molecular pharmaceutics

articles

High-Permeability Criterion for BCS Classification: Segmental/pH Dependent Permeability Considerations

Arik Dahan,*,‡ Jonathan M. Miller,§ John M. Hilfinger,|| Shinji Yamashita,⊥ Lawrence X. Yu,# Hans Lennernäs,▽ and Gordon L. Amidon§

Department of Clinical Pharmacology, School of Pharmacy, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel, Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, TSRL, Inc., 540 Avis Drive, Suite A, Ann Arbor, Michigan 48108, Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan, Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, Maryland 20857, and Department of Pharmacy, Biopharmaceutics Research Group, Uppsala University, S-75123 Uppsala, Sweden

Received May 17, 2010; Accepted August 10, 2010

Abstract: The FDA classifies a drug substance as high-permeability when the fraction of dose absorbed (F_{abs}) in humans is 90% or higher. This direct correlation between human permeability and F_{abs} has been recently controversial, since the β-blocker sotalol showed high F_{abs} (90%) and low Caco-2 permeability. The purpose of this study was to investigate the scientific basis for this disparity between permeability and F_{abs}. The effective permeabilities (P_{eff}) of sotalol and metoprolol, a FDA standard for the low/high P_{eff} class boundary, were investigated in the rat perfusion model, in three different intestinal segments with pHs corresponding to the physiological pH in each region: (1) proximal jejunum, pH 6.5; (2) mid small intestine, pH 7.0; and (3) distal ileum, pH 7.5. Both metoprolol and sotalol showed pH-dependent permeability, with higher P_{eff} at higher pH. At any given pH, sotalol showed lower permeability than metoprolol; however, the permeability of sotalol determined at pH 7.5 exceeded/matched metoprolol's at pH 6.5 and 7.0, respectively. Physicochemical analysis based on ionization, pK_a and partitioning of these drugs predicted the same trend and clarified the mechanism behind these observed results. Experimental octanol−buffer partitioning experiments confirmed the theoretical curves. An oral dose of metoprolol has been reported to be completely absorbed in the upper small intestine; it follows, hence, that metoprolol's P_{eff} value at pH 7.5 is not likely physiologically relevant for an immediate release dosage form, and the permeability at pH 6.5 represents the actual relevant value for the low/high permeability class boundary. Although sotalol's permeability is low at pH 6.5 and 7.0, at pH 7.5 it exceeds/matches the threshold of metoprolol at pH 6.5 and 7.0, most likely responsible for its high F_{abs}. In conclusion, we have shown that, in fact, there is no discrepancy between P_{eff} and F_{abs} in sotalol's absorption; the data emphasize that, if a compound has high fraction of dose absorbed, it will have high-permeability, not necessarily in the jejunum, but at some point along the relevant intestinal regions.

Keywords: Biopharmaceutics classification system; biowaiver; fraction dose absorbed; high-permeability criterion; intestinal permeability; oral drug absorption

10.1021/mp100175a
Published on Web 08/11/2010

Introduction

Since its publication in 1995, the biopharmaceutics classification system (BCS) has become an increasingly important tool in drug product development and regulation worldwide, by presenting a new paradigm in bioequivalence.[1] Bioequivalence (BE) is the most critical step that connects the physical drug product with the clinical properties claimed on its label, thus ensuring continuing quality of the innovative products and the generic products. Before the development of the BCS, the BE standard was essentially empirical, based on relative *in vivo* bioavailability (BA) studies, i.e. plasma levels, AUC and C_{max}. By revealing the fundamental parameters determining the *in vivo* oral drug absorption process, the BCS has enabled BE assurance through mechanistic tools, rather than empirical observation; if two drug products that contain the same active pharmaceutical ingredient (API) have a similar gastrointestinal (GI) concentration−time profile (i.e., *in vivo* GI release) under all luminal conditions, then a similar rate and extent of absorption is ensured for these products, and they will be necessarily bioequivalent.[2] Therefore, BE can be assured based on dissolution tests that provide the mechanistic proof for similar bioavailability, rather than empirical *in vivo* human studies. This is the scientific and mechanistic rationale provided by the BCS, for the regulatory waiver of *in vivo* BE.[3−6]

The U.S. Food and Drug Administration (FDA) has implemented the BCS to allow a waiver of *in vivo* BE testing for BCS class I, high-solubility, high-permeability drugs in IR fast-dissolving solid dosage forms. A drug substance is classified as high-permeability when the fraction of dose absorbed (F_{abs}) in humans is 90% or higher; based on extensive scientific research it was determined that an excellent correlation existed between the human jejunal permeability (P_{eff}) measured using intestinal perfusion and the fraction of dose absorbed obtained from pharmacokinetic or mass balance studies in humans.[7−11] Accordingly, the FDA guidance indicates that permeability classification can be determined directly by measuring the rate of mass transfer across human intestinal membrane, or indirectly by estimating the extent of drug absorption in human pharmacokinetic studies in comparison to iv reference dose. Alternatively, nonhuman systems capable of predicting the extent of drug absorption in humans can be used to classify a drug if appropriately validated (e.g., intestinal perfusion studies in rats and *in vitro* epithelial cell culture methods).[12]

This direct correlation between intestinal permeability and the fraction of dose absorbed in the high-permeability criterion has been recently debated; for a few drugs with high F_{abs}, low transepithelial permeability across Caco-2 cells was reported.[13] While evidence for carrier-mediated transport, mainly by hPEPT1, exists for a majority of these drugs, and hence the Caco-2 system is obviously not the optimal experimental method, the case of the β-blocker sotalol, with no evidence for carrier-mediated transport, represents a valid challenge to the BCS classification approach. Based on plasma levels following oral vs intravenous administration, the oral absorption of sotalol in humans has been shown to

* Corresponding author. Mailing address: P.O. Box 653, Beer-Sheva 84105, Israel. Tel: (972) 8 6479483. Fax: (972) 8 6479303. E-mail: arikd@bgu.ac.il.
‡ Ben-Gurion University of the Negev.
§ University of Michigan.
|| TSRL, Inc.
⊥ Setsunan University.
Food and Drug Administration.
▽ Uppsala University.

(1) Amidon, G. L.; Lennernas, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* **1995**, *12*, 413.
(2) Dahan, A.; Miller, J. M.; Amidon, G. L. Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. *AAPS J.* **2009**, *11*, 740–746.
(3) Lobenberg, R.; Amidon, G. L. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 3–12.
(4) Martinez, M. N.; Amidon, G. L. A mechanistic approach to understanding the factors affecting drug absorption: A review of fundamentals. *J. Clin. Pharmacol.* **2002**, *42*, 620–643.
(5) Yamashita, S.; Tachiki, H. Analysis of risk factors in human bioequivalence study that incur bioinequivalence of oral drug products. *Mol. Pharmaceutics* **2009**, *6*, 48–59.
(6) Yu, L. X.; Amidon, G. L.; Polli, J. E.; Zhao, H.; Mehta, M. U.; Conner, D. P.; Shah, V. P.; Lesko, L. J.; Chen, M. L.; Lee, V. H. L.; Hussain, A. S. Biopharmaceutics classification system: The scientific basis for biowaiver extensions. *Pharm. Res.* **2002**, *19*, 921.
(7) Amidon, G. L.; Sinko, P. J.; Fleisher, D. Estimating human oral fraction dose absorbed: a correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds. *Pharm. Res.* **1988**, *5*, 651–654.
(8) Knutson, T.; Fridblom, P.; Ahlstrom, H.; Magnusson, A.; Tannergren, C.; Lennernas, H. Increased understanding of intestinal drug permeability determined by the LOC-I-GUT approach using multislice computed tomography. *Mol. Pharmaceutics* **2009**, *6*, 2–10.
(9) Lennernas, H. Human intestinal permeability. *J. Pharm. Sci.* **1998**, *87*, 403–410.
(10) Lennernas, H. Animal data: the contributions of the Ussing chamber and perfusion systems to predicting human oral drug delivery in vivo. *Adv. Drug Delivery Rev.* **2007**, *59*, 1103–1120.
(11) Sun, D.; Lennernas, H.; Welage, L. S.; Barnett, J. L.; Landowski, C. P.; Foster, D.; Fleisher, D.; Lee, K.-D.; Amidon, G. L. Comparison of human duodenum and caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs. *Pharm. Res.* **2002**, *19*, 1400–1416.
(12) CDER/FDA. Guidance for industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system; Center for Drug Evaluation and Research: 2000.
(13) Chen, M.-L.; Yu, L. The use of drug metabolism for prediction of intestinal permeability. *Mol. Pharmaceutics* **2009**, *6*, 74–81.
(14) Deneer, V.; Lie-A-Huen, L.; Kingma, J.; Proost, J.; Kelder, J.; Brouwers, J. Absorption kinetics of oral sotalol combined with cisapride and sublingual sotalol in healthy subjects. *Br. J. Clin. Pharmacol.* **1998**, *45*, 485–490.

be nearly complete.[14−17] However, transepithelial transport studies across Caco-2 cell monolayers revealed low permeability for sotalol.[18−20]

The purpose of this study was to investigate the basis for this difference between sotalol's permeability in the tissue culture system and *in vivo* F_{abs} in humans. The effective permeability (P_{eff}) of sotalol was investigated in the *in situ* single-pass rat perfusion model, in three different intestinal segments. Metoprolol, a FDA reference drug for the low/high P_{eff} class boundary, was coperfused with sotalol for direct comparison. To maximize pseudophysiological conditions, the experiments were carried out at pHs corresponding to the physiological pH in each intestinal region: (1) proximal jejunum, pH 6.5; (2) mid small intestine, pH 7.0; and (3) distal ileum, pH 7.5. Theoretical physicochemical analyses based on ionization, pK_a and partitioning of sotalol and metoprolol were also performed, to further elucidate the mechanism behind the experimental data. Thus this work aims to determine whether sotalol's oral absorption represents a case of discrepancy between the fraction of dose absorbed, F_{abs}, and the intestinal permeability, P_{eff}.

Materials and Methods

Materials. Sotalol, metoprolol, phenol red, potassium phosphate monobasic and trifluoroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Potassium chloride, sodium phosphate dibasic and NaCl were obtained from Fisher Scientific Inc. (Pittsburgh, PA). Acetonitrile and water (Acros Organics, Geel, Belgium) were HPLC grade. Physiological saline solution was purchased from Hospira Inc. (Lake Forest, IL). All other chemicals were of analytical reagent grade.

Single-Pass Intestinal Perfusion Studies (SPIP) in Rats. All animal experiments were conducted using protocols approved by the University of Michigan Committee of Use and Care of Animals (UCUCA) and the Ben-Gurion University of the Negev Animal Use and Care Committee. Male Wistar rats (Charles River, IN) weighing 250−280 g were used for all perfusion studies. Prior to each experiment, the rats were fasted overnight (12−18 h) with free access to water. Animals were randomly assigned to the different experimental groups.

The procedure for the *in situ* single-pass intestinal perfusion followed previously published reports.[21−23] Briefly, rats were anesthetized with an intramuscular injection of 1 mL/kg ketamine−xylazine solution (9%:1%, respectively) and placed on a heated surface maintained at 37 °C (Harvard Apparatus Inc., Holliston, MA). The abdomen was opened by a midline incision of 3−4 cm. A proximal jejunal segment, midsmall intestinal segment, or a distal ileal segment of approximately 10 cm was carefully exposed and cannulated on two ends with flexible PVC tubing (2.29 mm i.d., inlet tube 40 cm, outlet tube 20 cm, Fisher Scientific Inc., Pittsburgh, PA). Care was taken to avoid disturbing the circulatory system, and the exposed segment was kept moist with 37 °C normal saline solution. All solutions were incubated in a 37 °C water bath. The isolated segment was rinsed with blank perfusion buffer at a flow rate of 0.5 mL/min in order to clean out any residual debris.

Three perfusion buffers, corresponding to the perfused segments jejunum, mid small intestine, and ileum, were prepared with different ratios of potassium phosphate monobasic and sodium phosphate dibasic, to give pHs of 6.5, 7.0, and 7.5. Similar ionic strength and osmolarity (290 mosm/L) were maintained in all buffers. At the start of the study, perfusion solution containing sotalol, metoprolol, and phenol red (0.05 mM) was perfused through the intestinal segment (Watson Marlow Pumps 323S, Watson-Marlow Bredel Inc., Wilmington, MA), at a flow rate of 0.2 mL/min. Phenol red was added as a nonabsorbable marker for water flux measurements. Metoprolol was coperfused as a reference standard for permeability in close proximity to the low/high permeability class boundary.[24] The perfusion buffer was first perfused for 1 h, in order to ensure steady state conditions. After reaching steady-state, samples were taken at 10 min intervals for 1 h. The pH of the collected samples was measured at the outlet, to verify that there was no pH change throughout the perfusion. All samples including perfusion samples at different time points, original drug solution, and inlet solution taken at the exit of the syringe

(15) Funck-Brentano, C. Pharmacokinetic and pharmacodynamic profiles of d-sotalol and d,l-sotalol. *Eur. Heart J.* **1993**, *14*, 30–35.
(16) Hanyok, J. Clinical pharmacokinetics of sotalol. *Am. J. Cardiol.* **1993**, *72*, 19A–26A.
(17) Poirier, J. M.; Jaillon, P.; Lecocq, B.; Lecocq, V.; Ferry, A.; Cheymol, G. The pharmacokinetics of d-sotalol and d,l-sotalol in healthy volunteers. *Eur. J. Clin. Pharmacol.* **1990**, *38*, 579–582.
(18) Alt, A.; Potthast, H.; Moessinger, J.; Sickmüller, B.; Oeser, H. Biopharmaceutical characterization of sotalol-containing oral immediate release drug products. *Eur. J. Pharm. Biopharm.* **2004**, *58*, 145–150.
(19) Bachmakov, I.; Werner, U.; Endress, B.; Auge, D.; Fromm, M. F. Characterization of beta-adrenoceptor antagonists as substrates and inhibitors of the drug transporter P-glycoprotein. *Fundam. Clin. Pharmacol.* **2006**, *20*, 273–282.
(20) Yang, Y.; Faustino, P. J.; Volpe, D. A.; Ellison, C. D.; Lyon, R. C.; Yu, L. X. Biopharmaceutics classification of selected beta-blockers: solubility and permeability class membership. *Mol. Pharmaceutics* **2007**, *4*, 608–614.
(21) Dahan, A.; Amidon, G. L. Segmental dependent transport of low permeability compounds along the small intestine due to P-glycoprotein: The role of efflux transport in the oral absorption of BCS class III drugs. *Mol. Pharmaceutics* **2009**, *6*, 19–28.
(22) Dahan, A.; Amidon, G. L. Grapefruit juice and its constituents augment colchicine intestinal absorption: Potential hazardous interaction and the role of P-glycoprotein. *Pharm. Res.* **2009**, *26*, 883–892.
(23) Kim, J. S.; Mitchell, S.; Kijek, P.; Tsume, Y.; Hilfinger, J.; Amidon, G. L. The suitability of an in situ perfusion model for permeability determinations: Utility for BCS class I biowaiver requests. *Mol. Pharmaceutics* **2006**, *3*, 686–694.
(24) Dahan, A.; Amidon, G. L. Small intestinal efflux mediated by MRP2 and BCRP shifts sulfasalazine intestinal permeability from high to low, enabling its colonic targeting. *Am. J. Physiol.* **2009**, *297*, G371–G377.

were immediately assayed by HPLC. Following the termination of the experiment, the length of each perfused intestinal segment was accurately measured.

The effective permeability (P_{eff}) through the rat gut wall in the single-pass intestinal perfusion studies was determined assuming the "plug flow" model expressed in the following equation:

$$P_{eff}\,(\mathrm{cm/s}) = \frac{-Q\ln(C'_{out}/C'_{in})}{2\pi RL}$$

where Q is the perfusion buffer flow rate, C'_{out}/C'_{in} is the ratio of the outlet and the inlet concentration of the tested drug that has been adjusted for water transport, R is the radius of the intestinal segment (set to 0.2 cm), and L is the length of the intestinal segment.

The net water flux in the single-pass intestinal perfusion studies was determined by measurement of phenol red, a nonabsorbed, nonmetabolized marker. The phenol red (0.05 mg/mL) was included in the perfusion buffer and coperfused with the tested drugs. The measured C_{out}/C_{in} ratio was corrected for water transport according to the following equation:

$$\frac{C'_{out}}{C'_{in}} = \frac{C_{out}}{C_{in}} \times \frac{C_{in\ phenol\ red}}{C_{out\ phenol\ red}}$$

where $C_{in\ phenol\ red}$ and $C_{out\ phenol\ red}$ are equal to the concentration of phenol red in the inlet and the outlet sample, respectively.

Analytical Methods. The simultaneous analysis of sotalol, metoprolol, and phenol red in the rat perfusion buffer was assayed using a high performance liquid chromatography (HPLC) system (Waters 2695 separation module) with a photodiode array UV detector (Waters 2996). Samples were filtered (Unifilter 96 wells microplate 0.45 μm filters, Whatman Inc., Florham Park, NJ), and aliquots of 10 μL were injected into the HPLC system. The HPLC conditions were as follows: XTerra, RP_{18}, 3.5 μm, 4.6 × 100 mm column (Waters Co., Milford, MA); a gradient mobile phase, going from 90:10 to 50:50% v/v aqueous/organic phase respectively over 15 min; the aqueous phase was 0.1% trifluoroacetic acid in water, and the organic phase was 0.1% trifluoroacetic acid in acetonitrile; flow rate of 1 mL/min at room temperature. The detection wavelengths were 275, 265, and 350 nm, and the retention times were 6.5, 9.5, and 12.0 min for metoprolol, phenol red, and sotalol, respectively. Separate standard curves were used for each experiment ($R^2 > 0.99$). The inter- and intraday coefficients of variation were <1.0 and 0.5%, respectively.

Physicochemical Analysis. The theoretical fraction extracted into octanol (f_e) was calculated using the following equation from Wagner and Sedman:[25]

$$f_e = \frac{f_u P}{1 + f_u P}$$

where P is the octanol−water partition coefficient of the un-ionized form of the drug and f_u is the fraction un-ionized drug at a given pH. f_u was calculated using the following equations:

$$f_u = 1 - |Z_c|$$

where Z_c is the net charge of the compound at a given pH. Z_c was calculated using the following equation:[26]

$$Z_c = \sum_{i=1}^{n} \frac{1}{1 + 10^{\mathrm{pH}-\mathrm{p}K_a(i)}} - \sum_{j=1}^{p} \frac{1}{1 + 10^{\mathrm{p}K_a(j)-\mathrm{pH}}}$$

where n and p are the integral numbers of basic and acidic groups on the molecules, respectively. $pK_a(i)$ is the negative log of the dissociation constants of basic moieties, and $pK_a(j)$ is the negative log of the dissociation constants of acidic moieties. Theoretical f_e and f_u as functions of pH were then calculated using literature experimental values for pK_a and Log P (Table 1).[27−29]

Experimental octanol−buffer partition coefficients for metoprolol and sotalol at pH 6.5, 7.0, and 7.5 were determined using a previously reported shake-flask method.[30]

Statistical Analysis. Animal experiments were $n = 4-6$. The data are presented as mean ± SD. To determine statistically significant differences among the experimental groups, the nonparametric Kruskal−Wallis test was used for multiple comparisons and the two-tailed nonparametric Mann−Whitney U test for two-group comparison where appropriate. A p value of less than 0.05 was termed significant.

Results

Sotalol vs Metoprolol Segmental/pH Dependent Permeability. The *in situ* rat permeability of sotalol and metoprolol in three small intestinal segments at the physi-

(25) Wagner, J. G.; Sedman, A. J. Quantitaton of rate of gastrointestinal and buccal absorption of acidic and basic drugs based on extraction theory. *J. Pharmacokinet. Pharmacodyn.* **1973**, *1*, 23–50.

(26) Miller, J. M.; Blackburn, A. C.; Shi, Y.; Melzak, A. J.; Ando, H. Y. Semi-empirical relationships between effective mobility, charge, and molecular weight of pharmaceuticals by pressure-assisted capillary electrophoresis: Applications in drug discovery. *Electrophoresis* **2002**, *23*, 2833–2841.

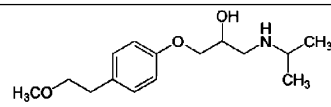
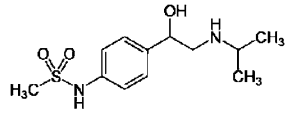
(27) Burgot, G.; Serrand, P.; Burgot, J. L. Thermodynamics of partitioning in the n-octanol/water system of some beta-blockers. *Int. J. Pharm.* **1990**, *63*, 73–76.

(28) Henchoz, Y.; Guillarme, D.; Martel, S.; Rudaz, S.; Veuthey, J.-L.; Carrupt, P.-A. Fast log P determination by ultra-high-pressure liquid chromatography coupled with UV and mass spectrometry detections. *Anal. Bioanal. Chem.* **2009**, *394*, 1919–1930.

(29) Shalaeva, M.; Kenseth, J.; Lombardo, F.; Bastin, A. Measurement of dissociation constants (pKa values) of organic compounds by multiplexed capillary electrophoresis using aqueous and cosolvent buffers. *J. Pharm. Sci.* **2008**, *97*, 2581–606.

(30) Miller, J. M.; Dahan, A.; Gupta, D.; Varghese, S.; Amidon, G. L. Quasi-equilibrium analysis of the ion-pair mediated membrane transport of low-permeability drugs. *J. Controlled Release* **2009**, *137*, 31–37.

Table 1. Sotalol and Metoprolol Physicochemical Properties

Compound	Chemical Structure	pK_a [29]	Log *P*
Metoprolol		9.51	2.2 [28]
Sotalol		8.38, 9.47	0.24 [27]

ological pH of each region is presented in Figure 1. Both metoprolol and sotalol showed segmental/pH dependent permeability, with higher P_{eff} at distal regions/higher pH. At any given segment/pH, sotalol showed lower permeability than metoprolol; however, the permeability of sotalol from the ileum at pH 7.5 exceeded/matched metoprolol's P_{eff} from the jejunum/mid small intestine at pHs 6.5 and 7.0, respectively.

To determine whether the permeabilities of sotalol and metoprolol presented in Figure 1 are pH-dependent as opposed to other segmental related factors, we have measured the drugs' P_{eff} in two additional perfusion conditions. Figure 2 compares the drugs' jejunal and ileal permeability at the physiological pH of the segment (6.5 and 7.5 respectively) vs the alternate pH, 7.5 and 6.5 respectively. It can be seen that the permeability followed the pH and not the segment; the drugs' P_{eff} values were equal when the perfusion buffer was at pH 6.5 irrespective of the segment being perfused. Similarly, perfusing the jejunum with pH 7.5 buffer resulted in P_{eff} equal to that from the ileum at pH 7.5. This experiment clearly indicates that, for sotalol and metoprolol, the segmental-dependent P_{eff} revealed in Figure 1 is completely pH-dependent and cannot be attributed to any other region related factors.

Physicochemical Analysis. Literature experimental pK_a and Log *P* values for metoprolol and sotalol are summarized in Table 1. The Log *P* value of metoprolol is ~2 orders of magnitude higher than that of sotalol, indicating significantly enhanced lipophilicity of the former. Both compounds possess a basic secondary amine functionality. Sotalol also has the acidic methanesulfonamide functionality, thus sotalol is an amphoteric molecule. The secondary amine pK_a of metoprolol has been determined to be 9.51.[29] This would suggest that, for sotalol, the pK_a of 9.47 is due to the secondary amine and the pK_a of 8.38 is due to the meth-

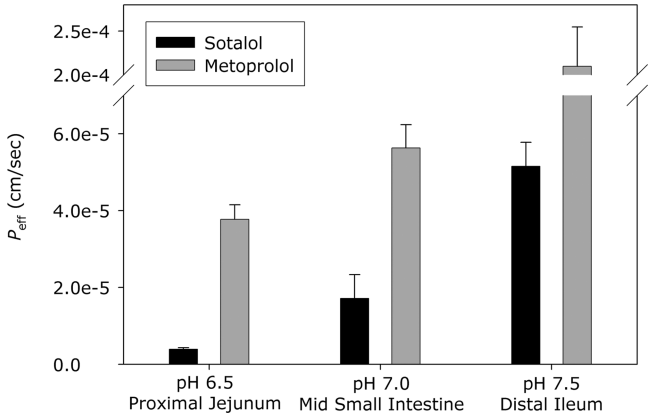


Figure 1. Effective permeability values (P_{eff}; cm/s) obtained for sotalol and metoprolol after *in situ* single pass perfusion to the rat proximal jejunum at pH 6.5, mid small intestine at pH 7.0, and distal ileum at pH 7.5. Data are presented as the mean ± SD; $n = 6$ in each experimental group.

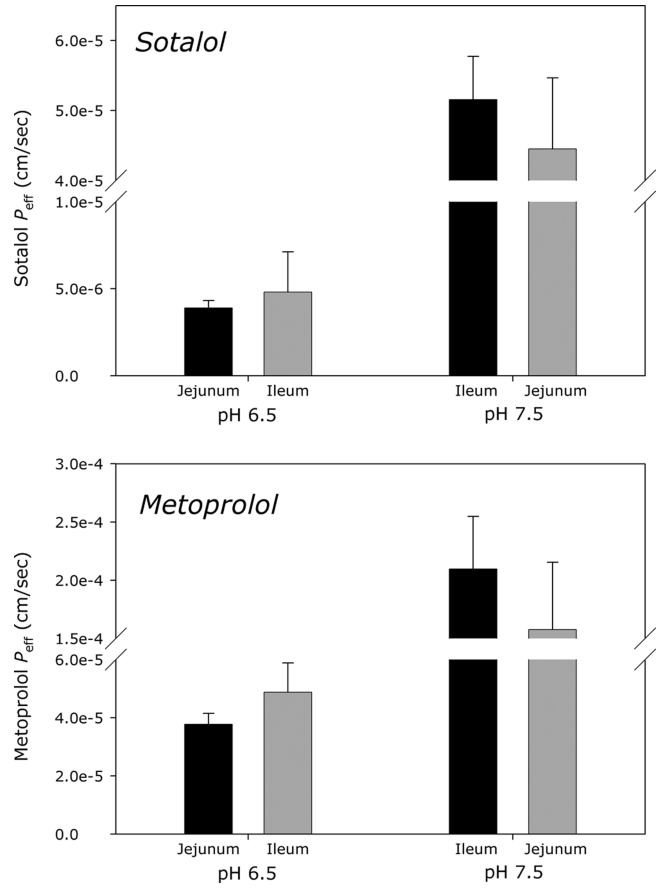


Figure 2. Comparison of the permeability values (cm/s) obtained for sotalol and metoprolol in the rat jejunum and ileum at the physiological pH of the segment (6.5 and 7.5 respectively; $n = 6$) vs the alternate pH, 7.5 and 6.5 respectively ($n = 4$). Data are presented as the mean ± SD.

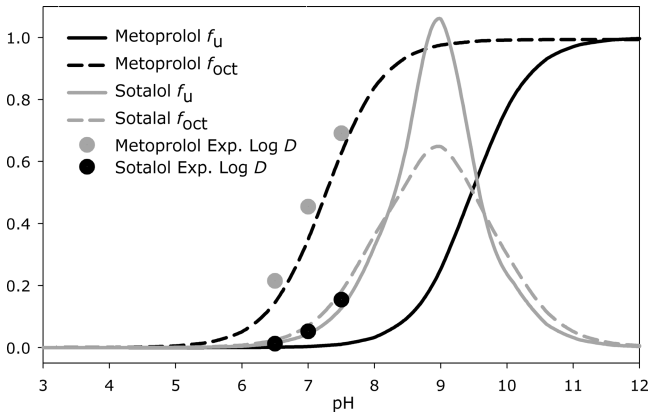


Figure 3. The theoretical fraction un-ionized (f_u) and fraction extracted into octanol (f_e) plots as a function of pH for metoprolol and sotalol, and experimental buffer−octanol partitioning of the drugs in the three pHs 6.5, 7.0, and 7.5 ($n = 3$).

anesulfonamide. However, the pK_a values of sotalol cannot be assigned definitively from the literature values.

The theoretical fraction un-ionized (f_u) and fraction extracted into octanol (f_e) plots as a function of pH for metoprolol and sotalol are presented in Figure 3. The f_u and f_e profiles were calculated using the experimental pK_a and Log P values in Table 1 as detailed in Materials and Methods. The f_u of metoprolol is zero at low pH due to protonation of the secondary amine, and as the pH rises, the f_u increases to give the sigmoidal profile. The f_e versus pH plot for metoprolol follows the same sigmoidal profile, except the f_e curve is shifted to lower pH values, with a shift magnitude of Log($P_B - 1$) at the midpoint of the f_e and f_u curves.[25,31] The f_u of sotalol is also zero at low pH, as protonation of both the secondary amine and the methanesulfonamide leads to an overall net positive charge. As pH rises, the f_u of sotalol gradually increases, reaching a maximum at the isoelectric point of sotalol (pH = 8.9). As pH is increased further above the isoelectric point, f_u gradually decreases again to zero as sotalol becomes more negatively charged due to deprotonation of both the secondary amine and the methanesulfonamide. The f_e versus pH curve for sotalol follows the same trend as the f_u plot, except the plateau value for the f_e curve is about 0.6. Since Log($P - 1$) of sotalol is near zero, there is essentially no shift between the f_u and f_e curves. Comparison between the theoretical f_e of sotalol and metoprolol at the one pH unit between 6.5 and 7.5 reveal the same trend observed in the rat permeability studies; at any given pH, f_e of metoprolol is higher than that of sotalol, however, sotalol's f_e at pH 7.5 matched that of metoprolol at 6.5. Experimental buffer−octanol partitioning of the drugs in the three pHs 6.5, 7.0, and 7.5 were in good agreement with theoretical plots.

(31) Winne, D. Shift of pH-absorption curves. *J. Pharmacokinet. Biopharm.* **1977**, *5*, 53–94.

Discussion

It is generally recognized that if a drug has ≥90% fraction dose absorbed, the drug must be highly permeable in the human intestine, although actual permeability data may not be available. The case of sotalol represented a challenge for this direct correlation between intestinal permeability and fraction dose absorbed, since sotalol has low Caco-2 permeability while ≥90% extent of absorption was reported for this β-blocker. The purpose of this paper was to investigate this apparent disparity in the intestinal absorption characteristics of sotalol.

At any given small intestinal segment/pH, sotalol's permeability was shown to be lower than that of metoprolol, suggesting a discrepancy between permeability and F_{abs} in the oral absorption of sotalol. However, a more thorough determination of sotalol's permeability profile in the intestine reveals that sotalol's permeability at pH 7.5 exceeds that of metoprolol at pH 6.5 and matches metoprolol's P_{eff} at pH 7.0. An IR oral dose of metoprolol has been shown to be completely absorbed in the upper small intestine, in both humans and animal models. Masaoka et al. have shown that, in rats, 90% of an oral dose of metoprolol is absorbed prior to the jejunum, and the rest, 10%, is absorbed in the upper jejunum.[32] In humans, Jobin et al. have used the intubation technique to show that 60% of a 100 mg metoprolol oral dose is absorbed from the duodenum, and an additional 20% is absorbed from the following 30 cm of the jejunum.[33] Overall, a nearly complete absorption of metoprolol dose was observed from as little as 50 cm out of ~3 m of small intestine. It follows, hence, that metoprolol's P_{eff} value at pH 7.5 is not likely physiologically relevant for an immediate release dosage form, rather the permeability at pH 6.5, the average pH of the human jejunum, represents the actual benchmark for the low/high permeability class boundary. Although sotalol's permeability was found to be low at pHs 6.5 and 7.0, at pH 7.5 it *exceeds* the threshold of metoprolol at pH 6.5, and actually matches metoprolol's P_{eff} at 7.0. Since 7.5 is the average pH in the human ileum, which accounts for approximately half of the small intestinal length, an oral dose of sotalol is under high-permeability conditions throughout a significant portion of the small intestinal residence time. This analysis reveals that no discrepancy between P_{eff} and F_{abs} is involved in sotalol's absorption; the data emphasize that if a compound has high fraction of dose absorbed, it will have high-permeability, not necessarily in the jejunum, but somewhere along the relevant intestinal absorbing regions. Sotalol's high permeability in the distal small intestine compensates for its low permeability in the proximal

(32) Masaoka, Y.; Tanaka, Y.; Kataoka, M.; Sakuma, S.; Yamashita, S. Site of drug absorption after oral administration: Assessment of membrane permeability and luminal concentration of drugs in each segment of gastrointestinal tract. *Eur. J. Pharm. Sci.* **2006**, *29*, 240–250.

(33) Jobin, G.; Cortot, A.; Godbillon, J.; Duval, M.; Schoeller, J.; Hirtz, J.; Bernier, J. Investigation of drug absorption from the gastrointestinal tract of man. I. Metoprolol in the stomach, duodenum and jejunum. *Br. J. Clin. Pharmacol.* **1985**, *19*, 97S–105S.

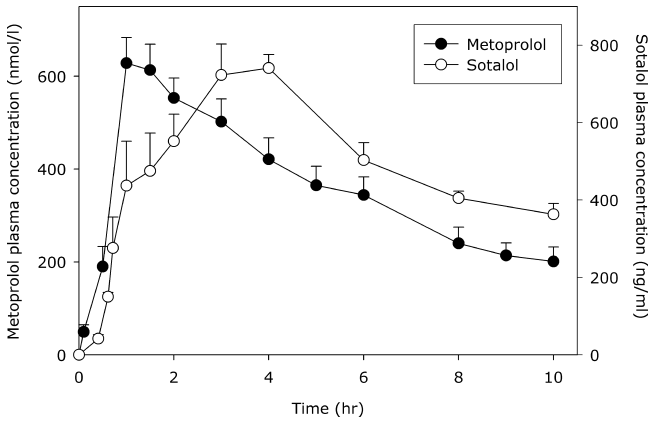


Figure 4. Human plasma concentration time profiles of metoprolol (●; $n = 12$) and sotalol (○; $n = 6$) following oral administration of 100 mg dose to healthy subjects. Data was digitalized using DigIt software (Simulations Plus Inc., Lancaster, CA) from Sandberg et al.[34] and from Poirier et al.[17]

segments, resulting in its high extent of absorption. The pharmacokinetic profiles of sotalol and metoprolol, specifically the time to reach maximal plasma concentration (t_{max}), support this analysis; Figure 4 illustrates human plasma profiles of sotalol ($n = 6$) and metoprolol ($n = 12$) following oral administration of a 100 mg dose to healthy subjects.[17,34] While metoprolol reaches maximal plasma concentration within ~1 h, certainly indicating rapid absorption from the proximal small intestine (following gastric emptying), sotalol maximal plasma levels are reached only after ~4 h, signifying absorption from the distal parts of the small intestine (elimination rates of the drugs were comparable, with $t_{1/2}$ ~6−7 h).

The underlying mechanism behind the distinctive intestinal permeability profile of sotalol could be demonstrated by the physicochemical analysis presented in Figure 3. For sotalol, the one pH unit between 6.5 and 7.5, which is the most relevant one within the small intestine, makes the whole difference; at pH 6.5, that is ~two pH units away from the lower pK_a (8.38, Table 1), the vast majority of the drug is positively charged, and considering sotalol's Log *P*, absorption under these conditions is low. As the pH rises, a significant fraction of the drug becomes neutral, followed by increased permeability. Comparison between the f_e vs pH plot of sotalol and metoprolol at the one pH unit between 6.5 and 7.5 revealed the same trend observed in the rat permeability studies; although at any given pH metoprolol's f_e is higher than that of sotalol, sotalol's f_e at pH 7.5 matched that of metoprolol at 6.5. Naturally, such a scenario may happen only for basic moieties; the permeability of an acidic moiety can only decrease as the pH rises, however, additional characteristics are required for that to occur. The extraction into octanol (f_e) curve of a basic drug is shifted to lower pH values than the f_u vs pH curve, with a shift magnitude of Log($P_B - 1$) at the midpoint of the f_e and f_u curves.[25,31] This shift is well illustrated by metoprolol curves in Figure 3, showing the two pH units between the f_u and the f_e vs pH ($P = 100$; Log($100 - 1$) = 1.99). For sotalol, with Log *P* near zero, there is essentially no shift, and the f_u and f_e curves are placed one on each other. Since the change in the f_u of sotalol is significant in the pH unit between 6.5 and 7.5, the f_e curve, which corresponds to the permeability profile, is also changing considerably throughout this narrow pH range. Overall, it is the combination of a basic moiety, with pK_a and Log *P* values in a critical range, that results in sotalol's unique permeability pattern. It should be noted that sotalol is an amphoteric drug, and above pH 8.9, the isoelectric point of sotalol, the fraction ionized starts to increase again (now with negative charge), followed by permeability decrease. However, the isoelectric point of sotalol is beyond the physiologically relevant pH range, and therefore sotalol can effectively be treated as a weak base.

The results presented in this paper directly relate to the BCS classification of sotalol. The FDA definition of high-permeability is based on human F_{abs}, stating that a drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose.[6,12,35] This link between F_{abs} and P_{eff} is strengthened by the results of this study, as we have shown that there is no disparity in the intestinal absorption of sotalol. Rather, the data stress that high F_{abs} accompanies high P_{eff}, not necessarily in the jejunum, but somewhere along the relevant intestinal regions. This finding points out a possible extension to the regulatory high-permeability criterion: taking metoprolol's jejunal P_{eff} at pH 6.5 as the benchmark for high-permeability, the results of this study suggest that if a compound matches/exceeds this threshold anywhere in the intestine, and not necessarily in the jejunum, it is a high-permeability compound.

The intestinal permeability along the human intestine may be segmental dependent in an, at present, largely unknown fashion. Given the lack of experimental data in humans, especially human ileal permeability, the best we can do today is evaluate animal segmental dependence that can give a

(34) Sandberg, A.; Blomqvist, I.; Jonsson, U.; Lundborg, P. Pharmacokinetic and pharmacodynamic properties of a new controlled-release formulation of metoprolol: a comparison with conventional tablets. *Eur. J. Clin. Pharmacol.* **1988**, *33*, S9–14.

(35) Polli, J. E.; Yu, L. X.; Cook, J. A.; Amidon, G. L.; Borchardt, R. T.; Burnside, B. A.; Burton, P. S.; Chen, M.-L.; Conner, D. P.; Faustino, P. J.; Hawi, A. A.; Hussain, A. S.; Joshi, H. N.; Kwei, G.; Lee, V. H. L.; Lesko, L. J.; Lipper, R. A.; Loper, A. E.; Nerurkar, S. G.; Polli, J. W.; Sanvordeker, D. R.; Taneja, R.; Uppoor, R. S.; Vattikonda, C. S.; Wilding, I.; Zhang, G. Summary workshop report: Biopharmaceutics classification system - implementation challenges and extension opportunities. *J. Pharm. Sci.* **2004**, *93*, 1375–1381.

(36) Dahan, A.; Sabit, H.; Amidon, G. L. Multiple efflux pumps are involved in the transepithelial transport of colchicine: Combined effect of P-gp and MRP2 leads to decreased intestinal absorption throughout the entire small intestine. *Drug Metab. Dispos.* **2009**, *37*, 2028–2036.

strong indication of the expected situation in humans.[21,32,36−39] In this report we address a controversy surrounding the high-permeability determination of sotalol, which we have shown to be due to segmental/pH dependence. Sotalol is a high permeability drug, based on the "gold" standard of human F_{abs}. The low sotalol Caco-2 permeability reported by Yang et al. ($\sim 4 \times 10^{-6}$ cm/s) was obtained with apical pH of 6.8,[20] and that reported by Alt et al. ($\sim 2 \times 10^{-6}$ cm/s) was obtained with apical pH of 6.5.[18] These studies demonstrate that while Caco-2 data may be valuable and can reduce the need for human and animal experiments, *in vitro* data must always be carefully evaluated in light of the various parameters that deviate from the (more complex) *in vivo* situation in humans.[40,41] We emphasize that segmental dependence may be important for many drugs and care must be taken in making a case for high permeability when making regulatory decisions. It should be noted that metoprolol, with nearly complete extent of absorption, is a conservative reference drug for the low/high permeability class boundary, and using a lower permeability drug as the threshold reference, e.g. labetalol ($F_{abs} = 90\%$), is generally acceptable.

In 2005, Wu and Benet have noticed that the high-permeability BCS class I and II drugs are eliminated by extensive metabolism (presumably having ready access to metabolizing enzymes within the hepatocytes), and observed that there may be a good correlation between the extent of drug metabolism and the permeability as defined in the BCS.[42] This Biopharmaceutics Drug Disposition Classification System (BDDCS) suggested that if the major route of elimination of a given drug is metabolism, then the drug is high-permeable, and if the major route of elimination is renal and biliary excretion of unchanged drug, then that drug should be classified as low-permeability.[43−45] Comparisons between the BCS and the BDDCS, asking whether a drug will possess the characteristics of high intestinal permeability inasmuch as the drug is metabolized extensively, have been the subject of extensive research and discussions.[2,13,44,46] With regard to the scope of this paper, sotalol represents a case in which the BCS and BDDCS are in disagreement; as shown in this paper, when more thoroughly evaluated, sotalol is a high-permeability compound according to the BCS, however it is poorly metabolized with over 80% urinary excretion of unchanged drug.[16,17] Sotalol, hence, would be misclassified as a class III compound according to the BDDCS. Thus, while the extent of drug metabolism may be useful in supporting permeability classification under certain circumstances, the case presented in this paper demonstrates that care must be taken in intestinal permeability/extent of absorption determinations.

Conclusions

In conclusion, the data presented in this report demonstrate that no discrepancy between P_{eff} and F_{abs} is involved in the intestinal absorption of sotalol, rather, the high fraction of dose absorbed is due to high-permeability, not necessarily in the jejunum, but somewhere along the relevant intestinal regions.

Acknowledgment. The opinions expressed in this article do not necessarily represent the views or policies of the U.S. Food and Drug Administration.

MP100175A

(37) Dahan, A.; West, B. T.; Amidon, G. L. Segmental-dependent membrane permeability along the intestine following oral drug administration: Evaluation of a triple single-pass intestinal perfusion (TSPIP) approach in the rat. *Eur. J. Pharm. Sci.* **2009**, *36*, 320–329.

(38) Narawane, M.; Podder, S. K.; Bundgaard, H.; Lee, V. H. L. Segmental differences in drug permeability, esterase activity and ketone reductase activity in the albino rabbit intestine. *J. Drug Targeting* **1993**, *1*, 29–39.

(39) Ungell, A. L.; Nylander, S.; Bergstrand, S.; Sjöberg, Å.; Lennernäs, H. Membrane transport of drugs in different regions of the intestinal tract of the rat. *J. Pharm. Sci.* **1998**, *87*, 360–366.

(40) Sun, D.; Yu, L.; Hussain, M.; Wall, D.; Smith, R.; Amidon, G. In vitro testing of drug absorption for drug 'developability' assessment: forming an interface between in vitro preclinical data and clinical outcome. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 75–85.

(41) Thiel-Demby, V. E.; Humphreys, J. E.; St. John Williams, L. A.; Ellens, H. M.; Shah, N.; Ayrton, A. D.; Polli, J. W. Biopharmaceutics classification system: Validation and learnings of an in vitro permeability assay. *Mol. Pharmaceutics* **2009**, *6*, 11–18.

(42) Wu, C.-Y.; Benet, L. Z. Predicting drug disposition via application of BCS: Transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm. Res.* **2005**, *22*, 11–23.

(43) Benet, L. Z. The drug transporter-metabolism alliance: uncovering and defining the interplay. *Mol. Pharmaceutics* **2009**, *6*, 1631–1643.

(44) Benet, L.; Amidon, G. L.; Barends, D.; Lennernäs, H.; Polli, J.; Shah, V.; Stavchansky, S.; Yu, L. The use of BDDCS in classifying the permeability of marketed drugs. *Pharm. Res.* **2008**, *25*, 483–488.

(45) Custodio, J. M.; Wu, C.-Y.; Benet, L. Z. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Adv. Drug Delivery Rev.* **2008**, *60*, 717–733.

(46) Takagi, T.; Ramachandran, C.; Bermejo, M.; Yamashita, S.; Yu, L. X.; Amidon, G. L. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol. Pharmaceutics* **2006**, *3*, 631–643.