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Biopharmaceutics Classification of Selected β-Blockers: Solubility and Permeability Class Membership

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Abstract: The purpose of this study was to determine the permeability and solubility of seven β-blockers (acebutolol, atenolol, labetalol, metoprolol, nadolol, sotalol, and timolol) and to classify them according to the Biopharmaceutics Classification System (BCS). Apparent permeability coefficients (P_{app}) were measured using the Caco-2 cell line, and the solubility was determined at 37 °C over a pH range of 1.0−7.5. The permeability coefficients ranged from 1.0×10^{-7} to 4.8×10^{-5} cm/s. On the basis of the in vitro permeability and solubility data observed in the study, labetolol, metoprolol, and timolol can be categorized as BCS Class I drugs, whereas acebutolol, atenolol, and nadolol belong to BCS Class III. The permeability coefficients in Caco-2 cells were consistent with the reported extent of intestinal absorption in humans for all drugs except sotalol. Sotalol displayed low permeability in the Caco-2 cell line, but the extent of intestinal absorption in humans is over 90%. The low permeability through the Caco-2 monolayers might be largely related to its low lipophilicity. In addition, the difference between the tightness of the intercellular junction in vivo and in vitro may partially contribute to this disparity in the sotalol permeability of in vivo and in vitro.

Keywords: Biopharmaceutics Classification System; β-blockers; Caco-2; permeability; solubility

Introduction

The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability.[1−2] According to this system, drug substances can be classified into four groups: (I) high solubility−high permeability, (II) low solubility−high permeability, (III) high solubility−low permeability, and (IV) low solubility−low permeability. In August 2000, the Food and Drug Administration (FDA) issued a guidance for industry on waivers of in vivo bioavailability and bioequivalence studies for immediate-release (IR) solid oral-dosage forms based on BCS.[3] A drug substance is considered highly soluble when the highest strength is soluble in 250 mL or less of aqueous media over the pH range of 1.0−7.5. The permeability classification is based directly on the extent of intestinal absorption of a drug substance in humans or indirectly on the measurements of the rate of mass transfer across the human intestinal membrane. Animal or in vitro models capable of predicting

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† This scientific contribution is intended to support regulatory policy development. The views presented in this article have not been adopted as regulatory policies by the Food and Drug Administration at this time.

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the extent of intestinal absorption in humans may be used as alternatives, for example, in situ rat perfusion models and in vitro epithelial cell culture models. A drug substance is considered highly permeable when the extent of intestinal absorption is 90% or higher.

Of the several in vitro models developed for studying intestinal absorption, the Caco-2 human colon carcinoma cell line has been widely utilized in the in vitro model system for evaluating the rate of intestinal drug absorption.[4−8] In the FDA laboratory, Volpe et al.[9] have evaluated the in vitro Caco-2 cell permeability assay according to the approach of method suitability outlined in the BCS guidance by using over 20 model drugs. The results showed that in vitro Caco-2 permeability is related to in vivo human intestinal absorption for the model drugs, and this relationship provides a means to distinguish between high- and low-permeability drug substances. These authors also recommended that the ability of a cell culture model to classify drugs be evaluated based upon suitability criteria described in the FDA guidance that use a number of model drugs and an internal standard for classification purposes. The high-permeability model labetalol, near the high/low permeability class boundary, was suggested to be a useful internal standard within their laboratory.[9] In this study, the permeability of seven selected β-blocker drugs was determined following the same approach, the solubility was determined, and their classification was established according to the BCS guidance.

Experimental Section

Materials. Acebutolol, atenolol, labetalol, metoprolol, nadolol, sotalol, timolol, palmitoylcarnitine (PCC), and fluorescein isothiocyanate-dextran (FITC-Dextran) were purchased from Sigma Chemical (St. Louis, MO). All solvents and buffer components were of reagent grade.

Cell Culture. The Caco-2 cell line (American Type Cell Collection, Manasas, VA) was grown in manner similar to that described previously.[9] Briefly, Caco-2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 0.1 mM nonessential amino acids, 100 U/mL penicillin, 0.1 g/mL streptomycin, 10 mM sodium bicarbonate, and 10% fetal bovine serum (FBS) at 37 °C in a 75 cm^2 culture flask (Corning Inc., Corning, NY) in a humidified atmosphere with 5% CO_2. Cells were harvested with trypsin-EDTA and seeded on polycarbonate filters (0.4 μm pore size, 1.13 cm^2 growth area) inside Transwell cell culture chambers (Costar, Corning, NY) at a density of approximately 75 000 cells/cm^2. All components of the culture medium and trypsin-EDTA were obtained from GIBCO (Grand Island, NY). The culture medium of 0.5 mL in the apical (AP) chamber and 1.5 mL in the basolateral (BL) chamber was replaced three times per week. Cells from passage number 54−58 were used for the transport studies.

Table 1. Drug Concentrations in Transport Studies[a]

drug	HDS (mg)	solvent	final concentration (mg/mL)	pK_a [b]	intrinsic log P [b]
acebutolol	400	water	1.6	9.12	1.19
atenolol	100	water	0.4	9.17	−0.026
FITC-Dextran	n/a	water	0.8		
labetalol	300	water	1.2	7.91	2.41
metoprolol	100	water	0.4	9.18	1.69
nadolol	160	ethanol	0.64	9.17	1.17
sotalol	240	water	0.96	9.19	0.37
sotalol	240	water	0.096	9.19	0.37
timolol	25	water	0.01	8.86	1.75

[a] HDS: highest dose strength. [b] Cited from ref 14.

Transport Studies in Caco-2 Monolayers. Drug-transport studies were conducted in Hank's balanced salt solution (HBSS, GIBCO) containing calcium and magnesium. The AP chamber contained HBSS (pH 6.8) supplemented with 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES, GIBCO), whereas the BL chamber contained HBSS (pH 7.4) supplemented with 25 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES, GIBCO). Drug stock solutions were prepared in deionized water except for nadolol, which was dissolved in ethanol. Drug stock solutions were prepared at 10-fold of the final working solution in water or 50-fold of the final working solution in ethanol. The final working solution was based on the drug's highest dose strength (HDS) dissolved in 250 mL. The drugs were diluted to their final concentrations in pH 6.8 buffer for AP to BL study or in pH 7.4 buffer for BL to AP study, respectively. The drug concentrations in transport studies are shown in Table 1. Caco-2 cell monolayers were used 19−22 days after initial seeding in the transport studies. Monolayers were equilibrated in transport medium (pH 7.4) for 60 min at 37 °C. The transepithelial electrical resistance (TEER) was measured for each monolayer using a Millicell-ERS apparatus (Millipore Corp., Bedford, MA). TEER values (Ω × cm^2) were calculated from the following equation

$$TEER = (R_{mono} - R_{blank}) \times A$$

where R_{mono} is the cell monolayer and filter resistance, R_{blank}

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is the filter resistance, and A is the filter surface area (1.13 cm^2). Monolayers with TEER values greater than 250 $\Omega \times cm^2$ were used in the study. For AP to BL transport studies, drug solution (0.5 mL, pH 6.8) was added to the AP chamber. The plates were agitated gently and incubated at 37 °C in a humidified atmosphere with 5% CO_2 during transport studies. The filter insert was transferred to a corresponding well in a new plate containing transport buffer in the BL chamber at 15, 30, 45, and 60 min. For BL to AP transport studies, drug solution (1.5 mL, pH 7.4) was added to the BL chamber. A 0.4 mL sample was collected from the AP chamber at each time point of 15, 30, 45, and 60 min and replaced with the same amount of fresh pre-warmed buffer. All samples were stored at −20 °C until HPLC analysis.

Transport Studies from Day 14's Caco-2 Monolayers. The procedure was the same as above except for the use of monolayers from day 14 regardless of TEER values. The aim of using day 14's monolayers for the transport studies was to test the possibility of involvement of paracellular pathway in the transport of sotalol, assuming that the tight junctions of Caco-2 monolayers are not fully formed at the early stage of monolayers.[10] All samples were stored at −20 °C until HPLC analysis.

Transport Studies in Caco-2 Monolayers with PCC. Following the procedure described above, after baseline measurements of TEER, 0.5 mL of the buffer was removed from the AP chamber and replaced with buffer containing 0.2 mM of PCC. Forty-five min after incubation at 37 °C, TEER was measured again. Then the buffer in the AP chamber was replaced with the buffer containing 0.2 mM of PCC and designed concentration of compounds. At discrete times, the filter insert was transferred to a corresponding well in a new plate containing1.5 mL of HBSS (pH 7.4). At the completion of study, the TEER was measured once again. All samples were stored at −20 °C until HPLC analysis.

Determination of Solubility. The solubilities of acebutolol, atenolol, labetolol, metoprolol, nadolol, sotalol, and timolol were determined in pH 1.0, 3.0, 4.0, 5.0, and 7.5 buffers in triplicate according to the method outlined in the BCS guidance.[3] The pH 1.0 buffer was comprised of 0.2 M potassium chloride and 1 N hydrochloric acid. The remaining buffers were prepared from the combination of 0.2 M monobasic potassium phosphate and 0.2 M dibasic potassium phosphate. Sufficient amount of drug substance was added to the 5 or 10 mL buffer to provide a potential concentration of solution being at least 2 times the drug's HDS in 250 mL. The solution pH was verified and adjusted, if necessary. After 24 h of incubation in a shaking water bath at 37 °C, aliquots of sample were carefully withdrawn, filtered with a nonadsorbing syringe filter (Acrodisc, Ann Arbor, MI), diluted if necessary, and quantified using HPLC. The volume required to dissolve the HDS was calculated by dividing the HDS by measured concentration. A drug substance was classified as highly soluble if the BCS volume was ≤250 mL of aqueous media over the pH range of 1.0−7.5.

Analytical Methods. All samples were analyzed by the HP-1090 (Hewlett-Packard, Wilmington, DE) or Agilent 1100 (Wilmington, DE) high-performance liquid chromatography (HPLC) system using Phenomenex C_{18} (5 μM, 250 × 4.6 mm, from Torrance, CA) or Waters Xterra RP_{18} (5 μM, 250 × 4.6 mm, from Fullerton, CA) column. The mobile phases for each drug were as follows: 20% acetonitrile, 80% phosphate buffer (20 mM, pH 3.0) containing 1% tetrahydrofuran for acebutolol, sotalol, and timolol; 10% acetonitrile, 90% phosphate buffer (10 mM, pH 3.0) for atenolol; 10% acetonitrile, 90% phosphate buffer (10 mM, pH 6.9) for FITC-dextran; 32% acetonitrile, 68% phosphate buffer (10 mM, pH 3.0) for labetalol; 20% acetonitrile, 80% phosphate buffer (10 mM, pH 3) for metoprolol; and 18% acetonitrile, 82% phosphate buffer (10 mM, pH 3.0) for nadolol. The flow rates of mobile phases were 1 mL/min. For ultraviolet detection, the wavelengths were 225, 214, and 280 nm for acebutolol, labetolol, and timolol, respectively. For fluorescence detection, the wavelengths of excitation and emission were 228 and 320 nm for atenolol, metoprolol, and nadolol; 490 and 515 nm for FITC-dextran, and 227 and 302 nm for sotalol.

Calculations. The apparent permeability (P_{app}) coefficient expressed in cm/s was calculated from the following equation

$$P_{app} = [V_R/(A \times C_0)][{}^{dC}/_{dt}]$$

where V_R is the volume in the receiver chamber (mL), A is the filter surface area (1.13 cm^2), C_0 is the initial concentration (mg/μL), and dC/dt is the initial slope of the cumulative concentration (μg/mL) in the receiver chamber with time (s).[9]

Results and Discussion

Analytical Methods and Validation. Isocratic HPLC methods with ultraviolet and fluorescence detection were used to detect model compounds (acebutalol, atenolol, FITC-dextran, labetalol, metoprolol, nadolol, sotalol, and timolol) in the study. The analytical assays were validated according to USP's validation of compendial methods.[11] The validation parameters utilized were accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range. The validation results for each model drug are shown in Table 2. In the study, accuracy and precision were assessed by using 15 determinations over three concentration levels (lowest, middle, and highest) covering the linear range and 5 replicates of each concentration for each drug. The accuracy of the method for all model drugs ranged from 99.7 to 100.8%. The precision of all of the analytical methods in the study was less than 3.2%. The specificity of the method

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Table 2. Validated Parameters of Analytical Methods

compound	detection limit (μg/mL)	quantitation limit (μg/mL)	linear range (μg/mL)	$r^{2\,a}$	accuracy (%) mean ± SD[b]	precision CV| (%)[c]
acebutolol	<0.25	0.25	0.25 ~ 256	0.9999	100.28 ± 0.18	0.53
atenolol	<0.31	0.31	0.31 ~ 20	1	99.81 ± 0.21	0.96
FITC-Dextran	<0.125	0.125	0.125 ~ 2	0.9998	99.69 ± 1.40	2.90
labetalol	<1.5	1.5	1.5 ~ 200	0.9986	100.23 ± 0.37	0.78
metoprolol	<0.31	0.31	0.31 ~ 40	0.9999	99.93 ± 0.56	0.57
nadolol	<0.125	0.125	0.125 ~ 36	0.9998	100.27 ± 0.19	1.12
sotalol	0.039	0.078	0.078 ~ 5	0.9999	100.81 ± 3.2	3.14
timolol	<0.78	0.78	0.78 ~ 100	1	99.88 ± 0.77	0.61

[a] Correlation coefficient obtained from six to nine concentrations and three replicates of each concentration. [b] The percentage of recovery obtained from fifteen determinations over three concentration levels of five each covering the low, middle and high concentration range. [c] The coefficient variation of 15 determinations over three concentration levels of 5 each covering the low, middle, and highconcentration range.

Table 3. Permeability Values of the Model Drugs

drug	$P_{app(ap\rightarrow bl)}$ ($\times 10^{-6}$ cm/s)[c] day 19~22[d]	$P_{app(ap\rightarrow bl)}$ ($\times 10^{-6}$ cm/s)[c] day 14[d]	$P_{app(ap\rightarrow bl)}$ ($\times 10^{-6}$ cm/s)[c] day 19[d] with PCC[e]	$P_{app(bl\rightarrow ap)}$ ($\times 10^{-6}$ cm/s)[c] ≥day 19~22[d]	extent of absorption (%)[f]
acebutolol	1.29 ± 0.25			2.19 ± 0.15	70
atenolol	1.01 ± 0.18	1.49 ± 0.28			50
FITC-Dextran	0.24 ± 0.02	0.47 ± 0.03	0.90 ± 0.27	0.12 ± 0.01	
labetalol	22.61 ± 0.68				90
metoprolol	36.86 ± 0.25	29.19 ± 1.75	48.08 ± 1.99	79.29 ± 2.88	>90
nadolol	0.56 ± 0.04				35
sotalol[a]	1.53 ± 0.29	2.46 ± 0.28	3.86 ± 0.28	1.42 ± 0.12	95
sotalol[b]	1.50 ± 0.51			1.27 ± 0.21	95
timolol	18.26 ± 1.22			52.6 ± 0.55	>90

[a] Initial concentration: 960 μg/mL. [b] Initial concentration: 96 μg/mL. [c] The value represents the mean ± SD. [d] Monolayer's age. [e] PCC: palmitoylcarnitine HCL. [f] Cited from ref 13.

was determined for each drug in the transport buffer HBSS. A minimum of seven standards was utilized to create each standard curve. For each assay, at least three standard curves were established in our study. The r^2 value of the linear regression was above 0.999 for all of the compounds.

Permeability of Selected Drugs. In transport studies using Caco-2 monolayers, apical solution contacts the cell surface directly because the mucin layer does not exist on the apical surface of Caco-2 cells. Thus, the pH value of the apical medium could have a critical effect on the membrane transport of drugs. Alternatively, the pH of the basolateral solution of Caco-2 monolayers corresponds to the pH of interstitial fluid in the villi of the small intestine. Under physiological conditions, the pH value in this region is considered to be neutral (pH = 7.4) because the extracellular fluids can be exchanged with the serum through the wall of the microvessels. Therefore, to mimic the drug transport in vivo, different pH values were applied for the apical medium (pH = 6.8) and basolateral medium (pH = 7.4) in our study.

Nineteen to twenty-two day-old Caco-2 monolayers from passage 54 to 58 were used for the transport studies. The TEER of the Caco-2 monolayers was between 310 and 410 ohms·cm^2, indicating integrity of the monolayers. Furthermore, FITC-dextran was used as the low-paracellular-permeability molecular marker for this study. The apparent permeability of FITC-dextran in the AP to BL direction across Caco-2 monolayers was $0.24 \pm 0.02 \times 10^{-6}$ cm/s ($n = 24$) and $0.12 \pm 0.01 \times 10^{-6}$ cm/s ($n = 9$) in the BL to AP direction (Table 3). The results indicated that the integrity of the monolayers was maintained for the duration of the study. The apparent permeability coefficients (P_{app}) of all of the selected compounds across Caco-2 monolayers were measured in the AP to BL and BL to AP directions. The $P_{app(ap\rightarrow bl)}$ coefficients are summarized in Table 3. The $P_{app(ap\rightarrow bl)}$ coefficients of β-blockers ranged from 0.1×10^{-6} to 37.6×10^{-6} cm/s. The $P_{app(ap\rightarrow bl)}$ and $P_{app(bl\rightarrow ap)}$ were not significantly different for acebutolol and sotalol, indicating a passive diffusion pathway for the transport of these compounds across the Caco-2 monolayers. However, a considerable difference was observed between the $P_{app(ap\rightarrow bl)}$ and $P_{app(bl\rightarrow ap)}$ for metoprolol and timolol. Data from the literature suggests that passive diffusion might be the principal mechanism of absorption for metoprolol and timolol.[12−14] Thus, it is quite likely that the change of medium

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pH in the donor chamber might cause the difference in P_{app} values. From Detroyer's report[15] we know that the pK_a values of the tested compounds are around 9.0. According to general pH partition theory,[16] the compounds added to the AP buffer (pH = 6.8) in the AP to BL study should have a higher dissociation degree than that of the compounds added to the BL buffer (pH = 7.4) in the BL to AP study. It is well known that the permeability of the undissociated molecule is higher than that of the dissociated molecule.[13] Similar phenomena were also observed by Yamashita.[17] These results suggest that the effects of medium pH on the permeability to Caco-2 monolayers largely depends upon both the dissociation condition in the solution and the intrinsic permeability of each drug.

The data indicates that the more lipophilic compounds among those studied showed the higher permeabilities. The compounds with log *P* values higher than 1.3, like metoprolol, labetalol, and timolol, exhibited high permeabilities, whereas the compounds with log *P* values less than 1.3, including acebutolol, atenolol, nadolol, and sotalol, appeared to have low permeability across Caco-2 monolayers. Several investigators have reported a similar correlation between octanol−water partition coefficients and membrane permeability.[18−20]

Comparison of Caco-2 Permeability to Human Intestinal Absorption. There was a rank−order relation between the extent of oral absorption (% f_a) in humans with the permeability across Caco-2 monolayers for six of the tested drugs including high- and low-permeability internal standards (Figure 1). Compounds with apparent permeability coefficients $>15 \times 10^{-6}$ cm/s exhibited complete absorption in humans (>90%). Other reports have shown a similar relationship between drug absorption in humans and permeability coefficients across Caco-2 cells.[9,21−25]

The in vitro permeability of sotalol did not correspond to its extent of intestinal absorption in humans. Sotalol is nearly completely absorbed after oral administration and undergoes no first-pass hepatic metabolism.[26] As a result, its absolute bioavailability is 90−100%.[26] However, the permeability of sotalol across Caco-2 monolayers was found to be very low in our study. It was about 32-fold lower than that of

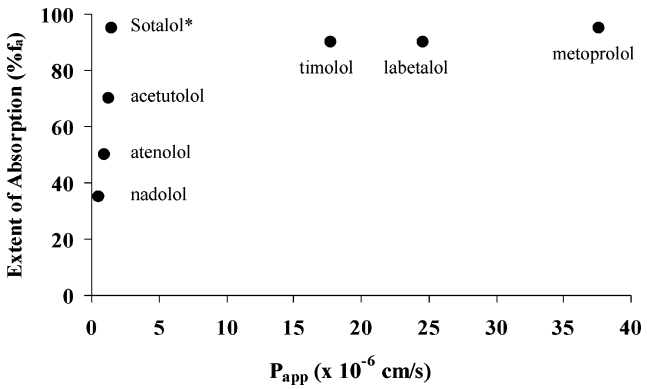

Figure 1. Extent of absorption in humans as a function of permeability coefficients of model drugs across Caco-2 monolayers.

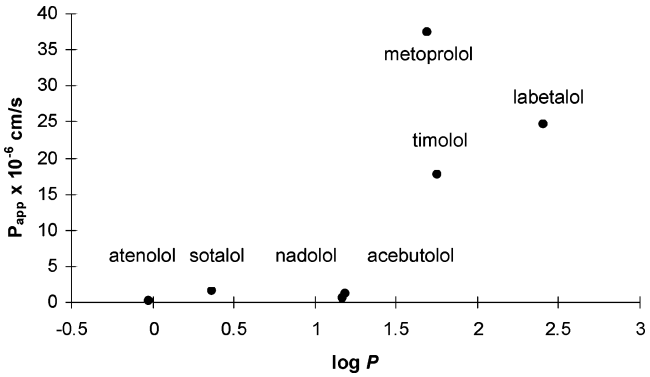

Figure 2. Relationship of the apparent permeability coefficients of model drugs across Caco-2 monolayers and the intrinsic octanol−water partition coefficients (log P).

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metoprolol. A similar result was observed by Irvine et al.,[27] where the cultured Caco-2 cells and MDCK cells were used to examine the permeabilities of sotalol and other drugs. In their research, it was found that the permeability of sotalol was about 32-fold lower than that of metoprolol through the Caco-2 cells and MDCK cells, respectively. Ranta et al.[28] found that the permeability of sotalol was about 10-fold lower than that of metoprolol across the corneal epithelial cells. They also found that the lipophilic compounds (e.g., alprenolol) permeated much faster than the hydrophilic compounds (e.g., atenolol). For sotalol, the low permeability through the Caco-2 monolayers might be largely related to its low lipophilicity. To investigate the possibility of the involvement of the active or efflux mechanisms, we conducted the transport studies for both directions, that is, AP to BL and BL to AP, at two concentration levels, respectively. The results clearly demonstrated that the apparent permeability coefficients across Caco-2 monolayers were independent of the initial concentrations and transport direction, indicating that active processes may not be involved. It should be noted that the media in GI tract is a complex medium of bile salts, lecithin, cholesterol and its ester, and a wide range of lipid materials.[29] It was also reported that some bile acids enhance the passive permeability of drugs to the intestinal membrane by decreasing its TEER through their chelating effect.[30] Moreover, a higher concentration (20 mM) of bile acid (taurocholic acid) was found to enhance the absorption of water-soluble drugs from the small intestine both in vivo and in vitro.[31,3231,32] However, due to the limitation of Caco-2 cells and transport medium, the results unlikely exclude the possibilities of paracellular pathway or other mechanisms involved in vivo.

Generally, hydrophilic molecules that are not recognized by a carrier cannot partition into the hydrophobic membrane and thus traverse the epithelial barrier via the transcellular pathway. The transport of hydrophilic molecules via the paracellular pathway is, however, restricted by the presence of tight junctions. The tightness of the intercellular junctional complex can be characterized in a leaky to tight epithelium by measuring the TEER. A leaky epithelium has a low TEER less than 100 Ω cm^2 (higher permeability), whereas an intermediate to tight epithelium has a higher TEER of 200−1000 Ω cm^2 (lower permeability).[33] The intestinal epithelium is composed of intercellular junctional complexes that vary in tightness along the length of the gastrointestinal tract: from leaky epithelium in the jejunum to a tighter epithelium in the colon. Like any simplified in vitro model, Caco-2 cells do not possess all of the properties of the intestinal epithelium in vivo. Caco-2 monolayers resemble colonic epithelia in their relative tightness with the TEER of over 250 Ω cm^2, especially with regard to tight junctions.

We hypothesize that the difference between the tightness of the intercellular junction in vivo and in vitro may partially contribute to the disparity of the sotalol permeability in vivo and in vitro. To prove our hypothesis, Caco-2 monolayers of day 14 with relatively low TEER values (220−290 Ω cm^2) were used to test the permeability of sotalol, atenolol, metoprolol, and FITC-dextran. As viewed from the permeability values in Table 3, the transport of hydrophilic marker, FITC-dextran, and hydrophilic drugs, sotalol and atenolol, was significantly increased, whereas the transport of metoprolol, a model drug of transcellular pathway, did not show any increase. The results suggest that a hydrophilic drug like sotalol is able to pass through the leaky intercellular junction via the paracellular pathway. To further confirm the finding, palmitoylcarnitine (PCC), an "opener" of tight junctions,[34,35] was used to test its effect on the permeability of sotalol, metoprolol, and FITC-dextran across Caco-2 monolayers. Caco-2 monolayers treated with 0.2 mM PCC showed dramatic increases in the transport of sotalol and FITC-dextran, but only a slight increase of metoprolol. This enhanced transport coincided with a drop in TEER (around 150 Ω cm^2), indicating that the tight junction was loosened to a certain extent. According to the previous studies,[34,35] the effect of 0.2 mM PCC on the TEER was reversible after

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Table 4. Solubility Test Results

drug	BCS volume[a] (mL) pH 1	pH 3	pH 4	pH 5	pH 7.5	solubility
acebutolol	163 ± 13	132 ± 8	132 ± 5	111 ± 28	98 ± 30	high
atenolol	212 ± 11	132 ± 7	141 ± 11	126 ± 3	109 ± 18	high
labetalol	107 ± 2	116 ± 1	113 ± 2	123 ± 6	115 ± 2	high
metoprolol	122 ± 6	125 ± 4	126 ± 4	122 ± 8	116 ± 4	high
nadolol	136 ± 4	133 ± 10	129 ± 2	140 ± 1	130 ± 7	high
sotalol	124 ± 14	127 ± 5	123 ± 8	122 ± 6	125 ± 1	high
timolol	131 ± 2	116 ± 1	140 ± 1	116 ± 1	126 ± 1	high

[a] BCS volume is calculated by dividing HDS by the measured concentration. The value represents the mean ± SD in triplicate.

Table 5. Biopharmaceutical Classification of the Selected β-blockers Based on Permeability and Solubility

drug	permeability	solubility
acebutolol	low	high
atenolol	low	high
labetalol	high	high
metoprolol	high	high
nadolol	low	high
sotalol	high[a]	high
timolol	high	high

[a] On the basis of available extent of absorption data. [Mis]classified as low permeability based on in vitro Caco-2 permeability data.

removing the PCC and did not appear to involve lysis of the apical membrane. Instead, PCC's effect appeared to be due to loosening of tight junctions. Likewise, the increased permeability of sotalol and FITC-dextran in this study may be attributed to the loosening of tight junctions by PCC. Certainly, clear elucidation of the reasons causing a big disagreement between the in vivo absorption and the in vitro permeability of sotalol requires further studies.

Biopharmaceutical Classification

The results of solubility of the selected drugs are shown in Table 4. According to the BCS guidance,[3] a drug substance is considered highly soluble when the HDS is soluble in ≤250 mL water over a pH range of 1−7.5. Therefore, all of the drugs in our study, acebutolol, atenolol, labetolol, metoprolol, nadolol, sotalol, and timolol, were considered highly soluble drugs.

A drug substance is considered highly permeable when the extent of intestinal absorption in humans is determined to be >90% of an administered dose, based on mass balance or in comparison to an intravenous reference dose. The permeability values in Caco-2 cells of the tested drugs in this study provide an accurate means to classify the extent of in vivo intestinal absorption, with the exception of sotalol. A clear high/low permeability boundary can be established from Figure 1 and drugs with apparent permeability coefficients $>18 \times 10^{-6}$ cm/s exhibited high absorption in humans (>90%). On the basis of this criterion, a classification of the model drugs into high and low permeability−solubility classes was established. This biopharmaceutical classification is presented in Table 5. The relationship among drug permeability, solubility, and absorption in humans suggests that drugs in the high permeability−high solubility group are generally absorbed completely. However, drugs in the low permeability−high solubility group have an extent of absorption between 35 and 70%. With the fact of almost complete extent absorption and over 90% bioavailability, we classified sotalol as a highly permeable compound, although it exhibited a significant disagreement between the in vitro permeability and the in vivo absorption.

High Permeability−High Solubility Group. This class of drugs includes labetalol, metoprolol, sotalol, and timolol. The absorption of these drugs is rapid and complete, with extents of absorption >90%.[14] However, systemic bioavailability of the drugs may be variable due to first-pass metabolism. The oral bioavailability of sotalol hydrochloride is 90−100%, whereas plasma levels following oral administration of metoprolol tablets or timolol tablets approximate 50% of the levels following intravenous administration, indicating about 50% first-pass metabolism.[13] The extensive first-pass metabolism results in only 25% bioavailability of labetalol.[13] In addition, the rate-limiting step to drug absorption for this class of drugs would be gastric emptying if dissolution is very rapid.

Low Permeability−High Solubility Group. Acebutolol, atenolol, and nadolol belong to this class of drugs for which the permeation of the drug across the intestinal membrane is the rate-limiting step in the absorption process. Both the rate and extent of absorption of this group of drugs may be highly variable because of differences in gastrointestinal transit, luminal contents, and membrane permeability, rather than dosage-form-related factors.[1,29]

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