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Original article

Determination of lamivudine and zidovudine permeability using a different *ex vivo* method in Franz cells

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ABSTRACT

Introduction: The major processes that control the absorption of orally administered drugs are dissolution and gastrointestinal permeation. These processes depend on two main properties: solubility and permeability. Based on these characteristics, the Biopharmaceutical Classification System (BCS) was proposed as a tool to assist in biowaiver and bioavailability prediction of drugs. **Methods:** The purpose of the present study was to evaluate the permeability of lamivudine (3TC) and zidovudine (AZT) using a different *ex vivo* method in Franz cells. A segment of jejunum was inserted in a Franz cells apparatus, in order to assess drug permeability in the apical–basolateral (A–B) and basolateral–apical (B–A) directions. Each drug was added to the donor chamber, collected from the acceptor chamber and analyzed by HPLC. Fluorescein (FLU) and metoprolol (METO) were used as low and high permeability markers, respectively. **Results:** The apparent permeability (P_{app}) results for the A–B direction were: $P_{app\ FLU\ A–B} = 0.54 \times 10^{-4}$ cm·s^{-1}, $P_{app\ METO\ A–B} = 7.99 \times 10^{-4}$ cm·s^{-1}, $P_{app\ 3TC\ A–B} = 4.58 \times 10^{-4}$ cm·s^{-1} and $P_{app\ AZT\ A–B} = 5.34 \times 10^{-4}$ cm·s^{-1}. For the B–A direction, the P_{app} results were: $P_{app\ FLU\ B–A} = 0.56 \times 10^{-4}$ cm·s^{-1}, $P_{app\ METO\ B–A} = 0.25 \times 10^{-4}$ cm·s^{-1}, $P_{app\ 3TC\ B–A} = 0.24 \times 10^{-4}$ cm·s^{-1} and $P_{app\ AZT\ B–A} = 0.19 \times 10^{-4}$ cm·s^{-1}. **Discussion:** For the A–B direction, the P_{app} results of fluorescein and metoprolol show low and high permeability, respectively, indicating that the membranes were appropriate for permeability studies. For the A–B direction, the P_{app} results of 3TC and AZT suggest that these antiretroviral drugs have permeability values close to metoprolol. Nevertheless, for the B–A direction the P_{app} results do not suggest efflux mechanism for any of the drugs. Thereby, the different *ex vivo* methods using Franz cells can be successfully applied in drug permeability studies, in particular for drug biopharmaceutical classification.

1. Introduction

Oral drug administration is the most common route for drug intake, mainly due to the convenience, safety, low cost and good acceptance by patients (Balimane, Chong, & Morrison, 2000; Corá et al., 2011; Lennernäs & Abrahamsson, 2005; Pretorius & Bouic, 2009).

The active ingredient in a solid dosage form must be soluble in biological fluids and should be able to cross the biological membrane in physiological conditions (Balimane et al., 2000; Dahan, West, & Amidon, 2009). Thus, solubility and permeability characteristics are important processes for drug absorption and, consequently, for its bioavailability (Amidon, Lennernäs, Shah, & Crison, 1995).

Drug absorption takes place especially in the small intestine, mostly in the duodenum and proximal jejunum portions, through enterocytes (Balimane et al., 2000; Fagerholm, Johansson, & Lennernäs, 1996; Hillgren, Kato, & Borchardt, 1995; Lennernäs, 1998). These cells are connected to each other by tight junctions, which are considered the main physical barriers for drug permeation, together with lipid membrane characteristics (Artursson, Ungell, & Löfroth, 1993; Deferme, Annaert, & Augustijns, 2008).

Absorption of drugs is a dynamic and complex process that occurs by different pathways, such as: passive transcellular and paracellular, active uptake transporters (including influx and efflux mechanisms) and endocytosis (Balimane, Han, & Chong, 2006; Balimane et al., 2000; Cook & Shenoy, 2003; Corá et al., 2011; Deferme et al., 2008; Kerns et al., 2004). About 80–95% of drugs are absorbed by passive diffusion and this route is affected by physicochemical properties such as lipophilicity, hydrogen bonding, pK_a, and molecular weight (Cao et al., 2006; Kerns et al., 2004).

Therefore, based on aqueous drug solubility and permeability, the Biopharmaceutical Classification System (BCS) was proposed by Amidon et al. (1995). This system allows the classification of drugs into four classes: high solubility and high permeability (class I), low solubility and high permeability (class II), high solubility and low permeability (class III) and, low solubility and low permeability (class IV).

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Additionally, BCS is widely used as a tool for developing new pharmaceutical formulations and assisting in biowaiver for class I drugs (Amidon et al., 1995). Thereafter, there was a considerable increase in permeability studies using different methods for drug biopharmaceutical classification purposes.

According to the US FDA guidance on BCS (Food and Drug Administration, 2000), the permeability classification can be directly determined by measuring the rate of mass transfer across the human intestinal membrane or, indirectly, by estimating the extent of drug absorption in pharmacokinetics studies in humans in comparison to a reference dose administered by intravenous route. Alternatively, different systems can be used for oral drug absorption prediction in humans and also for classification of drugs according to their permeability. Such models include artificial membranes and *in vitro* cell cultures (PAMPA, Caco-2 and MDCK), intestinal perfusion studies (*in situ* methods) and *ex vivo* studies using intestinal segments from animals or humans (Balimane et al., 2006; Food and Drug Administration, 2000).

In vitro methods such as PAMPA (parallel artificial membrane permeability assay) and cell cultures such as Caco-2 (human colon carcinoma cell line) or MDCK (Madin–Darby canine kidney cells) monolayers are simple tests for permeability studies. Nevertheless, PAMPA only allows the study of the passive transcellular diffusion pathway, since this model does not present tight junctions (paracellular pores) or transporters, and thus underestimates the permeability of drugs with specific characteristics (substances that need to be transported by carriers or through paracellular route, for example). Furthermore, there are no metabolism processes in PAMPA (Balimane et al., 2000; Balimane et al., 2006; Deferme et al., 2008; Kerns et al., 2004; Li et al., 2008). Cell cultures of Caco-2 and MDCK present limitations such as: expense of cell culture, variable expression of transporters and metabolizing proteins, contamination risk, long cell growth cycle, lack of standardized protocols, use of serum proteins and co-solvents, influence of unstirred water layer (UWL) and high implementation costs (Balimane et al., 2000; Deferme et al., 2008; Kerns et al., 2004; Li et al., 2008; Masungi et al., 2008; Reis, Sinko, & Serra, 2010).

Intestinal perfusion studies are closer to *in vivo* conditions. Limitations of this method are associated with the technical complexity of studies in anesthetized animals making the method of very low throughput. The permeability measurement is based on disappearance of the drug from the intestinal lumen as the only indication of absorption and a large number of animals are needed for a statistical significance (Balimane et al., 2000).

In the literature, *ex vivo* permeability methods have demonstrated a good correlation with *in vivo* absorption data from humans due to the structural and physiological similarities between rats and humans. In addition, rat gut expresses the vast majority of influx and efflux carriers which are found *in vivo* (Balimane et al., 2000; Cao, Yu, & Sun, 2008; Grass, 1997; Jain, Duvvuri, Kansara, Mandava, & Mitra, 2007; Lane, Levis, & Corrigan, 2006; Lennernäs, 2007; Pretorius & Bouic, 2009; Ruan et al., 2006; Trapani et al., 2004; Tukker, 2000; Yao & Chiou, 2006; Zakelj, Sturm, & Kristl, 2006).

Methods that use isolated intestinal tissues from animal models are some of the most commonly used. In an *ex vivo* method using a diffusion cell, two compartments are defined: one donor chamber and one acceptor chamber. The intestinal segment is kept between the chambers and the rat gut is the most used part to establish a correlation between rats and humans (Cao et al., 2008; Pretorius & Bouic, 2009).

The advantages of using diffusion cells are: (i) minor tissue handling, (ii) possibility of continuous sample collection, (iii) small amount of drug required, and (iv) similarities between rat and human tissues (Cao et al., 2008; Lennernäs, 2007).

Franz cells are widely used in skin permeation studies or even in supporting Caco-2 cell monolayers (Grabovac & Bernkop-Schnürch, 2006; Herai, Gratieri, Thomazine, Bentley, & Lopez, 2007; Lopez, Collett, & Bentley, 2000; Röpke et al., 2002; Sandri et al., 2007). There are few studies for orally administered drugs using the diffusion cell, which is a vertical system (according to a solution flux direction) and presents a donor chamber and an acceptor chamber, where the intestinal segment is placed between the chambers (Hamid, Katsumi, Sakane, & Yamamoto, 2009; Nicolazzo & Finnin, 2008; Ong & Heard, 2009; Pretorius & Bouic, 2009; Röpke et al., 2002).

Lamivudine (3TC) and zidovudine (AZT) are nucleoside reverse transcriptase inhibitors largely prescribed for HIV (human immunodeficiency virus) treatment (Aymard, Legrand, Trichereau, & Diquet, 2000; Balint, 2001; Checa, Soto, Hernández-Cassou, & Saurina, 2005). *Ex vivo* permeability data of these antiretroviral drugs are scarce in the literature, but some permeability values can be found using alternative techniques. Quevedo and Briñon (2009) studied AZT permeability using everted gut and they reported a permeability value of 0.05×10^{-4} cm·s^{-1} (Quevedo & Briñon, 2009).

Based on these conditions, the purpose of the present study was to evaluate the intestinal permeability of the antiretroviral drugs 3TC and AZT using a different *ex vivo* method in Franz cells.

2. Materials

2.1. Chemicals and reagents

Active pharmaceutical ingredient (API) working standards of 3TC and AZT were kindly donated by Fundação para o Remédio Popular (FURP, São Paulo, Brazil) and Cristália Produtos Químicos Farmacêuticos (São Paulo, Brazil). Fluorescein (FLU) and metoprolol (METO) were used as low and high permeability markers, respectively and were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chromatographic grade acetonitrile, methanol, potassium phosphate monobasic and orthophosphoric acid were purchased from Merck (Darmstadt, Germany). High purity deionized water was obtained from a Milli-Q purification system (Millipore, MA, USA).

NaCl, KCl, $CaCl_2$, $MgSO_4$, $NaHCO_3$, KH_2PO_4, and D-glucose were used for Krebs-Ringer solution preparation.

2.2. Experimental animals

All the procedures involving animals in the present work were firstly approved by the São Paulo University Ethics Committee under protocol number 180 (Ethics Committee on Animal Use of the Faculty of Pharmaceutical Sciences, University of São Paulo, Brazil).

Male albino Wistar rats weighing 250–300 g were considered for all permeability studies. Prior to each experiment, rats were fasted for 18 h with free access to water (Dahan et al., 2009; Jain et al., 2007; Kim et al., 2006; Langguth et al., 1997; Sutton, Rinaldi, & Vukovinsky, 2001).

3. Methods

3.1. Krebs-Ringer solution

The Krebs-Ringer solution composition was NaCl (7.0 g·L^{-1}), KCl (0.35 g·L^{-1}), $CaCl_2$ (0.28 g·L^{-1}), $MgSO_4$ (0.28 g·L^{-1}), $NaHCO_4$ (2.1 g·L^{-1}), KH_2PO_4 (0.16 g·L^{-1}), and D-glucose (5.05 g·L^{-1}). All components were solubilized in purified water (Milli-Q) and pH was adjusted to 6.8 for both sides (luminal and basolateral) (Sharma, Chawla, & Panchagnula, 2002).

3.2. Preparation of excised intestinal segments

After the fasting period, animals were sacrificed by decapitation. The jejunum was excised and placed in an ice-cold Krebs-Ringer solution for 20 min in order to reach the equilibrium (Legen & Kristl, 2003). The intestine segment was carefully opened, avoiding any handling damage, and thus it was inserted in the Franz cells previously