension of metabolism are very important tools in the early stages of the development and regulatory stages of new products. However, until now, there was no clear understanding between the interplay among these classification systems. Therefore, the main objective of this work was to make a comparison of concepts of BCS and BDDCS to understand what are the key factors that allow for the integration of these biopharmaceutics classification systems. Also, the suitability of an in situ single-pass intestinal perfusion assay in rats (SPIP) development was assessed by us to determine the limit between high and low permeability following what the FDA BCS guidance suggests. An excellent correlation was found between the values of permeability obtained by applying SPIP assays and the extensions of the metabolism of the set of compounds studied in this work, with the exception of three compounds that showed disparity between their permeability coefficients (P_{eff}), obtained herein by SPIP, and their metabolism (acetazolamide, azithromycin, and efavirenz). Discrepancies allowed us to elucidate the interrelationship between BCS and BDDCS.



KEYWORDS: *biopharmaceutics classification system (BCS), biopharmaceutics drug disposition classification system (BDDCS), acetazolamide, azithromycin, clopidogrel, efavirenz*

INTRODUCTION

The bioavailability (F) of a drug dose orally administered is obtained by individual drug fractions that survive the different barriers they have to overcome from the gut lumen until reaching the systemic circulation.[1] Within the many factors affecting the drug oral bioavailability are those related to the course of the drug issues through the gut wall because the extent of the drug absorption is governed, among other issues, by the drug effective permeability (P_{eff}) across the intestinal mucosa.[2]

In order to predict the in vivo pharmacokinetic behavior of a drug dosage form, Amidon et al. proposed the biopharmaceutics classification system (BCS), which is supposed to predict the in vivo oral absorption for the disposition of drugs based on the permeability and solubility parameters, categorizing them into four classes.[3] The BCS is considered a valuable tool during the development of a new medicine and in helping to make decisions from a regulatory point of view because this classification system enables waivers in in vivo bioavailability (BA)/bioequivalence (BE) testing on the basis of in vitro dissolution assays for IR drugs Class 1 and 3.[4]

In the same line of work, Wu and Benet established criteria of the solubility and extent of metabolism classified drugs into four groups. The authors observed that highly permeable compounds, Class 1 and Class 2 according to the BCS, are usually eliminated mainly by metabolism, while poorly permeable compounds, Class 3 and Class 4 according to the BCS, are eliminated primarily as an unchanged drug by renal and biliary excretion.[5] On the basis of these observations, the authors proposed the biopharmaceutics drug disposition classification system (BDDCS), considering their aqueous solubility and extent of metabolism instead of considering the drug oral permeability.[6]

The differences between both classification systems (BCS and BDDCS) lie fundamentally in the parameters that allow the drug classification (solubility and extent of intestinal permeability (BCS) or extent of metabolism (BDDCS)). The BCS postulated that the extent of the drug absorption could be predicted by determining drug intestinal permeability, because drug absorption across the gastrointestinal tract (GIT) correlates well with the drug P_{eff} rate, accounting that the extensive drug oral absorption (≥85%) is indicative of high drug membrane permeability across the intestinal epithelium.

The P_{eff} of a drug can be determined directly by measuring the amount of drug that crosses the intestinal epithelium by

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Table 1. Chromatographic Conditions for the Drugs Assayed

drug	flow (mL/min)	mobile phase	injection volume (μL)	λ (nm)	temperature (°C)	t_R (min)
ACZ	1.0	AcN/MeOH/water (3:2:95)	50	265	40	10.72
acyclovir	1.0	AcN/MeOH/water (3:2:95)	50	254	35	4.39
allopurinol	1.0	AcN/MeOH/water (3:2:95)	50	254	40	4.56
atenolol	1.0	AcN/MeOH/KH_2PO_4 (7.5:7.5:85)	50	230	25	5.51
atorvastatin	0.5	phosphoric acid 0.1%/ACN	30	238	25	6.87
AZM	1.0	phosphate buffer/MeOH (20:80)	20	210	30	7.37
carvedilol	1.0	phosphate buffer/ACN	30	240	50	4.28
chloramphenicol	1.0	MeOH/water (65:35)	50	280	25	4.07
clopidrogel	1.0	phosphate buffer/AcN (35:65)	30	254	25	6.50
EFA	1.0	NH_4COOCH_3/AcN (40:60)	30	252	30	5.17
ezetimibe	0.8	phosphate buffer ()	30	232	30	4.55
glibenclamide	1.0	AcN/water (53:47)	30	231	30	4.80
glimepiride	1.0	AcN/water (53:47)	30	231	30	7.05
ibuprophen	1.2	AcN/MeOH/KH_2PO_4 (7.5:7.5:85)	50	222	25	17.25
metoprolol	1.0	AcN/KH_2PO_4 (25:75)	50	230	40	5.43
propranolol	1.0	NH_4COOCH_3/AcN (30:70)	50	230	40	5.45
risperidone	1.0	phosphate buffer/AcN/MeOH (55:20:25)	30	276	30	4.88
theophiline	1.0	MeOH/water (50:50)	50	270	25	
verapamil	1.0	NH_4COOCH_3/AcN (30:70)	50	230	40	7.31

using the in situ method of single-pass intestinal perfusion (SPIP) in animal models, with rat being the most used because good correlations were observed between the P_{eff} determined in rats by this methodology and the human oral fraction of dose absorbed (f_a), especially for compounds absorbed by a passive mechanism across the gut wall.[7] However, for BCS classification proposes, the US Food and Drug Administration (FDA) guidance for industry recommends to establish the suitability of in vitro permeability or in situ animal perfusion methods using a set of reference drugs.[8]

The BDDCS assumes that the extent of the drug metabolism may predict high (≥70% of extent of metabolism) versus low (drug overall extent of metabolism ≤30%) intestinal permeability, and the extent of the metabolism of drugs may be used as a surrogate for the intestinal membrane permeability determination and as an alternative to foretell the drug disposition after its oral administration.[5,6] Also, it was proposed by Amidon et al., to the regulatory agencies, to consider the extent of drug metabolism as an alternative method for classifying those drugs as Class 1 (BCS) with an extent of metabolism ≥90%, being that these drug candidates waiver of in vivo studies of bioequivalence.

Herein, this work was undertaken to compare the concepts of BCS and BDDCS to investigate the oral drug disposition predictive ability of the two classification systems by using a set of compounds with different absorption characteristics across the TGI. Also, we assessed the suitability of the SPIP method development by us in rats to establish the permeability classification in accord with the FDA BCS guidance, and finally, to apply the concepts of the BCS and BDDCS systems in some drug case studies.

MATERIALS AND METHODS

Chemicals. The model drugs used were acetazolamide (ACZ) and metoprolol (Sigma 99%, USA), acyclovir (provided by Elea Argentina), allopurinol, atenolol, atorvastatin, azithromycin (AZM), carvedilol, chloramphenicol, clopidogrel (CLO), efavirenz (EFA), ezetimibe, glibenclamide, glimepiride, ibuprofen, propranolol, risperidone, theophylline, and verapamil (purchased from Parafarm, Buenos Aires, Argentina). The HPLC solvents methanol and acetonitrile were purchased from (Sintorgan, Buenos Aires, Argentina, and J.T. Baker, Mexico) and were all HPLC grade. Chemicals and solvents were of analytical grade, and water was generated by a Millipore Milli-Q water purification system.

Data Sets. Pharmacokinetic and oral absorption data of the of the set of drugs investigated in this work were compiled from published human studies.

SPIP Studies in Rats. *Solutions.* The perfusion buffer solution was composed of 20.1 mM $Na_2HPO_4{\cdot}12H_2O$, 47.0 mM KH_2PO_4, and 101.0 mM NaCl (PBS) with pH 6.3 ± 0.1 at 37 °C. Drug-perfused solutions were prepared by dissolving each drug in PBS pH 6.3 or in DMSO/PBS pH 6.3 (being the final concentration of the organic solvent in the buffer solution less than 0.5% (v/v)), and they were placed in a water bath at 37 °C. Because a volume of 250 mL was taken to obtain the highest concentration that a drug could reach in the intestinal lumen, drug-perfusion solutions were elaborated by dissolving the highest dose strength in 250 mL of the perfusion buffer solution, except for acyclovir, allopurinol, carvedilol, ibuprofen, metoprolol, propranolol, risperidone, theophylline, and verapamil, due to their low solubility in the perfusion medium, which was why the following marketed doses were considered: acyclovir 200, allopurinol 100, carvedilol 25, ibuprofen 200, metoprolol 100, propranolol 40, risperidone 2, theophylline 100, and verapamil 80 mg. Thus, the final drug concentrations were ACZ 966, acyclovir 787, allopurinol 402, atenolol 403, atorvastatin 166, AZM 840, carvedilol 166, chloramphenicol 1016, CLO 11, EFA 2382, ezetimibe 0.11, ibuprofen 802, giblenclamide 20, glimepiride 0.72, metoprolol 396, propranolol 159, risperidone 8.59, theophiline 400, and verapamil 318 μg/mL.

Perfusion Experiments. The SPIP experimental protocol used in this study was the one previously published by us, which was approved by the Chemistry Faculty of the National University of Córdoba Animal Care Committee in accordance with the "Guide for the Care and Use of Laboratory Animals".[9]

The net water flux in the gut segment assayed was determined by applying the density corrected gravimetric

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Table 2. Physicochemical and Pharmacokinetics Parameters of the Tested Compounds

drug	f_a reported values	f_a estimated by applying eq 4	f_M reported values	P_{eff} ± DS (cm/s) experimental values obtained herein	Log $P_{o/w}$ reported values	Log $D_{7.4}$ reported values	pK_a reported values
ACZ	1.00	0.99	0.01	$(2.79 \pm 0.15) \times 10^{-4}$	−0.26[34]	−0.85[47]	7.2[34]
acyclovir	0.21	0.91	0.14	$(6.00 \pm 0.04) \times 10^{-5}$	−1.8[41]	−1.8[41]	2.23[51]
allopurinol	0.9	0.80	0.80	$(3.95 \pm 0.46) \times 10^{-5}$	−0.55[34]	0.1[48]	10.2[48]
atenolol	0.52	0.83	0.10	$(4.40 \pm 1.40) \times 10^{-5}$	0.16[37]	−1.72[39]	9.54[51]
atorvastatin	0.97	0.99	0.99	$(2.59 \pm 0.05) \times 10^{-4}$	6.36[36]	1.61[36]	4.5[53]
AZM	1.00	0.97	0.10	$(9.04 \pm 4.78) \times 10^{-5}$	3.97[38]	0.50[50]	7.34[54]
carvedilol	0.9	0.98	0.99	$(1.69 \pm 1.70) \times 10^{-4}$	4.14[38]	2.40[50]	7.8[55]
chloramphenicol	0.9	0.71	0.90	$(3.48\ 1.40) \times 10^{-5}$	1.14[34]	1.08[39]	5.5[56]
clopidrogel	0.92	0.99	0.90	$(1.20 \pm 0.34) \times 10^{-4}$	2.50[45]	3.4[50]	4.6[50]
EFA	0.67	0.81	0.99	$(2.7 \pm 0.70) \times 10^{-5}$	4.7[50]	4.7[40]	
ezetimibe	0.93	0.99	0.99	$(4.01 \pm 1.22) \times 10^{-4}$	4.52[43]	4.51[43]	9.75[53]
glibenclamide	0.95	0.99	0.99	$(4.80 \pm 1.10) \times 10^{-4}$	4.1[44]	2.7[43]	5.9[57]
glimepiride	1.00	0.99	0.99	$(1.28 \pm 0.51) \times 10^{-4}$	3.97[46]	2.8[43]	6.8[58]
ibuprophen	1.00	0.99	0.99	$(5.47 \pm 1.27) \times 10^{-4}$	4.13[38]	1.07[41]	4.34[51]
metoprolol	0.95	0.88	0.95	$(5.16 \pm 0.80) \times 10^{-5}$	1.88[42]	−0.16[35]	9.51[51]
propranolol	0.93	0.96	0.99	$(7.70 \pm 2.90) \times 10^{-5}$	3.12[37]	1.26[35]	9.51[51]
risperidone	0.97	0.99	0.99	$(1.52 \pm 0.41) \times 10^{-4}$	3.11[42]	2.29[49]	8.24, 3.11[52]
theophyline	0.97	0.99	0.90	$(2.44 \pm 1.68) \times 10^{-4}$	−0.02[34]	−0.04[41]	8.62[51]
verapamil	0.98	0.99	0.96	$(7.64 \pm 5.30) \times 10^{-5}$	3.79[40]	2.29[39]	8.81[51]

method.[10] The P_{eff} (cm/s) was determined considering the "plug flow" model according to the following equations:

$$P_{eff} = \frac{-Q_{in} \ln\left(\frac{C_{out(corr)}}{C_{in}}\right)}{2\pi RL} \quad (1)$$

$$C_{out(corr)} = C_{out} x \frac{Q_{out}}{Q_{in}} \quad (2)$$

$$V = \pi R^2 L \quad (3)$$

where Q_{in} (mL/min) is the flow rate of the perfusion solution that enters the intestinal segment, Q_{out} (mL/min) is flow rate (mL/min) of the perfusion solution at the exit of the intestinal segment in each time interval, $C_{out(corr)}$ is the corrected concentration (μg/mL) of drug in the exiting solution, C_{in} denotes the drug concentration measured in entering perfusate, C_{out} is the drug concentration (μg/mL) of the solution that leaves the intestinal segment, V is the volume of the gut segment perfused (mL), L is the length of the gut segment (cm), and R is the radius of the gut segment (0.18 cm).

HPLC Analysis. The HPLC system consisted of a Jasco chromatograph, equipped with a quaternary pump, and a Jasco multiple wavelenght detector (Jasco UV-2077 Plus). Chromatographic separations were performed on a C18 Restek (15 cm × 4.5 mm × 5 μm) and on a C8 Restek (15 cm × 4.5 mm × 5 μm). A Phenomenex security guard fusion RP (4 × 30 mm) guard column was also employed. The chromatographic conditions for drugs are displayed in Table 1. The calibration curves were prepared in the intestinal perfusion buffer solution. The HPLC methods were validated according to the parameters of linearity, precision, and accuracy. The specificity of the method was established by using intestinal perfusion solutions collected from different rats.

Correlation between Rat Effective Permeability Coefficients (P_{eff}) and the Fraction of Oral Dose Absorbed in Humans (f_a) or the Extent of Metabolism (f_M). The P_{eff} values obtained from the in situ SPIP assays were fitting with the published f_a data by the least-square method using the following equation:[11]

$$f_a = 1 - e^{(-P_{eff} x b)} \quad (4)$$

where P_{eff} is the effective intestinal permeability coefficient (cm/s), and f_a (%) is the oral fraction absorbed in humans. Also, P_{eff} coefficients were fitted with f_M values by the least-square method by using the following equation:[12]

$$f_M = 1 - e^{(-P_{eff} x b)} \quad (5)$$

RESULTS AND DISCUSSION

Suitability Evaluation of the in Situ SPIP in Rats. Table 2 lists the P_{eff} values obtained herein from 19 tested compounds by applying our SPIP assay in rats. The correlation between the P_{eff} values determined in this study and the extent of the intestinal absorption of drug in humans (f_a) was established (Figure 1A). Without considering within the correlation of the acyclovir data, a relatively high correlation (R^2= 0.8587) between the rat P_{eff} values and the oral f_a in humans was obtained, reflecting the predictive capacity of the human absorption from our SPIP method.

By applying eq 4 and setting an $f_a \geq 85\%$ specified in the FDA guide for good oral absorbed drugs,[8] a value of 6.2×10^{-5} cm/s was established as the in-house cutoff to the high/low permeability boundary. This value was comparable with that obtained for metoprolol ($P_{eff} = 5.2 \times 10^{-5}$ cm/s) by applying our SPIP conditions, which was included in this work as a high permeability standard substance for high-permeability based on the criterion of the BCS classification.

With the goal to investigate the robustness of the in situ SPIP method standardized herein in rats, results of P_{eff} coefficients obtained in this study were compared with P_{eff} reported values obtained in situ by SPIP in rats and in vitro apparent permeability coefficients (P_{app}) obtained using MCDK (Madin−Darby canine kidney) cell monolayers, which are used by the pharmaceutical industry as an industrial standard for use in BDDCS classification system of atenolol, a

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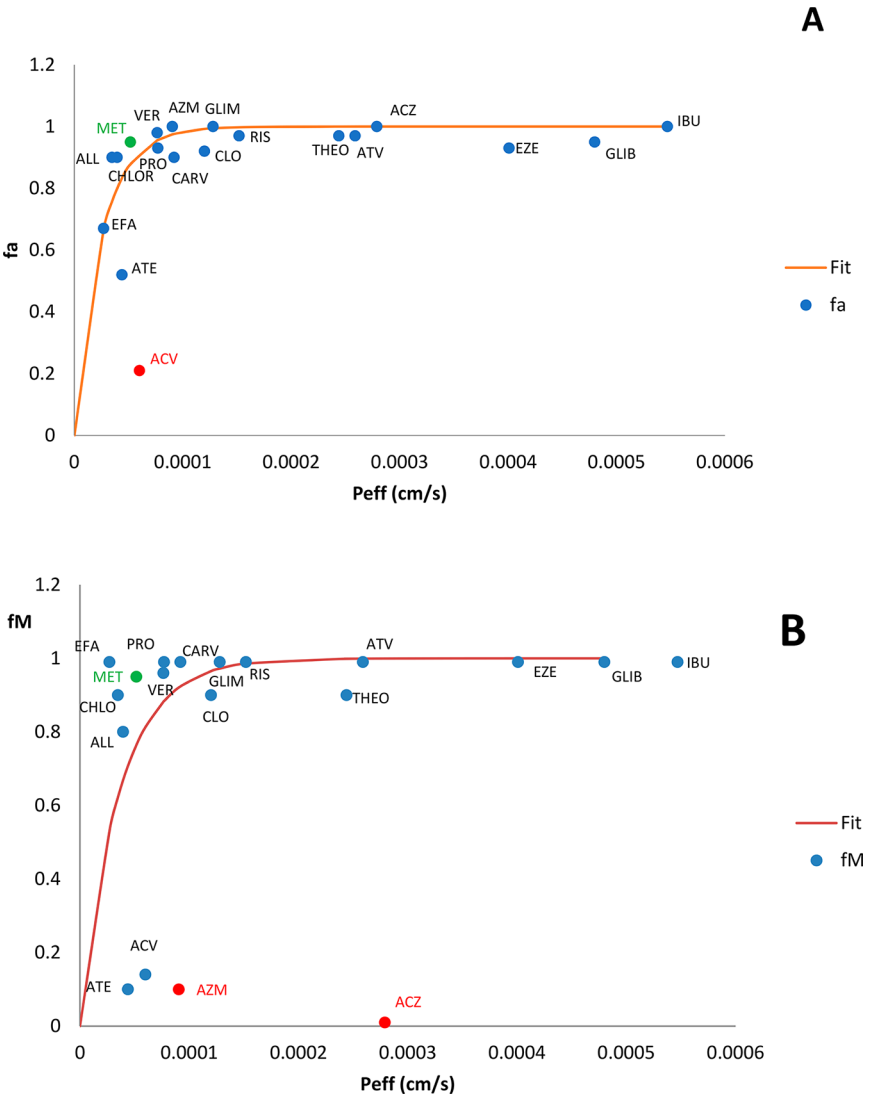

Figure 1. Correlation plot between the (A) fraction of dose absorbed (f_a) or the (B) fraction of drug metabolized (f_M) versus the effective permeability coefficient (P_{eff}) determined by SPIP in rats. ACZ, acetazolamide; ACV, acyclovir; ALL, allopurinol; ATE, atenolol; ATV, atorvastatin; AZM, azithromycin; CARV, carvedilol; CHLOR, chloramphenicol; CLO, clopidogrel; EFA, efavirenz; EZE, ezetimibe; GLIB, glibenclamide; GLIM, glimepiride; IBU, ibuprofen; MET, metoprolol; PRO, propranolol; RIS, risperidone; THEO, theophylline; and VER, verapamil.

Table 3. Permeability Values of Some Compounds Obtained in Situ by SPIP in Rats in This Study and Reported Permeability Values Obtained in Vitro with MDCK Cells

drug	experimental values obtained in this study of $P_{eff} \pm$ DS (cm/s) obtained by SPIP (apical pH = 6.3)	reported values of $P_{eff} \pm$ DS (cm/s) obtained by SPIP	reported values of $P_{eff} \pm$ DS (cm/s) obtained with MDCK cells (apical pH= 7.4)[60]
atenolol	$(4.40 \pm 1.4) \times 10^{-5}$	$(0.3 \pm 0.2) \times 10^{-5}$[61] (apical pH = 7.2) $(2.1 \pm 1.3) \times 10^{-5}$[62] (apical pH 6.5)	$(0.12 \pm 0.36) \times 10^{-5}$
metoprolol	$(5.16 \pm 0.8) \times 10^{-5}$	$(5.32 \pm 0.54) \times 10^{-5}$[59] (apical pH = 6.5) $(2.7 \pm 0.7) \times 10^{-5}$ (apical pH = 7.2) $(6.3 \pm 1.6) \times 10^{-5}$[62] (apical pH = 6.5)	$(4.10 \pm 0.12) \times 10^{-5}$
propranolol	$(7.70 \pm 2.9) \times 10^{-5}$	$(4.1 \pm 1.0) \times 10^{-5}$[61] (apical pH = 7.2) $(8.90 \pm 0.39) \times 10^{-5}$[63] (apical pH = 7.4)	$(4.47 \pm 1.90) \times 10^{-5}$

low permeability marker; propranolol, a high permeability compound; and metoprolol, a high/low permeability boundary substance (Table 3). We observed that P_{app} values from MDCK cells were lower than P_{eff} values obtained in situ with the SPIP method. There are different reasons that can explain these results, such as the size of the surface for absorption,

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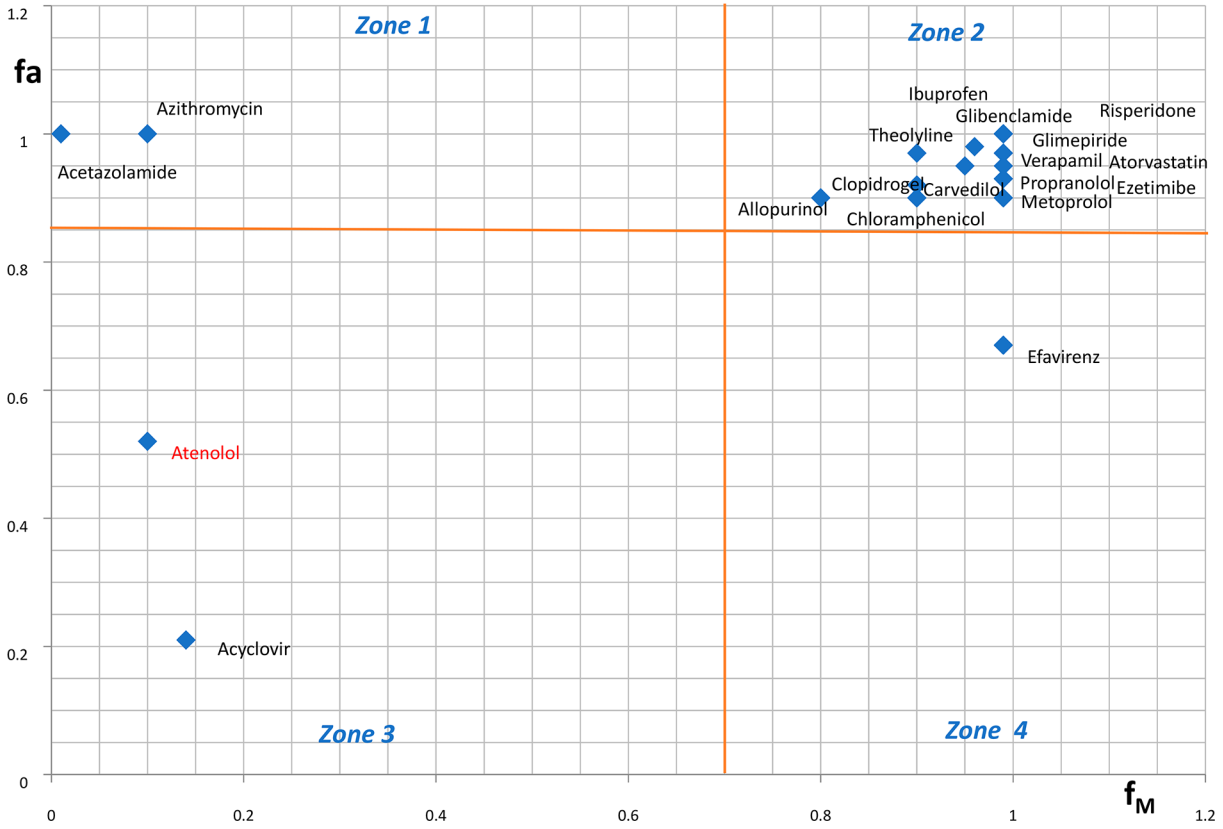

Figure 2. Correlation plot between the fraction of dose absorbed (f_a) versus the fraction of drug metabolized (f_M).

morphological and physiological conditions, setup experimental conditions, etc. P_{eff} values obtained herein were relatively close to P_{eff} values found in the literature. Thus, the in situ standardized SPIP method used here was appropriate for the permeability assessment of drugs.

Prediction of the Extent of Metabolism (f_M) for High Drug Absorption Using P_{eff} Coefficients. Figure 1B shows a graph correlating the extent of metabolism (f_M) of 19 standard compounds (Table 2) versus their P_{eff} values obtained by applying our SPIP assay in rats. Without considering within the correlation the AZM and ACZ data, the best curve fitting showed a sigmoidal correlation between these two parameters with an R^2 of 0.5848, indicating a moderate correlation. By introducing a P_{eff} value of 6.2×10^{-5} cm/s, established herein as the limited boundary for a f_a value of ≥85%, an extent of metabolism of ∼0.76 was obtained for predicting an extent of absorption ≥85%. This value is similar to that value of 0.7 proposed by Benet.[13]

Correlation between the Extent of Absorption (f_a) and the Extent of Metabolism (f_M). Figure 2 depicts a graph correlating the human fraction of the dose absorbed (f_a) of 19 compounds tested versus their extent of metabolism (f_M). By setting a cut off limit value for a high dose fraction absorbed (f_a) of 0.85 and a cut off limit of a high fraction of extension of metabolism of 0.7, as proposed by Benet et al.,[14] this graph could be divided into four areas; which we will call zone 1, high f_a and low f_M; zone 2, high f_a and high f_M; zone 3, low f_a and low f_M; and zone 4, low f_a and high f_M. From this figure, it can be observed that zones 2 and 3 involve highly absorbed and extensively metabolized or lowly absorbed and poorly metabolized drugs, respectively. In these cases, the drug elimination criteria successfully predicted the absorption characteristics of drugs located in these areas. Nonetheless, the extent of metabolism fails to predict the drug absorption for those drugs that fall into zone 1 (BDDCS false negative) or zone 4 (BDDCS false positive). Considering the 19 compounds herein studied, two compounds were located in zone 1 (ACZ and AZM) and one compound in zone 4 (EFA).

ACZ, AZM, CLO, and EFA Case Studies. In situ SPIP assays in rats were conducted to obtain the P_{eff} values of 19 training compounds, and within this set were found ACZ, AZM, CLO, and EFA. Until now, the permeability classification of these compounds remained to be clarified; therefore, herein data were collectively analyzed from their P_{eff}, which were obtained in this work from SPIP in rats, and their extent of metabolism (f_M), which was obtained from literature (Table 2), as predictive parameters of their oral disposition in the base of criteria's BCS and BDDCS for gaining insight into their biodisposition after oral administration.

CLO showed a good correlation between the P_{eff}, which was obtained herein from SPIP assays, and its reported f_M (Figure 1B). It is a particular drug extensively metabolized with a very complex metabolic process. This prodrug undergoes a large metabolism in the liver through a hydrolytic mechanism by hepatic esterase's (∼85%), yielding an inactive carboxylic acid derivative, and a quantitatively minor metabolic pathway through the hepatic CYP enzyme system (∼15%).[15,16] It was found by a mass balance study that an oral single dose of 75 mg of ^{14}C-labeled CLO, yielding a radioactivity fecal recovery of ∼46%, was obtained; although, it should be noted that metabolites and/or degradation products eliminated in feces are not yet well characterized. Also, a cumulative urinary excretion of ∼41% of the dose was found in the study of Lins et al.,[17] indicating a total excretion of ∼92% of the oral CLO

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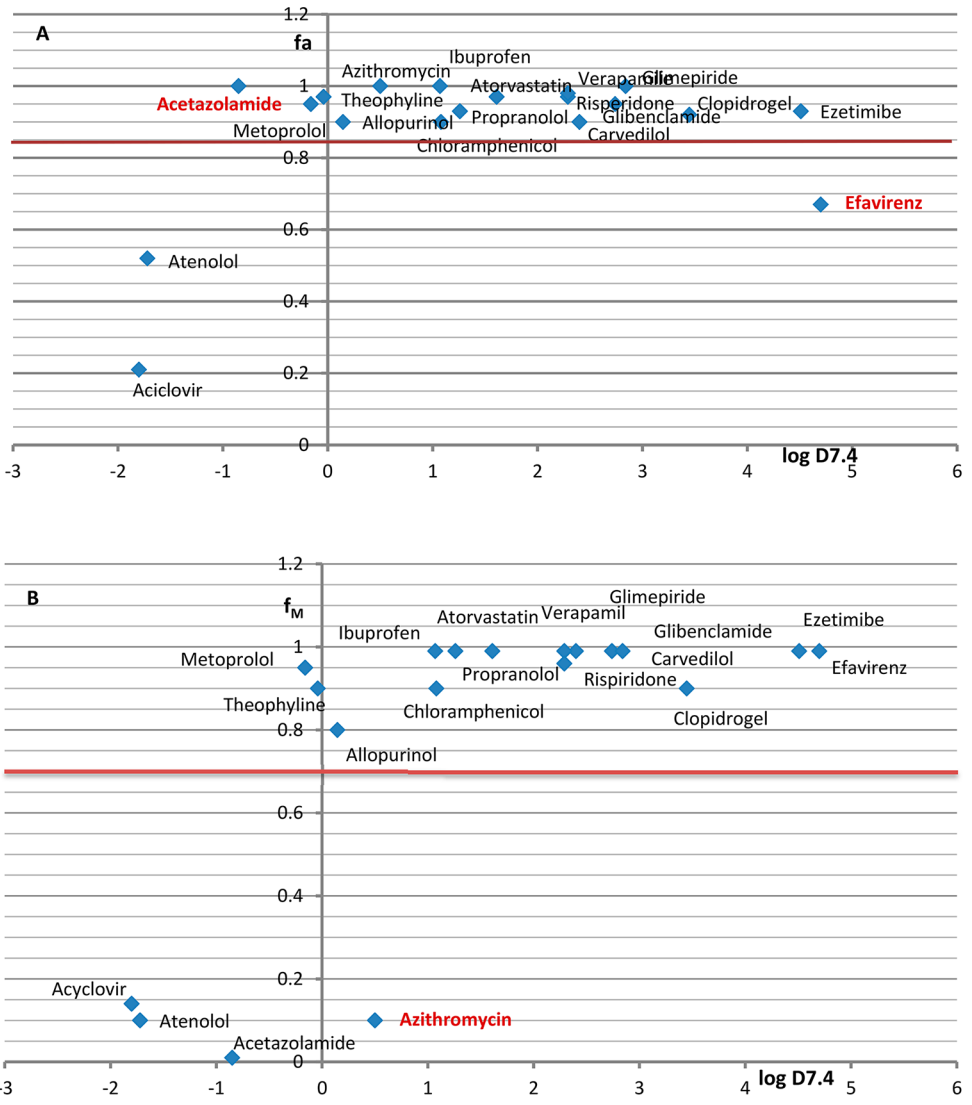

Figure 3. Correlation plot between the (A) fraction of dose absorbed (f_a) or the (B) fraction of drug metabolized (f_M) versus the octanol/buffer pH 7.4 distribution coefficient (log $D_{7.4}$).

dose. This last value was considered herein as its absorbed dose fraction (f_a); therefore, this drug was located in zone 2 of Figure 2, with a high f_a and high f_M, and it was also considered a "high permeability drug" according to the BCS criteria. Thus, taking into account that CLO is practically insoluble in water at neutral pH, is extensively metabolized, and herein proved to have a high permeability; it is proposed to classify this compound as a BCS and BDDCS class 2 drug.

The EFA's absolute bioavailability in humans is unknown due to a lack of an intravenous formulation; however, a value of 0.67 of f_a after its administration through the oral route was estimated by Takano et al. from clinical absorption data,[18] although this drug was associated with a high variability in both absorption and disposition processes. EFA is principally metabolized by oxidation via the cytochrome P450s (CYPs) and less than 1% of a dose administrated orally is eliminated in urine as unchanged EFA.[19] In this study, it was found that EFA exhibited a moderate permeability, with a P_{eff} value less than the P_{eff} cutoff permeability boundary found herein and also lower than the P_{eff} value obtained here for metoprolol by applying our SPIP developed method and included in this study as a standard of high permeability.

Figure 2 displays that EFA was located in zone 4, with a high f_M and low f_a. Due to this, the drug has an aqueous solubility of <20 ng/mL, and it is extensity metabolized; EFA should be a BDDCS class 2 drug. However, EFA demonstrated herein to be a poorly permeable drug, thus according to criteria of the BCS, this drug should be a BCS class 4 drug.

The BDDCS predicts that those compounds that are ≥90% metabolized by phase 1 and 2 hepatic processes are expected to be ≥90% absorbed. However, here we find a discrepancy in the EFA's classification between the two classification systems (BCS and BDDCS). EFA showed low permeability but high metabolism, so it may be considered as a BDDCS false positive; in this case, the EFA's metabolism was not able to predict the EFA's extent of absorption. A possible explanation for this discrepancy may be that EFA is a substrate of the breast cancer resistance protein (BCRP/MXR) known as ABCG2, which is highly expressed in the GIT and can efflux EFA into the intestine, impairing its permeability.[20] This is in agreement with the Benet's hypothesis that the extent of the drug metabolism correlates well with passive transcellular permeability rate.[6]

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In opposition, herein we found two drugs (AZM and ACZ) that were shown to be highly permeable on the base of the BCS criteria, but they are poorly metabolized. These drugs were located in zone 1 of Figure 2, with low f_M and high f_a, thus they were BDDCS false negatives.

Despite of the low AZM absolute bioavailability (BA) of ∼37%, this compound was reported to be rapidly absorbed after its oral administration if it was not inactivated by gastric acid.[21,22] AZM elimination occurs primarily in the feces as the unchanged drug following excretion into the bile, and only ∼6% of the dose is found in urine as an unchanged drug.[23]

It was found in this work that AZM exhibited a high intestinal permeability coefficient (Figure 1, Table 2). This was in agreement with Iskaidek and Arafat, who estimated the f_a of AZM to be ∼1.00 from plasma mean concentration profiles by using the Simcyp Program and classified AZM as a class 1 drug from their proposed salivary excretion classification system (SECS).[24]

The low recovery of AZM in urine (∼6%) and its biliary elimination as an unchanged drug could suggest a poor oral absorption according to BDDCS criteria. However, for a drug to be eliminated into bile, it must first be taken up into hepatocytes, and once inside the hepatocytes, the parent drug may be subjected to its elimination toward the bile, but for drugs to access the liver, they must cross the intestinal epithelium. Therefore, for drugs to undergo extensive biliary excretion, they would expect an almost total absorption after their administration. On the other hand, it should be pointed out, that although AZM does not conform to Lipinski rules (rule of 5, Ro5), because this drug has a molecular mass (MW) of ∼700 Da, a number of hydrogen-bond acceptors (HBA) (∼10), and a polar surface area (PSA) of ∼200 Å,[25,26] it is predicted to have a poor permeability or absorption; however, herein, AZM showed a good absorption. This discrepancy was explained by Lipinski based on that this class of drugs shows good oral absorption because they are substrates of physiological transporters that may favor their permeability.[27] Therefore, among the potential explanations for the discrepancy between the P_{eff} value obtained herein for AZM and its low extension of metabolism is that AZM absorption could be mediated via facilitated uptake by intestinal transporters like organic anion-transporting polypeptide (OATP) transporters due to AZM having two strongly basic ternary amine (cationic) centers and/or others intestinal transporters expressed in the intestine, or due to the slow exit of AZM from tissues because of its great affinity by tissues, yielding high tissue levels and relatively low but prolonged blood concentrations.[28] In addition to this, the biliary and intestinal excretion of AZM is mediated by canalicular transporters, such as the efflux protein P-glycoprotein (Pgp), one of the main transporters from hepatocytes in the liver.[29,30]

Because of AZM's favorable in situ permeability, and the fact that AZM is not metabolized and is extensively eliminated unchanged into bile, the BDDCS predicts it to have a poor permeability. Because the commercial solid dosage forms of AZM are either the monohydrate or dehydrate, and crystalline forms of this compound are characterized by low aqueous solubility,[31] AZM should be classified as a BCS class 2 but as a BDDCS class 4.

The other drug found in zone 1 of Figure 2, with a high f_a and low f_M, was ACZ. It is well absorbed through the GIT and completely excreted unchanged in urine.[32,33] Herein, ACZ exhibited high permeability, where its P_{eff} value obtained from the SPIP was higher than the P_{eff} value established herein as the boundary of high permeability and greater than the corresponding value of metoprolol. This result was in line with the reported oral bioavailability >90% for this compound. Moreover, in a previous publication, we demonstrated that the ACZ's high permeability was associated with an epithelial barrier disruption.[9] Therefore, ACZ herein was found to be a BCS high permeability compound, although having poor metabolism, probably because of the hydrophilic nature of this compound (log P ∼ −0.26) that may decrease its affinity for hepatic metabolic enzymes by preventing its metabolism.[34]

Considering the ACZ's low aqueous solubility (0.72 mg/mL),[35] this drug should be classified as BCS class 2 and BDDCS class 4; therefore, they were found to be BDDCS false negative.

Correlation between the Extent of Absorption (f_a) or the Extent of Metabolism (f_M) versus the Octanol/Buffer pH 7.4 Distribution Coefficient (log $D_{7.4}$). Figure 3 and Table 4 show that there is an interplay among transport, metabolism processes, and lipophilicity characteristics of drugs; where the rule of thumb is that the most lipophilic compounds are generally well absorbed, due to the increase in the transmembrane diffusion due to greater lipophilicity of the drug, and metabolized, because the drug metabolism appears to be driven by a relatively high lipophilicity because the more hydrophilic compounds are eliminated more efficiently from excretory tissues.

Table 4. Fraction of Dose Absorbed (f_a), Fraction of Metabolized (f_M). and Logarithm of Distribution Partition *n*-Octanol/Buffer pH 7.4 Coefficients (log $D_{7.4}$) of the Studied Compound Set

Drug	f_a	f_M	lipophilicity (log $D_{7.4}$)
ACZ	↑	↓	↓
acyclovir	↓	↓	↓
allopurinol	↑	↑	↑
atenolol	↓	↓	↓
atorvastatin	↑	↑	↑
AZM	↑	↓	↑
carvedilol	↑	↑	↑
chloramphenicol	↑	↑	↑
clopidrogel	↑	↑	↑
EFA	↓	↑	↑
ezetimibe	↑	↑	↑
glibenclamide	↑	↑	↑
glimepiride	↑	↑	↑
ibuprophen	↑	↑	↑
metoprolol	↑	↑	↑
propranolol	↑	↑	↑
risperidone	↑	↑	↑
theophiline	↑	↑	↑
verapamil	↑	↑	↑

Figure 3 displays clear relationships between the logarithm of octanol/buffer pH 7.4 distribution coefficient (log $D_{7.4}$) and the fraction of absorbed dose (f_a) or the fraction metabolized (f_M), ratifying the postulate that drug permeability and metabolism correlate well with increased lipophilicity. In general, compounds displayed well absorption as log $D_{7.4}$ increased (Figure 3A). This can be explained because increasing lipophilicity promotes passive diffusion through lipid membranes. A similar trend was followed with the

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relationship between log $D_{7.4}$ and f_M (Figure 3B). Nevertheless, there were several notable exceptions among the compound set studied. EFA and ACZ showed an inverse relation between f_a and log $D_{7.4}$ (Figure 3A).

EFA, a lipophilic compound, showed a low f_a, while ACZ, a hydrophilic drug, displayed a high f_a. These discrepancies may be explained because these compounds are transported across the intestinal epithelium by a different mechanism than just simple passive diffusion. In the transport of EFA, active transporters are possibly involved, in addition to its passive component. ACZ, despite being a hydrophilic compound, has a high intestinal permeability due to this drug being able to disrupt the epithelial barrier to produce a leaky gut, with an unexpected high permeability.[9]

On the contrary, AZM, a poorly metazolized drug, although it has an adequate lipophilicity, is eliminated mainly in bile as the intact parent drug, probably because of its low affinity by CYP3A4 in humans and explained also by the possibility that this compound is eliminated into bile by Pgp.[30]

CONCLUSIONS

In this study, a correlation between f_a in humans with the effective permeability coefficient (P_{eff}) obtained by using the SPIP method in rats for 19 training compounds was found. A high/low permeability boundary with a P_{eff} value of >6.2 × 10^{-5} cm/s was established for those drugs exhibiting high absorption in humans (>85%). This value was comparable with that obtained for metoprolol (5.16 ± 0.8) × 10^{-5} cm/s, which is included herein as a high permeability standard. Thus, SPIP may be a good predictor for BCS classifications but may not be suitable for BDDCS classifications due to the involvement of other absorption mechanisms in this system instead of simple passive diffusion.

Herein, of the 19 training compounds studied, 16 (~79%) were classified as highly permeable on the BCS criteria; however, only 14 (~68%) underwent extensive metabolism, and 2 compounds (~16%), which were classified as poorly permeable on the base of the BCS, were poorly metabolized.

A very good fitting was not found between the extent of absorption in humans (and intestinal permeability) and the extent of metabolism of the drug set studied, which were the main compounds that came out remarkably from this correlation of the four drugs.

The two notable outliers that broke the rule of highly permeable drugs being eliminated from metabolism were AZM and ACZ. AZM is a lipophilic drug, and it is primarily eliminated unchanged into the bile, while ACZ is a hydrophilic drug mainly eliminated unchanged in urine. These compounds exhibit different oral absorption process. The AZM's absorption is probably related to the possibility of the involvement of an active influx mechanism, while ACZ, in spite of its hydrophilic nature, is able to overcome the passive permeability problems through a non-passive or pseudo-passive mechanism by membrane disruption of the intestinal epithelium, as it was proposed by Mora et al.[9]

Conversely, within the set of compounds studies herein, we found a poorly metabolized drug exhibiting low permeability, EFA. The low absorption of this compound could be attributed to an efflux mechanism in the GIT.[20]

Consequently, from the results found herein, we postulated that the correlation between the permeability and extension of metabolism yields good results when the drug crosses the intestinal epithelium through a simple passive diffusion mechanism, which is in agreement with the postulate by Benet et al.

Discrepancies found in both BCS and BDDCS can be attributed to mechanisms other than the simple passive diffusion transport of drugs across cell membranes and/or the intervention of active transport systems, as well as by the action of some drugs on the intestinal epithelium, which causes the disruption of the gut mucosa, increasing the drug permeability.

The joint analysis of both classification systems (BCS and BDDCS) is therefore a powerful tool to infer the involvement of oral drug disposition systems other than simple passive diffusion, especially at the beginning of the development of new drug products, as through in vitro studies of solubility, permeability, and metabolism. On the basis of the criteria of the BCS and BDCCS, it is possible to understand the mechanisms involved in the absorption and disposition processes of drugs.

AUTHOR INFORMATION

Corresponding Author

*E-mail: glagra@fcq.unc.edu.ar; Tel. +54-351-5353865.

ORCID

Gladys Ester Granero: 0000-0003-1051-2539

Notes

The authors declare no competing financial interest.

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