In Situ Perfusion Model in Rat Colon for Drug Absorption Studies: Comparison with Small Intestine and Caco-2 Cell Model

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ABSTRACT: Our aim is to develop and to validate the *in situ* closed loop perfusion method in rat colon and to compare with small intestine and Caco-2 cell models. Correlations with human oral fraction absorbed (Fa) and human colon fraction absorbed (Fa_colon) were developed to check the applicability of the rat colon model for controlled release (CR) drug screening. Sixteen model drugs were selected and their permeabilities assessed in rat small intestine and colon, and in Caco-2 monolayers. Correlations between colon/intestine/Caco-2 permeabilities versus human Fa and human Fa_colon have been explored to check model predictability and to apply a BCS approach in order to propose a cut off value for CR screening. Rat intestine perfusion with Doluisio's method and single-pass technique provided a similar range of permeabilities demonstrating the possibility of combining data from different laboratories. Rat colon permeability was well correlated with Caco-2 cell-4 days model reflecting a higher paracellular permeability. Rat colon permeabilities were also higher than human colon ones. In spite of the magnitude differences, a good sigmoidal relationship has been shown between rat colon permeabilities and human colon fractions absorbed, indicating that rat colon perfusion can be used for compound classification and screening of CR candidates. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3136–3145, 2015

Keywords: colon absorption; fraction absorbed; permeability; absorption; colonic drug delivery; in vitro models; Caco-2 cells; site-specific absorption

INTRODUCTION

The small intestine is the main site of absorption, because of its anatomical, physiological, physicochemical environment, and biopharmaceutical features.[1] Traditionally, the colon has been considered less important than the small intestine for drug absorption. Anatomically, colon is shorter and wider than small intestine; colonic lumen has no extra surface area provided by villi; moreover, epithelial cell layer junctions are tighter in colon than in small intestine. So, the available surface for drug absorption in colon is smaller and morphologically less well equipped than that of the small intestine.[1–4] Differences also affect the expression of efflux and uptake transporters. Intestinal drug transporters are predominant in the jejunum and ileum[5,6] and, according to Drozdzik et al.[7] the transporter protein abundance was similar in the jejunum and ileum but markedly different in the colon. In fact the major transporter protein in the small intestine (around 50%) was the uptake carrier PEPT1, and in the colon, the major transporter was the basolateral efflux carrier ABCC3.[8] In addition, the gastrointestinal tract is populated with a large number of bacteria and most of them reside in the large intestine; the stability of a drug to the microbiota is clinically relevant; the metabolism can transform a drug in pharmacologically active, inactive, or toxic. Many drugs are substrates for colonic bacteria, which could result in degradation and influence their bioavailability.[9,10] These arguments also support that the permeability and the absorptive capacity for drugs of the colonic membrane are considered lower than the small intestine. However, this is not always so simple. Distal regions of intestine, including the colon, can significantly contribute to the overall absorption as well. Several studies have demonstrated that some drugs show fairly high permeability in colon.[11–14]

Colon has one main advantage for absorption: the transit time of drugs. The colonic transit time is longer than 24 h, whereas the small intestinal transit time is shorter about 2–5 h.[15,16] Thus, colonic absorption can be beneficial to ensure the performance of oral controlled release (CR) products. As CR formulations are designed to release the drug during 12–24 h, it is obvious that drug absorption in colon is a prerequisite for oral CR development.[3,13] Despite the fact that there are various *in vitro* and *in situ* models to predict the absorption and permeability in small intestine, there is less knowledge regarding the large intestine.[1,17] There is a need for exploratory *in vivo* studies to clarify regional drug absorption along the intestine, and especially from the colon in order to validate animal or *in vitro* biopharmaceutical colon models as these models would have a clear impact for the study of oral CR formulations.[4]

It is well known that oral drug absorption is defined mainly by two parameters: solubility and permeability. These parameters are the basis for the Biopharmaceutics Classification System (BCS) for immediate-release (IR) products developed by Amidon et al.[18] The premise of BCS is that the rate and extent of drug absorption depends on the solubility and permeability

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of the drug. The applicability of BCS for CR formulations and colonic absorption has been discussed.[19–21] Some authors have remarked that to extend the BCS model to the CR formulations with a simple measure of permeability is not adequate,[21] but on the contrary, Tannergren et al.[1] demonstrated that compounds with high permeability had good absorption in colon, whereas low permeability drugs presented unfavorable colon absorption, that is, permeability data could be used for early assessment of the colonic absorption potential during CR development.

The first purpose of this work is to develop and to validate the technique of *in situ* perfusion in rat colon based on Doluisio's method and to correlate the colon permeability values with permeability data obtained in small intestine to characterize the segmental differences in the rat model. Moreover, in order to obtain a simpler model for screening drug absorbability in colon, the second objective is to compare the animal model with an *in vitro* model based on Caco-2 monolayers. For those purposes, 16 model drugs with different permeability characteristics have been selected and the permeability has been assessed in both segments of the rat gastrointestinal tract: small intestine and colon, and in Caco-2 monolayers. Small intestine/colon and *in situ/in vitro* correlations and the comparison with literature *in vivo* data will be used to apply a BCS approach to colon absorption in order to propose a cut off value for screening during CR product development.

MATERIALS AND METHODS

Antipyrine, caffeine, carbamazepine, metoprolol, naproxen, theophylline, verapamil, amoxicillin, atenolol, cimetidine, codeine, colchicine, furosemide, ranitidine, terbutaline, and valsartan were purchased from Sigma–Aldrich. Methanol, acetonitrile, and water were HPLC grade. All other chemicals were of analytical reagent grade.

Table 1 summarizes the contribution of transporters that can affect the permeability of the test compounds and some physicochemical properties.[1,6,22–42] In this work, a high concentration of drugs (500 μM) was used to ensure saturating conditions in order to get the diffusional component of the transport and a negligible contribution of the transporters.

Absorption Studies

Male Wistar rats were used in accordance with 2010/63/EU directive of September 22, 2010 regarding the protection of animals used for scientific experimentation. The Ethics Committee for Animal Experimentation of the University of Valencia approved the experimental protocols (Spain, code A1330354541263).

The absorption rate coefficient and the permeability value in the whole small intestine of each compound were evaluated by *in situ* "close loop" perfusion method based on Doluisio's Technique.[43] Male Wistar rats (body weight, 250–300 g) fasted 4 h and with free access to water were used for these studies. Rats were anesthetized using a mixture of pentobarbital (40 mg/kg) and butorphanol (0.5 mg/kg). A midline abdominal incision was made. The intestinal segment was manipulated in order to minimize any intestinal blood supply disturbances. The bile duct was always tied up in order to avoid drug enterohepatic circulation and the presence of bile salts in lumen. The perfusion technique consists of creating an isolated compartment in the intestinal segment of interest, with the aid of two syringes and two three-way stopcock valves. Two incisions were performed in the intestine, the first one at the beginning of the duodenal portion and the second one at the end of the ileum portion just before the cecum sac. Surgical ligature to a catheter was placed at the duodenal incision. In order to remove all intestinal contents, the small intestine was copiously flushed with a physiologic solution: isotonic saline (pH 6.9) with 1% Sörensen phosphate buffer (v/v), 37°C. After that, the end of the segment was tied up and connected to a second catheter. Both catheters were connected to a glass syringe using a stopcock three-way valve. The intestinal segment was carefully placed back into the peritoneal cavity and the abdomen was covered with a cotton wool pad avoiding peritoneal liquid evaporation and heat losses. Once this system is set up, the intestinal segment is an isolated compartment where the drug solution (10 mL) can be introduced and sampled with the aid of the syringes and stopcock valves. The samples were collected every 5 min up to a period of 30 min.

The absorption rate coefficient and the permeability value in the colon were evaluated with a similar technique to that used for the whole small intestine.[3] The colon was flushed with a physiologic solution: isotonic saline (pH 7.5) with 1% Sörensen phosphate buffer (v/v), 37°C. The drug solution (5 mL) was introduced in the isolated compartment and it was sampled at fixed times.

Drug solutions for experiments in colon and intestine were prepared in isotonic saline buffered with Sörensen phosphate buffer (pH 7.0). For furosemide and valsartan, 1% DMSO (dimethyl sulfoxide) was used to achieve complete dissolution of these compounds.

At the end of the experiments, the animals were euthanized. In order to separate solid components (mucus, intestinal contents) from the samples, they were centrifuged for 5 min at 2991 g. All samples were analyzed by HPLC.

The absorption rate coefficient (K_a) and permeability values (P_{app}) for 16 drugs were determined in two segments of gastrointestinal tract: entire small intestine and colon ($n = 5$–7). The perfusion solution of each drug was prepared at 500 μM. After dissolving the drugs, the pH of the solution was readjusted to 7.0.

The reduction in the volume of the perfused solutions at the end of the experiments was significant (up to 20%), and a correction became necessary in order to calculate the absorption rate constants accurately. Water reabsorption was characterized as an apparent zero-order process. A method based on direct measurement of the remaining volume of the test solution was employed to calculate the water reabsorption zero-order constant (k_o). The volume at the beginning of the experiment (V_0) was determined on groups of three animals, whereas the volume at the end (V_t) was measured on every animal used. The concentration in the samples was corrected as

$$C_t = C_e\,(V_t/V_o) \tag{1}$$

where C_t represents the concentration in the gut that would exist in the absence of the water reabsorption process at time t, and C_e represents the experimental value. The C_t values (corrected concentrations) were used to calculate the actual absorption rate coefficient.[44]

The absorption rate coefficients (k_a) of compounds were determined by nonlinear regression analysis of the remaining

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