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Research Paper

Investigating drug absorption from the colon: Single-pass vs. Doluisio approaches to in-situ rat large-intestinal perfusion

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ABSTRACT

Traditionally, the colon is considered a secondary intestinal segment in the drug absorption process. However, in many cases the role of colonic drug permeability cannot be overlooked. The purpose of this research was to compare colon permeability data obtained using two different rat perfusion methods the single-pass intestinal perfusion (SPIP) approach and the closed-loop (Doluisio) perfusion model. A list of 14 structurally diverse model drugs was constructed, and their rat colon permeability was studied using the two methods. The two sets of results were compared to each other, and were evaluated vs. in-vitro, ex-vivo, and in-vivo literature values. The SPIP and the Doluisio results exhibited good correlation between them ($R^2 = 0.81$). The best correlation of both sets was obtained with transport studies across Caco-2 monolayers ($R^2 \sim 0.9$), as well as the sigmoidal fit vs. human fraction of dose absorbed (F_{abs}) data. On the other hand, Ussing chambers data, as well as lipophilicity (Log P) data, resulted in weak correlation to the in-situ results. In conclusion, the single-pass intestinal perfusion (SPIP) and the Doluisio (closed-loop) perfusion models were found to be equally convenient and useful for obtaining validated colon permeability values, although more human colonic F_{abs} data are needed for a better understanding of colonic drug permeability and absorption.

1. Introduction

Traditionally, the colon is considered a secondary intestinal segment in the drug absorption process (Masaoka et al., 2006; Fagerholm 2007; Wolk et al., 2014). However, in many cases the role of colonic drug permeability cannot be overlooked. For instance, controlled release dosage forms are typically designed to release the drug over 12–24 hours, and since the small intestinal transit time is 3–4 hours, majority of the dose will become available to the intestinal lumen only in the colon, where the transit time is typically 24–48 hours (Tannergren et al., 2009; Dahlgren et al., 2016). Likewise, rectal dosage forms deliver their drug content directly to the large intestine, making the colonic permeability a key factor for these drug products. In inflammatory bowel disease (IBD), and other medical conditions, the original pathogenesis and some of the major pathological manifestations are focused in the colon, and when a targeted drug therapy is desired, colonic absorption is an important parameter (Dahan et al., 2010; Wolk et al., 2013; Dahan et al., 2014). Overall, oftentimes colonic drug permeability cannot be disregarded, and indeed, this parameter receives increasing attention over the past years (Sjöberg et al., 2013; Lozoya-Agullo et al., 2015a; Zur et al., 2015; Dahlgren et al., 2016).

There is a lack of validated models to predict the permeability and absorption of drugs in colon. Direct measurements of in-vivo colonic permeability in humans are difficult to obtain (Lennernäs, 2014a, 2014b), yet intubation technique was recently used to acquire such data (Dahlgren et al., 2016a, 2016b). Permeability data obtained from human colonic tissue mounted in the Ussing chamber is a scientifically sound method that has also been recently published (Rozehnal et al., 2012; Sjöberg et al., 2013). Transport studies across Caco-2 cell monolayers (Rubas et al., 1996; Hilgendorf et al., 2000), as well as parallel artificial membrane

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permeability assay (PAMPA) (Bujard et al., 2017), have been also evaluated for their ability to predict colonic drug permeability, often with limited success. Better yet, perfusion studies in the rat have been emerged as a reliable and efficient experimental method, which allows the assessment of drug permeability from various intestinal segments (Lozoya-Agullo et al., 2015a; Zur et al., 2015).

The aim of this work was to compare colon permeability data obtained using two different rat perfusion methods: the single-pass intestinal perfusion (SPIP) model and the closed-loop Doluisio rat perfusion method. These two experimental methods, which correlate disappearance of drug from the intestinal lumen with drug permeability, exhibit significant mechanistic differences between them. For instance, different kinetic conditions (steady-state in the SPIP vs. non-steady state in the Doluisio method), as a result of the experimental setting: while the Doluisio method tracks the loss of drug during the initial 30 minutes of the study, the SPIP approach allows a 60 minutes acclimation period, followed by the drug loss measurments. Moreover, in the SPIP approach each drug molecule gets only one passage through the colon, whereas in the Doluisio method the tested drug solution remains within the colon the entire experiment. A list of 14 diverse model drugs was constructed, and their colon permeability was studied using both SPIP and the Doluisio methods. The two sets of results were compared to each other, and were evaluated vs. in-vitro, ex-vivo, and in-vivo literature values. While we have recently presented analysis concerning the small intestine (Lozoya-Agullo et al., 2015b, 201b), this is the first time to thoroughly examine colon permeability using these perfusion approaches, and overall, this work provides a deeper understanding of colon permeability.

2. Materials and Methods

2.1. Materials

Paracetamol, antipyrine, atenolol, caffeine, carbamazepine, cimetidine, codeine, colchicine, digoxin, furosemide, hydrocortisone, metformin, metoprolol, and pravastatin were purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile, methanol, ethanol and water (Merck KGaA, Darmstadt, Germany) were UPLC grade. All other chemicals were of analytical reagent grade.

2.2. The Model Drugs

A list of 14 model drugs was constructed: Paracetamol, antipyrine, atenolol, caffeine, carbamazepine, cimetidine, codeine, colchicine, digoxin, furosemide, hydrocortisone, metformin, metoprolol, and pravastatin. This list was chosen while making sure to include drugs with diverse permeability characteristics: low, moderate, and high permeability, as well as passively vs. actively absorbed drugs, as will be discussed hereinafter.

2.3. Single-Pass Rat Colon Perfusion

The single-pass colon perfusion studies were performed according to Protocol IL-08-01-2015 approved by the Ben-Gurion University of the Negev Animal Use and Care Committee. Male Wistar rats (280–320 g, Harlan, Israel) were housed and handled according to Ben-Gurion University of the Negev Unit for Laboratory Animal Medicine Guidelines.

The *in situ* single-pass colon perfusion studies followed previously published reports (Beig et al., 2012; Fairstein et al., 2013; Zur et al., 2014). Rats were anesthetized with 1 mL/kg ketamine:xylazine (9%:1%, respectively) injection, placed on a heated surface (37 °C; Harvard Apparatus Inc., Holliston, MA), followed by a 3 cm midline abdominal incision. The colon (~6 cm) was cannulated on two ends, and was rinsed with blank perfusion buffer warmed to 37 °C.

At the beginning of the experiment, the perfusion solution (10 mM MES buffer, 135 mM NaCl, 5 mM KCl, 290 mOsm/L, pH 6.5) containing the investigated drug, was perfused (0.2 mL/min) through the isolated colon (Watson Marlow 205S, Watson-Marlow Bredel Inc., Wilmington, MA). This buffer was first perfused for 1 hour to ensure steady state, followed by 1 hour of perfusion with samples taken every 10 minutes, that were immediately assayed by UPLC. With both perfusion methods, the pH is measured in each collected sample (i.e. over time), as this may affect the permeability of ionizable drugs.

The effective permeability (P_{eff}; cm/s) through the rat colon membrane was calculated according to the following equation (Fine-Shamir et al., 2017):

$$P_{eff} = \frac{-Qln(C'_{out}/C'_{in})}{2\pi RL} \quad (1)$$

where the perfusion flow rate (Q; 0.2 mL/min), the ratio of the outlet vs. inlet drug concentration (C'_{out}/C'_{in}) that has been corrected for water transport *via* the gravimetric method (Beig et al., 2015, 2016, 2017), and the colon radius (R) and length (L) are accounted for.

2.4. Doluisio (Closed-Loop) Rat Colon Perfusion

Male Wistar rats were used in accordance with 2010/63/EU directive of 22 September 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 coefficients and the permeability values of the 14 drugs studied in this work were determined in colon (n = 6-7) using *in situ* "closed loop" perfusion method based on the Doluisio's Technique (Doluisio et al., 1969a, 1969b), modified to the colon as previously described (Lozoya-Agullo et al., 2015a, 2016a). Briefly, the rats were anesthetized using a mixture of pentobarbital (40 mg/kg) and butorphanol (0.5 mg/kg). An isolated compartment (~6 cm) in the large intestine was created from the beginning of the colon, just after the cecum sac, to the end of the colon, with the aid of two syringes and two three-way stopcock valves. Before doing the second incision, the large intestine was flushed with warm (37 °C) isotonic saline, 1% Sörensen phosphate buffer (v/v). At the end of the surgical procedure and throughout the experiment, the abdomen was covered with a cotton wool pad avoiding peritoneal liquid evaporation and heat loss. The drug solution (5 mL) was introduced into the colon compartment, and at each sampling time point (i.e. every 5 minutes) all of the solution is pumped out of the intestinal segment into one of the syringes, the sample is taken (100 μL), and the solution is then placed back into the intestinal segment. Sampling is done alternatively in proximal and distal syringe, thus ensuring the mixing of the solution. The withdrawn samples were centrifuged for 5 minutes at 5000 rpm, followed by HPLC analyses. Administered drug solutions were prepared in isotonic (290 mOsm/L) saline buffered with Sörensen phosphate buffer (66.6 mM).

There is a reduction in the volume of the perfused solutions at the end of the experiment; therefore, a volume correction becomes 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) is compose from the volume of the drug solution (5 mL) plus the residual volume that was determined on a group of three animals and was found to be