A Sensitive Medium-Throughput Method to Predict Intestinal Absorption in Humans Using Rat Intestinal Tissue Segments

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Received 12 September 2014; revised 9 December 2014; accepted 8 January 2015

Published online 17 February 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24372

ABSTRACT: A range of *in vitro, ex vivo,* and *in vivo* approaches are currently used for drug development. Highly predictive human intestinal absorption models remain lagging behind the times because of numerous variables concerning permeability through gastrointestinal tract in humans. However, there is a clear need for a drug permeability model early in the drug development process that can balance the requirements for high throughput and effective predictive potential. The present study developed a medium throughput screening Snapwell (MTS-Snapwell) *ex vivo* model to provide an alternative method to classify drug permeability. Rat small intestine tissue segments were mounted in commercial Snapwell™ inserts. Unidirectional drug transport (A–B) was measured by collecting samples at different time points. Viability of intestinal tissue segments was measured by examining transepithelial electric resistance (TEER) and phenol red and caffeine transport. As a result, the apparent permeability ($P_{app;}$ $\times 10^{-6}$ cm/s) was determined for atenolol (10.7 ± 1.2), caffeine (17.6 ± 3.1), cimetidine (6.9 ± 0.1), metoprolol (12.6 ± 0.7), theophylline (15.3 ± 1.6) and, ranitidine (3.8 ± 0.4). All drugs were classified in high/low permeability according to Biopharmaceutics Classification System showing high correlation with human data ($r = 0.89$). These findings showed a high correlation with human data (r = 0.89), suggesting that this model has potential predictive capacity for paracellular and transcellular passively absorbed molecules. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2807–2812, 2015

Keywords: Biopharmaceutics classification system (BCS); *ex vivo*; *in vitro* models; intestinal absorption; permeability; rat intestine segments

INTRODUCTION

It is widely acknowledged that highly attrition rate in the drug discovery process is mainly related to poor pharmacokinetic properties during absorption, distribution, metabolism, and excretion (ADME) phases.[1] It would be more efficient if drug permeability performance could be assessed during the less costly, earlier stages of the drug discovery & development (DDD) process and, in parallel with bioactivity-guided assays. Although, several *in silico*, *in vitro*, *in vivo* and *ex vivo* methods are currently used, highly predictive human intestinal absorption models remain to be developed because of profusion of variables affecting permeability through gastrointestinal tract in humans.[2]

For the prediction of potential human intestinal absorption, great emphasis is currently placed on excised tissue permeability models such is Ussing chambers used to measure drug transport along rat intestinal segments.[3] Also, include original expression of influx and efflux transporters found *in vivo* tissues.[3] In addition, there is interplay between many absorption mechanisms such as passive and carried-mediated diffusion[4] allowing for high correlation between both process, *i.e.* the fraction of drug absorbed (F_a) in humans and the apparent permeability (P_{app}) in rat tissues models. Moreover, e*x vivo* permeability systems using tissue segments can be a valuable tool regarding morphological and physiological characteristics that closely mimics features of the *in vivo* intestinal epithelium. It is also recognized by the US Food and Drug Administration (FDA) as most ideal one for preclinical human intestinal permeability.[5] For instance, it provides both metabolism capacity and expression of a mucus layer on the luminal side working as a protective coating to reduce damage by sample cosolvents. This feature is often useful in DDD phases.

Despite these advantages, maintenance of *ex vivo* tissue integrity and viability requires close attention and highly controlled experimental conditions, in order to avoid miscalculation of drug transport, membrane retention and low throughput.[6–8] Briefly, tissue segments should be continuously oxygenated with carbogen mixture (95%O_2, 5% CO_2) and kept under stirring in physiological buffer, at 37°C, reducing the unstirred water layer and, at high-humidity level incubations to avoid sample evaporation.

In this context, it is clear the need for drug permeability models that can be used early in DDD phases providing a reasonably high throught and predictive potential. Here, the medium throughput screening (MTS-Snapwell) model is showed as a new alternative *ex vivo* model to classify drugs in high and low permeability, using female rat small intestine tissue segments mounted in commercial Snapwell™ inserts, under stirring and *sink conditions*.

MATERIALS AND METHODS

Chemicals and Reagents

Metoprolol tartrate, caffeine, theophylline, atenolol, cimetidine, ranitidine hydrochloride, sodium chloride, potassium chloride, monobasic sodium phosphate, magnesium sulfate heptahydrate, calcium chloride dihydrate and sodium bicarbonate were

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Journal of Pharmaceutical Sciences, Vol. 104, 2807–2812 (2015)

purchased from Sigma–Aldrich (St. Louis, Missouri). Sodium hydroxide and dibasic sodium phosphate anhydrous were purchased from J.T. Baker (Mexico City, Mexico), glacial acetic acid from Vetec (Rio de Janeiro, RJ, Brazil), and orthophosphoric acid from Scharlau (Barcelona, Spain), whereas HPLC chromatographic grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany).

Rat Excised Tissue Segments

The study protocol for the use of rat intestine tissues were approved by the Ethics Committee on Animal Use at the Federal University of Goiás under number 013/2011. Female *Wistar* rats (200 ± 25g) were housed under controlled conditions at a temperature of 22°C, 12-h light/dark cycle and access to standard food and tap water *ad libitum* for a minimum acclimatization period of one week. Prior to each experiment, animals were fasted for 12 h with free access to water.[9,10]

Female *Wistar* rats were anesthetized with ketamine/xilazyne (90 and 7.5 mg/kg). The small intestine was excised, washed and kept in an ice-cold oxygen-saturated Krebs–Ringer bicarbonate (KRB) solution. Proximal jejunal tissue (the first 12 cm from stomach) was excised and placed in beakers containing ice-cold KRB (10°C), which was continuously gassed with an 95% O_2 and 5% CO_2. Next, the seromuscular layer was carefully stripped off from the membrane and cut into 20 mm segments. During this procedure, tissues were kept moist using KRB (10°C). Rat jejunal segments were then mounted with the basolateral side set above the polycarbonate filter membrane (Isopore™ 0.4 μm, 13 mm; Millipore, Darmstadt, Germany). Using acrylic disks (02) with an internal diameter of 3 mm over and above the filter membrane (Fig. 1) and with the mucosal membrane facing upward, the permeability apparatus was set-up. Next, it was tightly mounted and mechanically sealed inside the donor chamber (Snapwell™ device). Then, it was checked for any leakage using 1 mL KRB solution. The MTS-Snapwell system was completed by filling the receiver (2 mL) and donor (0.4 mL) compartments with KRB solution.

Viable intestinal segments (transepithelial resistance, TEER > 30 Ω cm^2), as described under *Viability and Barrier Integrity*, were mounted on the permeation apparatus and submitted to a preincubation period (30 min) under a controlled atmosphere (95% O_2 and 5% CO_2) inside a humidified incubator (MCO-18AC; Sanyo Scientific, UK) at 37°C and gentle shaking (60 rpm) for equilibration. KRB blank solution was then aspirated off from the donor compartment and replaced with drug solutions (400 μL) at variable concentrations (0.30–12.68 mM). Drug doses assayed were defined as the highest human commercial dose divided by 250 mL, as described in FDA guide.[5] During drug incubation, unidirectional drug transport from apical to basolateral side was measured by collecting samples at different time points (0, 20, 30, 40, 60, 90, and 120 min). Sampling volume was immediately replaced with the same volume of fresh prewarmed (37°C) KRB solution. Samples were analyzed as described in *Analytical Methods*.

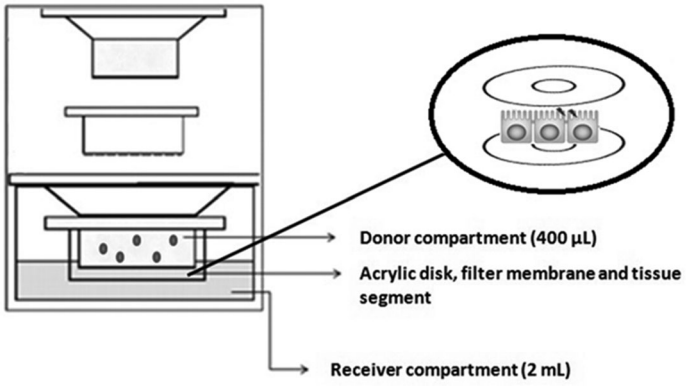

Figure 1. Schematic representation of Snapwell™ adaptation using rat intestine tissue segments.

For each drug, the apparent permeability coefficient (P_{app}) was calculated according to the following equation (Eq. (1) [11–13,35]:

$$P_{app}\,(\mathrm{cm/s}) = \frac{dQ}{dt}\left(\frac{1}{C_0 A}\right) \quad (1)$$

Where dQ/dt is the appearance rate of the drug in the receiver compartment, C_0 is the initial concentration of the drug in the donor compartment, and A is the surface area (0.1 cm^2) of the tissue segment.

Viability and Barrier Integrity

Viability of intestinal tissue segments mounted in the MTS-Snapwell system was assessed by measuring the transport of marker molecules (e.g., phenol red and mannitol) and TEER during incubation time (120 min) at 37°C with 95% O_2, and 5% CO_2. TEER was measured at three different time points (0, 30, and 120 min). Additionally, potential damage to the barrier function for commonly used cosolvents (DMSO 1% and EtOH 1%) for poorly water-soluble drugs was evaluated.[14–16]

The integrity of the intestinal barrier function was measured by linearity of mucosal to serosal transport of permeability markers over 2 h. Caffeine was evaluated as a highly absorbed marker transported by passive transcellular mechanism, in addition to phenol red as a nonabsorbable one.

Analytical Methods

Drug samples were analysed by HPLC–DAD (Infinity 1260; Agilent Technologies) with an ACE 5 C18 column (100 × 4.6 mm^2, 5 μm), using compendial methods from the United States and Brazilian Pharmacopeia. The chromatographic conditions employed are described in Table 1.

Data Analysis

TEER values in the presence and absence of cosolvents were compared using Student's t-test for two independent samples at the 5% significance level (Microsoft Excel 2010; Action®). Results were shown as arithmetic mean ± standard deviation (SD).

Potential correlation between P_{app} values obtained from the MTS-Snapwell model and P_{app} values from published data (Ussing chamber, rat perfusion, or % F_a in humans) were plotted. Linear regression was performed and expressed as a measurement of the goodness of fit (R^2) on predicting human drug absorption.

 DOI 10.1002/jps.24372