Capric Acid Absorption in the Presence of Hydroxypropyl-β-Cyclodextrin in the Rat Ileum using the *In Situ* Single-Pass Perfusion Technique

RICHARD V. HYMAS, NORMAN F. H. HO, WILLIAM I. HIGUCHI

Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah

Received 29 July 2014; revised 22 September 2014; accepted 22 September 2014

Published online 12 November 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24212

ABSTRACT: The purpose of the present study was to gain quantitative mechanistic insight into the role cyclodextrin carriers may play in the intestinal absorption of highly lipophilic molecules. The physical model approach was employed to investigate capric acid absorption in the rat ileum using the *in situ* single-pass method with 2-hydroxypropyl-β-cyclodextrin (HPB) present in the perfusate. Two physical models were examined: the flat surface model in which the intestinal wall was treated as a hollow, smooth, circular cylinder, and the villus model in which the intestinal surface allowed for the presence of villi. Capric acid absorption was found to be essentially 100% aqueous boundary layer controlled at low HPB concentrations and increasingly membrane controlled at the higher HPB concentrations. Theoretical calculations based on the experimental data and model parameters were found to be consistent with: at low HPB concentrations, capric acid was mainly absorbed at the villus tips and there was very little capric acid penetration into the intervillus space; in contrast, at 50 mM HPB, there was considerable capric acid penetration into the intervillus space, this corresponding to around a 4.5-fold increase in the accessible area for absorption when compared with 0 mM HPB. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2832–2844, 2015

Keywords: solubility; absorption enhancer; bioavailability; cyclodextrins; permeability; epithelial delivery/permeability; permeation enhancers; intestinal absorption; ileum; passive diffusion/transport

INTRODUCTION

Although there has been much progress from the practical standpoint in the area of oral drug formulation development involving cyclodextrins,[1–5] there has been relatively little progress from the standpoint of obtaining a quantitative mechanistic understanding of the interplay of key variables involved in the intestinal absorption enhancement by cyclodextrins. Recently, we developed a physical model approach involving a model which we called the flat surface model (FSM) that should represent an important step toward the mechanistic understanding of the transport of highly lipophilic, ionizable drug molecules between two aqueous compartments separated by a lipophilic membrane, and where a carrier such as a cyclodextrin is present in the aqueous phase.[6] Capric acid permeability coefficients predicted by the FSM equations describing the permeant transport behavior in the presence of a carrier were found to be in good agreement with the experimental results for capric acid transport between two aqueous compartments separated by a silicone polymer membrane over wide ranges of pH and the 2-hydroxypropyl-β-cyclodextrin (HPB) concentrations. The good agreement of experimental data with the FSM predictions over the range of conditions demonstrates the rigor of the approach and the appropriateness of extending the applications of the FSM approach to other transport situations and conditions. The FSM was able to quantitatively demonstrate the interplay of key variables such as solution pH, capric acid p*K*a, caprate species-HPB binding constants, aqueous boundary layer (ABL) thickness, diffusion coefficients, and the capric acid lipophilicity and to demonstrate the relative importance of each of these variables for a given set of transport conditions.

A main purpose of establishing the FSM approach in this previous study[6] was to establish a sound physical model framework that could be used to probe the effects of cyclodextrins on the transport of lipophilic molecules across biological membranes, more specifically the intestinal membrane. Accordingly, the aim of the present study was to gain insight into the role of cyclodextrin carriers in improving the oral bioavailability of highly lipophilic molecules by applying the FSM approach to results from intestinal absorption experiments involving the rat ileum. Capric acid and HPB are used to represent a typical, highly lipophilic weak acid molecule and a carrier molecule, respectively. The interplay of key variables, their relative importance under various experimental conditions, and the mechanism by which cyclodextrins may improve oral bioavailability were to be examined. An additional aim of this study was to extend the FSM to allow for a more detailed examination of the rat ileal intestinal absorption problem. To this end, a villus model (VM) that takes into account the villus architecture of the ileum is also presented. Each of the models is examined in light of the experimental data for capric acid absorption in the rat ileum at pH 7.4 in the presence of 0–50 mM HPB.

STRATEGY

The Experimental Method and the Estimation of Key FSM Parameters

The technique of *in situ* single-pass perfusion of an isolated rat ileal segment and local mesenteric venous blood collection is

Correspondence to: William Higuchi (Telephone: +801-519-2578; Fax: +801-585-1270; E-mail: whiguchi@pharm.utah.edu)

This article contains supplementary material available from the authors upon request or via the Internet at http://onlinelibrary.wiley.com/.

Journal of Pharmaceutical Sciences, Vol. 104, 2832–2844 (2015)

Table 1. Parameter Values used in Model Calculations

Parameter	Symbol	Value
Effective aqueous boundary layer thickness (cm)	h_{ABL}	0.117(±0.02)
Capric acid intrinsic permeability coefficient (FSM case) (cm s^{-1})	$P_{i,FSM}$	3.5(1.7–10)
Capric acid intrinsic permeability coefficient (VM case) (cm s^{-1})	$P_{i,VM}$	0.48(0.15–2.1)
Capric acid (HA) or caprate ion (A^-) aqueous diffusion coefficient (cm^2 s^{-1})	D^f	$6.9(\pm0.5) \times 10^{-6}$
A^-·HPB or HA·HPB diffusion coefficient (cm^2 s^{-1})	D^*	$2.9(\pm0.2) \times 10^{-6}$
Taurocholate diffusion coefficient (cm^2 s^{-1})		5.6×10^{-6}[a]
HA·HPB binding constant (mM^{-1})	K^u	7.5(±0.3)
A^-·HPB binding constant (mM^{-1})	K^-	2.5(±0.2)
Buffer solution pH	pH	7.40(±0.01)
p*K*a of capric acid	p*K*a	4.53(±0.03)

Diffusion coefficients, binding constants, and p*K*a are from the previous study.[6] Intrinsic membrane permeability coefficient and aqueous boundary layer thickness were determined in the present study.

[a] From Ref. 7.

used.[7,8] As the perfusing permeant (i.e., capric acid) solution flows down the rat ileum, taken as a cylinder with a smooth inner surface, at a constant rate, the permeant is absorbed across the epithelial cells into a blood sink. The perfusion flow rate is strictly controlled using a perfusion pump, and the exit concentration of the permeant and the osmolarity of the perfusate are assayed at regular time points. Mesenteric venous cannulation provides the means for the determination of the permeant appearance rate in the blood draining the intestinal segment. This allows for a more sensitive determination of steady-state flux as compared with methods that determine the permeant flux from the disappearance kinetics in the lumen. The gut length and the inside diameter are measured for the flat surface area determination (flat surface area refers to treating the intestinal gut segment as a hollow, smooth circular cylinder), and the mean permeant concentration in the lumen is determined from initial and exit concentrations of the permeant. With the mean luminal concentration, membrane surface area, and appearance rate of permeant in the blood, the permeant flux (J_b) and the total permeability coefficient (P_T) are directly determined. Further details are explained in the methods and data treatment sections.

Key parameters of the FSM developed in the previous study[6] were intended for application in the intestinal perfusion studies. With the exception of the ABL thickness (h_{ABL}) and intrinsic membrane permeability coefficient (P_i), the parameters determined in the previous study are applicable to the intestinal perfusion of capric acid in the presence of HPB and are listed, along with the appropriate h_{ABL} and P_i for the present study, in Table 1. The h_{ABL} is dependent upon the hydrodynamics and therefore must be determined experimentally under the same experimental conditions as those of the perfusion experiments in this study. The h_{ABL} was determined to be 0.117 cm by a best-fitting procedure (as described in detail in the results section) involving the experimental data over the range of HPB concentrations at pH 7.4. Taurocholate, which is actively absorbed in the rat ileum and which is completely ABL controlled,[7] was used to confirm the h_{ABL} thickness. A value of 0.102 cm was obtained using tracer levels of taurocholate, which was consistent with the h_{ABL} determined previously in our laboratory (0.109 cm) for the same perfusate flow rate.[7] The best-fit h_{ABL} of 0.117 cm was chosen for the purposes of this study. The estimation of P_i was initially thought to be problematic. Although capric acid alone transport across the silicone polymer membrane was shown in the previous study[6] to be essentially membrane controlled at pH 7.4, preliminary intestinal perfusion experiments indicated that capric acid alone absorption was 100% ABL controlled at pH 7.4 in the rat ileum and therefore P_i was thought to be insensitive to quantification. However, as will be seen, the HPB carrier investigation itself provided a means for obtaining a reasonably good estimate of P_i because, as was shown in the previous study, with increasing HPB concentration capric acid transport becomes increasingly membrane controlled. Thus, the capric acid transport experiments in the presence of varying concentrations of HPB (0–50 mM) provided a means for determining both h_{ABL} and P_i by best fitting of both parameters to the entire range of experimental data.

In the present *in situ* perfusion experiments, the buffered condition of pH 7.4 was regarded to hold, not only in the bulk luminal solution, but also across the ABL and in the intervillus spaces, that is, no significant pH gradients are expected to exist in the ileum for two reasons. Firstly, the buffer capacity (β) of 0.057 M sodium phosphate at pH 7.4 is 3.13×10^{-2} M.[9] Caprate (5×10^{-5} M cold and 0.4×10^{-5} M radiolabeled), corresponds to a total caprate concentration (C_T) of 5.4×10^{-5} M. Therefore any pH gradient (ΔpH) should be insignificant:

$$\Delta\text{pH} = -\frac{[H^+]}{\beta} \cong -\frac{C_T}{\beta} = -0.0017 \quad (1)$$

Secondly, the microclimate pH about the rat ileal membrane surface, owing to the continuous flow of alkalinized secretions from intervillus secretory cells, has been found to be pH 7.3,[10,11] which is essentially the same as the pH of the phosphate buffer used in the present study with the rat ileum. In contrast, the secretory cells in the rat jejunum would maintain a more acidic microclimate pH 6.0 environment; in the latter case, there may be the potential to sustain pH gradients if the luminal solution pH was buffered at pH 7.4,[12] and for this latter more complex situation, a physical model has yet to be written. On a somewhat different but related issue, the open-ended intraluminal perfusion of pH 7.4 buffered solution of capric acid and HPB mixtures from an infinite reservoir at 0.2 mL/min (or linear flow velocity of 1.6 cm/min) would also preclude the existence of any longitudinal pH gradient along the 10 cm ileal segment as well as any significant accumulation of secretions in the perfusate with time.