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Excised segments of rat small intestine in Ussing chamber studies: A comparison of native and stripped tissue viability and permeability to drugs

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ABSTRACT

Excised rat intestinal tissue mounted in an Ussing chamber can be used for intestinal permeability assessments in drug development. The outer layer of the intestine, the serosa and part of the muscle layer, is traditionally removed since it is considered a barrier to the diffusion of nutrients and oxygen as well as to that of pharmaceutical substances. However, the procedure for removing the serosal-muscle layer, i.e. stripping, is a technically challenging process in the pre-experimental preparation of the tissue which may result in tissue damage and reduced viability of the segment. In this study, the viability of stripped and native (non-stripped) rat small intestine tissue segments mounted in Ussing chambers was monitored and the apparent permeability of the tissue to a set of test compounds across both tissue preparations was determined. Electrical measurements, in particular the potential difference (PD) across the intestinal membrane, were used to evaluate the viability. In this study, there were no differences in initial PD (health status of the tissue) or PD over time (viability throughout the experiment) between native and stripped rat jejunum segments. Overall, there were also no significant differences in permeability between stripped and native rat intestinal tissue for the compounds in this study. Based on these results, we propose that stripping can be excluded from the preparation procedures for rat jejunal tissue for permeability studies when using the Ussing chamber technique.

1. Introduction

The majority of drug products designed for systemic pharmacological activity are administered orally (Lennernäs and Abrahamsson, 2005; Mrsny, 2012). One of the key parameters for assessing the systemic bioavailability of a drug is the permeability of the intestinal epithelia to the drug, which determines the rate and extent of intestinal drug absorption (Amidon et al., 1995). The Ussing chamber is an *in vitro* system applied for measuring the apparent permeability (P_{app}) of a compound over an excised tissue segment (Rozehnal et al., 2012; Sjöberg et al., 2013; Ungell et al., 1998). A good correlation between rat jejunum permeability and the fraction of drug absorbed in the human intestine after oral drug administration has been shown for compounds with low intestinal metabolism (Bueters et al., 2013; Fagerholm et al., 1996; Zhao et al., 2003). In the Ussing chamber procedure, intestinal tissue is resected from an anaesthetized animal and carefully prepared to minimize tissue stress and damage (Polentarutti et al., 1999). The wall of the intestine consists of four layers: mucosa, submucosa, muscularis propria (circular and longitudinal muscle layer) and serosa, from the apical to the basolateral side (DeSesso and Jacobson, 2001; Sleisenger et al., 2010). The absorption process takes places at the mucosal surface, and nutrients and pharmaceutical substances enter the circulation through blood vessels situated in the submucosa (Sleisenger et al., 2010). Part of the muscle layer and the entire serosal layer is traditionally removed in order to improve access for nutrients and oxygen to the mucosa, and also to remove an absorption barrier that is not present in the

Abbreviations: ABL, aqueous boundary layer; BCS, biopharmaceutics classification system; D, diffusion coefficient; h_l, thickness of serosal-muscle layer; KBR, Krebs-bicarbonate-Ringer solution; P_{app}, apparent permeability; $P_{app.stripped}$, stripped tissue apparent permeability; $P_{app.native}$, native tissue apparent permeability; PD, potential difference; PD_0, initial PD; $\Delta PD_{\%}$, relative change in PD; SCC, short-circuit current.

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in vivo situation. The procedure for removing the serosal-muscle layer, i.e. stripping, is a technically challenging technique that takes time and is associated with risks of tissue stress and damage and, in addition, the quantity of muscle layer removed can vary between preparations (Clarke, 2009). In accordance with the guiding principles of the Three Rs in the use of animals in research (replacement, refinement and reduction) it is important to ensure maximal and optimal use of animal tissues (Russell and Burch, 1959). While it is undisputed that the thick serosa and longitudinal muscular layers need to be removed from human or pig intestinal specimens before placement in the Ussing chamber, there have been studies where native (non-stripped) rodent (mouse and rat) tissue was used (Clarke, 2009; Nejdfors et al., 2000; Pantzar et al., 1994; Söderholm et al., 1998). These examples primarily involved mouse tissue, since the thin murine serosal-muscle layer is considered a negligible diffusion barrier (Clarke, 2009). Anatomically, rat intestinal wall is similar to that of the mouse and the same principles might apply for rat studies (Nejdfors et al., 2000). However, no report has conclusively shown the implications of the serosal-muscle layer for rat jejunal viability and drug permeability in the Ussing chamber (Bajka et al., 2003; Binder and Rawlins, 1973; Nejdfors et al., 2000). Consequently, a systematic documentation of the effects of the stripping procedure on the viability and permeability of rat jejunum tissue is warranted.

The aim of this study was to investigate whether the stripping procedure can be excluded from the pre-experimental preparation of rat intestinal tissue intended for permeability experiments in an Ussing chamber. Viability and integrity were evaluated using electrical parameters and the implications for permeability were evaluated using a set of test compounds with diverse physicochemical properties and different fractions absorbed in humans.

2. Materials and methods

2.1. Chemicals

Chemicals and final concentrations (mM) used for the Krebs-bicarbonate-Ringer solution (KBR) were NaCl (108) and $Na_2HPO_4 \times 2H_2O$ (1.8) from Merck (Darmstadt, Germany), and KCl (4.7), $MgSO_4 \times 7H_2O$ (1.2), KH_2PO_4 (0.6), $NaHCO_3$ (16), $CaCl_2 \times 2H_2O$ (1.25), glucose (11.5), Na-pyruvate (4.9), Na-fumarate (5.4) and Na-L-glutamate (4.9) from Sigma Aldrich (St. Louis, Missouri, USA). At 37° C and under CO_2/O_2 (5/95%) gas, the KBR pH was 7.4. Ketoprofen, atenolol, metoprolol, enalaprilat, diclofenac, fexofenadine, trimethoprim, dextromethorphan and testosterone were purchased from Sigma Aldrich (St. Louis, Missouri, USA) and candesartan was obtained from AstraZeneca (Mölndal, Sweden). Warfarin, which was used as the internal standard for bioanalysis, and the solvents DMSO and acetonitrile were purchased from Sigma Aldrich (St. Louis, Missouri, USA).

2.2. Test compound selection

The absorption of pharmaceutical substances is related to their physicochemical properties, such as lipophilicity and molecular size (Winiwarter et al., 1998). The serosal-muscle layer could in theory affect the permeation of substances through the intestinal tissue differently depending on their molecular characteristics. For this reason, the nine test compounds were chosen to represent a selection of classes in the Biopharmaceutics Classification System (BCS), which classifies pharmaceutical substances according to their intestinal permeation and solubility (Amidon et al., 1995). The BCS classes for the selected test compounds (ketoprofen, atenolol, metoprolol, enalaprilat, diclofenac, fexofenadine, trimethoprim, dextromethorphan, testosterone and candesartan) are displayed in Table 1.

2.3. Ussing chamber setup and tissue preparation

A modified Ussing chamber made at AstraZeneca Mölndal was used (Sjöberg et al., 2013). Male Wistar rats (body weight ~300 g) with free access to food and water were anaesthetized with isoflurane® and a segment of the jejunum was excised through laparotomy. The intestinal segment was immediately rinsed with ice-cold oxygenated KBR (pH 7.4) and placed in a beaker containing KBR on ice which was constantly oxygenated using O_2/CO_2 (95/5%) gas. After a 30 min equilibration period, pieces of tissue approximately 2 cm in length were cut and opened along the mesenteric border. Areas with evident Peyers patches were not used for the experiments. The intestinal pieces were carefully flattened out and pinned to the floor of a container filled with ice cold and oxygenated KBR. The outer serosal-muscle layer was then gently removed from the tissue using forceps. It is worth noting that the exact degree of muscle-layer stripping can vary slightly, (Fig. 1), but only visually acceptable stripped samples were taken forward into the experiments. For each experiment four segments

Table 1
Physicochemical properties [logP, $logD_{7.4}$, molecular radius (r), and molecular weight (Mw)], and biopharmaceutics classification system (BCS) class and diffusion coefficient in the aqueous boundary layer (D_{ABL}) and the serosal-muscle layer (D_l) for the investigated compounds.

Compound	logP[a]	$logD_{7.4}$[b]	r[c] ($\times 10^{-10}$ m)	Mw[c] (g/mol)	BCS[d]	D_{ABL}[e] ($\times 10^{-6}$ cm²/s)	D_l[f] ($\times 10^{-6}$ cm²/s)
Atenolol	0.45	−1.76	3.97	266	III	8.23	0.46
Candesartan	4.56	0.54	4.48	440	IV	7.29	1.89
Dextromethorphan	3.59	2.17	4.03	271	II	8.09	2.35
Diclofenac	4.37	1.44	3.84	296	II	8.50	2.30
Enalaprilat	0.47	−0.27	4.26	348	IV	7.67	0.41
Fexofenadine	4.83	1.23	4.91	502	III	6.65	1.67
Ketoprofen	3.19	−0.16	3.82	254	II	8.54	2.39
Metoprolol	1.74	−0.47	4.03	267	I	8.10	1.61
Trimethoprim	1.12	0.47	3.97	290	II	8.23	1.09

[a] Acquired from ALOGPS 2.1 (Tetko et al., 2005).
[b] Acquired from ChEMBL data base (Gaulton et al., 2012).
[c] Acquired from chemicalize.org (ChemAxon, 2014).
[d] BCS class (Chuasuwan et al., 2009; Custodio et al., 2008; Lindenberg et al., 2004; Sjögren et al., 2013).
[e] Estimated by the Stoke-Einstein equation.
[f] Estimated according to Pruijn et al. (Pruijn et al., 2005).