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Original article

Standardization of an *ex vivo* method for determination of intestinal permeability of drugs using everted rat intestine apparatus

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ABSTRACT

Introduction: Everted gut sac of rat intestine is a paradigm widely employed for determination of absorption kinetics of drugs along with evaluation of effects of absorption enhancers. Since its inception in 1954, it has been optimized to enhance tissue survival and use, but it still suffers the limitation of small serosal compartment size and lack of validity of single experiment. **Methods:** The aim of the present work was to standardize a new *ex vivo* model to study drug absorption using a specially designed glass apparatus, everted segment of rat intestine, and three absorption markers [paracellular (atenolol), transcellular (metoprolol and propranolol)]. To validate a single experiment phenol red was used as non-absorbable marker. **Results:** The mean apparent permeabilities (Papp) for the markers were found to be $0.054 \pm 0.024 \times 10^{-4}$ cm/s (atenolol), $0.84 \pm 0.14 \times 10^{-4}$ cm/s (metoprolol), and $1.64 \pm 0.16 \times 10^{-4}$ cm/s (propranolol); wherein data from only those experiment was used, which showed negligible absorption of phenol red. **Discussion:** The model is simple to establish, gives excellent absorption kinetics, and most importantly provides a way to validate the experiment simultaneously. The proposed method can be used in all kinds of drug absorption studies, especially biopharmaceutical investigations studying absorption enhancement strategies.

1. Introduction

The everted gut sac of the rat small intestine was originally described by Wilson and Wiseman (1954). It can be used to determine transport of various compounds from intestine like sugars, amino acids, drugs, and evaluate the performance of novel drug delivery systems with high reliability and reproducibility (Barthe, Woodley, & Houin, 1999; Barthe, Woodley, Kenworthy, & Houin, 1998). Oxygenated tissue culture media (TC 199) and specific preparation techniques ensure tissue viability for up to 2 h (Barthe et al., 1998; Gandia, Lacombe, Woodley, & Houin, 2004). The technique can be used to study drug transport across the intestine and into the epithelial cells. Earlier the technique was widely used to study uptake of liposomes, proteins, macromolecules etc. (Rowland & Woodley, 1981a, 1981b, 1981c); but recently it was reported to be useful to quantify the paracellular transport of hydrophilic molecules, and estimation of the effect of absorption enhancers (Leppert & Fix, 1994).

The well known limitations of the everted gut sac model are morphological damages to intestinal tissue while everting (Balimane, Chong, & Morrison, 2000); presence of muscularis mucosa (Le Ferrec et al., 2001); small closed serosal compartment (Gandia et al., 2004). Secondly, the validation of experiment cannot be made simultaneously as no parameters are set to measure the integrity of mucosal barrier like transepithelial electrical resistance measurement (Barthe et al., 1999). In order to overcome some of these limitations a new apparatus was used by Appaji (1980). In the present investigation, we have attempted to standardize the use of this apparatus, which employs everted segment of rat intestine for permeability estimation. We have taken special care to ensure the validity of one experiment, which was one of the major drawbacks of methods employing everted rat intestine.

For standardization, we have used metoprolol, propranolol, atenolol, and phenol red. These drugs represent the biopharmaceutical classification system, as they belong to class I to class IV respectively. In addition, propranolol and metoprolol are absorbed via the transcellular route (Brouwers, Mols, Annaert, & Augustijns, 2010; Hilgendorf et al., 1999); atenolol by paracellular route (Brouwers et al., 2010); and phenol red is employed as a non-absorbable marker (Zakeri-Milani et al., 2007).

2. Materials

2.1. Chemicals and reagents

Atenolol, propranolol, and metoprolol were gifted by Ipca Laboratories Limited, Ratlam, India. Phenol red was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade. Freshly double distilled water, filtered through 0.45 mm nylon filter (47 mm) (Pall Life Sciences, Mumbai, India) in Millipore unit (USA), was used throughout the experiments.

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2.2. Experimental animals

Male Albino Wistar rats (150–200 g) born and reared in the Animal House of College of Pharmacy, IPS Academy, Indore, M.P. from a stock originally purchased from Sudhakar Rao Naik Institute of Pharmacy, Pusad, Maharashtra were used for the study. Animals were acclimatized to laboratory conditions 1 week before starting the experiment; they were given free access to water and standard rat diet (Trimurti Industries, Maharashtra, India) except during experimentation.

2.3. Everted rat intestine apparatus

The design of the glass apparatus used for the present study is depicted in Fig. 1a. The apparatus consists of two cylindrical glass tubes; one (110 × 17 mm) joined to other (48 × 17 mm) via J-shaped tapering end. Both the tubes are held together by a glass joint on the upper end. On the lower ends of both tubes a bulge is given for proper mounting of tissue. The dimensions of the apparatus (110 × 49 × 17 mm) are such that it can be conveniently set up in a 250.0 ml glass beaker. After mounting the everted intestinal segment on the apparatus and setting it in the beaker; the inside of the glass tubes serve as the mucosal compartment and the beaker serves as the serosal compartment (Fig. 1b).

3. Methods

3.1. Drug analysis by RP-HPLC

Drug analysis was carried out using an HPLC system (Shimadzu LC IOAT, Tokyo, Japan) attached to solvent delivery module with a low pressure gradient pump (LC 10AT VP), Rheodyne injector port (7725i, 20 µl loop), 15 cm C-18 column (Supelcosil®, Merck, India), and, UV/Vis photodiode array detector (SPD-M10A). The data interpretation was done with CSW (Chromatographic Work Station) CLASS-VP version 1.6 software (Shimadzu, Tokyo, Japan). We employed a previously reported method with slight modification (Zakeri-Milani, Valizadeh, Azarmi, Jalali, & Tajerzadeh, 2006) for drug analysis. The mobile phase for analysis of metoprolol, propranolol, and phenol red was a mixture of 55% methanol and 45% of 0.05 M KH_2PO_4 adjusted to pH 6.0, to which 0.2% (v/v) triethylamine was added. The mobile phase for analysis of atenolol was a mixture of 10% methanol and 90% of 0.05 M KH_2PO_4 aqueous solution adjusted to pH 6.0. The mobile phase was pumped in isocratic mode at a flow rate of 1.0 ml/min at ambient temperature. The UV detection was accomplished at 223 nm and samples of 20 µl were injected using Hamilton syringe on to the column.

3.2. Isolation and eversion of the intestine

The rat was sacrificed humanely by cervical dislocation and the abdomen was opened by midline incision and the intestine was carefully maneuvered to identify the ileocaecal junction. A 7 cm segment, 5–6 cm distant to the ileocaecal junction was excised, and it was removed of the mesenteric attachments carefully without damaging the intestinal architecture. The intestinal segment was transferred to a petri dish containing Kreb's medium (118.0 mM Nacl, 4.7 mM Kcl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$. $7H_2O$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 5.5 mM glucose). It was cleaned with Kreb's solution and then gently everted using a glass rod. A 3.0 cm everted segment was then used for permeability experiments.

3.3. Permeability determination

After mounting the tissue, the glass apparatus was placed in a 250.0 ml beaker containing 100 µg/ml drug solutions. The mucosal side of the intestine was perfused with Kreb's solution. This assembly (beaker and apparatus with tissue) was placed on a magnetic stirrer and a magnetic bead was allowed to rotate at 25 rpm in beaker and the temperature was maintained at 37 °C with adequate aeration. The samples were collected at different time points (at every 5 min for 1 h) and analyzed by HPLC for estimation of permeability.

3.4. Histopathology

After completion of the experiments, a small piece of the everted intestine was fixed with 10% formalin for 24 h and then dehydrated

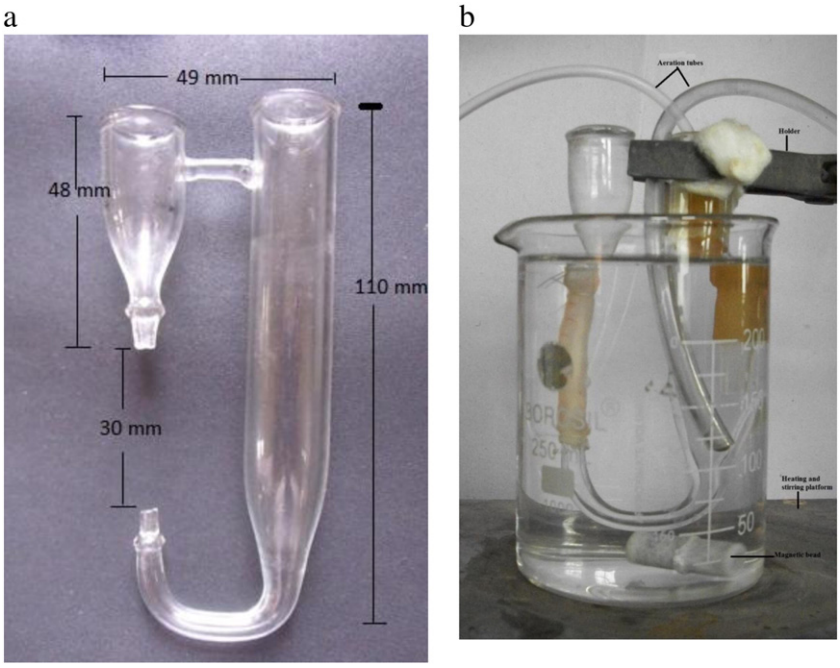

Fig. 1. Photographs of the apparatus (a) with dimensions; and (b) complete set up.