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Drug gastrointestinal absorption in rat: Strain and gender differences

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ABSTRACT

Predictive animal models of intestinal drug absorption are essential tools in drug development to identify compounds with promising biopharmaceutical properties. *In situ* perfusion absorption studies are routinely used in the preclinical setting to screen drug candidates. The objective of this work is to explore the differences in magnitude and variability on intestinal absorption associated with rat strain and gender.

Metoprolol and Verapamil absorption rate coefficients were determined using the *in situ* closed loop perfusion model in four strains of rats and in both genders. Strains used were Sprague–Dawley, Wistar-Han, Wistar-Unilever, Long-Evans and CD⁎IGS.

In the case of Metoprolol only CD⁎IGS and Wistar Unilever showed differences between males and females. For Verapamil, Wistar Han and Sprague–Dawley strains do not show differences between male and female rats.

That means that in these strains permeability data from male and female could be combined. In male rats, which are commonly used for permeability estimation, there were differences for Metoprolol permeability between Sprague–Dawley (with lower permeability values) and the other strains, while for Verapamil Sprague–Dawley and Wistar-Han showed the lower permeability values. In conclusion, the selection of rat's strain and gender for intestinal absorption experiments is a relevant element during study design and data from different strains may not be always comparable.

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1. Introduction

Rat as animal model has been used in preclinical drug development in many research areas from physiological to psychological research (Asem et al., 2015; Doluisio et al., 1969; Ghofrani et al., 2015; Gonzalez-Alvarez et al., 2007; Gundogdu et al., 2012; Hadar et al., 2015; Ikeda et al., 2015; Rato et al., 2015; Sato et al., 2015). Laboratory rats belong to the specie *Rattus norvegicus* (brown rat) which is bred and kept for scientific research. Most of the rat strains are derived from the albino Wistar rat, which is still widely used (Chiu et al., 2015; Etxeberria et al., 2015; Gonzalez-Alvarez et al., 2009; Liu et al., 2014; Lozoya-Agullo et al., 2015; Tugcu-Demiroz et al., 2014). Other common strains are the Sprague Dawley and the Long-Evans (Gokcek-Sarac et al., 2014; Mogil et al., 2000).

Predictive animal models of intestinal drug absorption are essential tools in drug development to identify compounds with promising biopharmaceutical properties. *In situ* perfusion absorption studies in rat are routinely used in the preclinical setting to screen drug candidates and this experimental model has demonstrated good predictive ability for human intestinal absorption (Lennernas, 1997, 2007; Sjogren et al., 2014).

In order to reduce the number of animals in absorption studies and to obtain predictive mathematical and *in silico* models, it would be desirable the combination of data from different laboratories and literature reports. One of the potential problems is the use of different experimental techniques and different rat strains. There are two main perfusion techniques broadly used to obtain intestinal permeability values in rat: single pass perfusion method and Doluisio's technique/closed loop method (Dahan and Amidon, 2009; Dahan et al., 2009; Doluisio et al., 1969; Hu and Amidon, 1988; Incecayir et al., 2013; Lozoya-Agullo et al., 2015). In spite of their differences in experimental settings, both methods give comparable results when the same rat strain is used with standardized protocols (Lozoya-Agullo et al., 2015). On the other hand,

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for absorption screening, Wistar (Han and Unilever) and Sprague Dawley are commonly used. Another issue related with rat strain is the fact that different rat strains are routinely used for various preclinical analyses, from toxicokinetic assays to teratogenic or pharmacological ones. As any systemic effect of a new drug administered by the oral route is related with its intestinal absorption it would be interesting to select the same strain for all the preclinical tests, if possible. Nevertheless, there is not much information regarding differences on intestinal drug absorption in rat strains and also in both genders, male versus female. In order to design experimental assays in a more rational and efficient way it is necessary to know at what extent different rat strains could be interchangeably used for characterizing drug absorption and pharmacological effects.

The objective of this work is to explore differences in magnitude and variability on intestinal absorption associated with rat strain and gender. This information would allow to data combination from different laboratories thus contributing to the 3 R's policy and it also would be useful to indicate whether the same strain can be used for pharmacological/toxicological and pharmacokinetic testing and if there are relevant differences associated to gender that could be extrapolated to humans.

2. Material and methods

2.1. Compounds assayed

Metoprolol, Verapamil, Acetonitrile and Methanol were purchased from Sigma (Barcelona, Spain).

2.2. Rat strains

Rat strains commonly used for nutrition and drug absorption studies were selected: Wistar Han (RjHan: **WI**), Wistar Unilever (HsdCpb: **WU**), Sprague Dawley (RjHan:**SD**(CD)), Long-Evans (RjOrl: **LE)** and CD∗IGS (Crl:**CD**(SD)).

2.3. Experimental technique

The absorption experiments were performed using an *in situ* loop technique previously described by Doluisio et al. (1969). The procedures used throughout the experiments were previously validated in our laboratory to adapt them to our experimental conditions. The study was approved by the Scientific Committee of the Faculty of Pharmacy at the University of Valencia [A133035 4541263] and followed the guidelines described in the EC Directive 86/609, the Council of the Europe Convention ETS 123 and Spanish national laws governing the use of animals in research (Real Decreto 223/1988, BOE 67, 18-3-98: 8509-8511).

Fasted rats weighing ∼250 g with free access to water were used in these studies. Rats were anesthetized using a mixture of Diazepam (1.67 mg/kg, Valium, Roche), Ketamine (50 mg/kg, Ketolar, Parke-Davis), and Atropine (1 mg/kg, Atropina sulfato, Braun), and placed on heated surface maintained at 37 °C. A midline abdominal incision was made and the small intestine was exposed. The bile duct was ligated in order to avoid drug enterohepatic circulation and the presence of bile salts in lumen. The method consists of creating a small intestinal compartment with the aid of two syringes and two three-way stopcock valves. Two incisions were made in the intestine, the first at the beginning of the duodenal segment, and the second at the end of the ileum segment, just before the cecum, and the entire small intestine was cannulated through these incisions (Casabó et al., 1987; Ferrando et al., 1999). Care was taken to avoid disturbance of the intestinal blood supply. In order to remove all intestinal contents, the small intestine was thoroughly flushed with a warm physiologic isotonic solution. The catheters were then connected to a glass syringe using a stopcock three way valve. The intestine was carefully placed back into the peritoneal cavity and the abdomen was covered with cotton wool pads to prevent peritoneal liquid evaporation and heat loss. This set up ensures the isolation of the small intestine, and drug solution can be introduced and sampled with the aid of the syringes and stopcock valves. To take a sample, the luminal content is pushed out from one syringe to the other. This procedure is done alternatively from the proximal syringe to the distal one, assuring the mixing of the solution in the intestinal lumen. The samples were collected every 5 min up to a period of 30 min (Bermejo et al., 1999; Ruiz-García et al., 1999).

Water absorption flux throughout the experiment may be significant, and hence must be accounted for (Tuğcu-Demiröz et al., 2014). A method based on direct measurement of the remaining solution volume was employed to calculate the water reabsorption zero order constant (k_0). For each tested compound, the initial volume (V_0) was determined on groups of three animals, while the endpoint volume (V_t) was measured on every animal used. The drug concentration in the samples was corrected as: $C_t = C_e(V_t/V_0)$, where C_t represents the concentration in the absence of water reabsorption at time t, and C_e the experimental value. The corrected concentration, C_t, was then used for absorption rate coefficient calculations (Martín-Villodre et al., 1986).

The absorption rate coefficients (k_a) of the compounds were determined by nonlinear regression analysis of the remaining concentrations in the intestinal lumen (C_t) versus time, as it is represented in Eq. (1):

$$C_t = C_0 \cdot e^{-k_a \cdot t} \quad (1)$$

These absorption rate coefficients were then transformed into permeability values (P_{eff}) using the relationship: $P_{eff} = k_a \cdot R/2$, where R is the effective radius of the intestinal segment, calculated from the area/volume relationship considering a 10 mL volume and an intestinal length of 100 cm.

The drug test solutions (100 μM Metoprolol and 100 μM Verapamil) were prepared in an isotonic saline matrix adjusted to pH 7.0 with 1% Sörensen phosphate buffer (v/v). Drug solutions were pre-warmed at 37 °C before administration into the intestinal lumen. The drug solution was perfused into the loop and then the entire intestine was restored and placed inside the abdominal cavity. The body temperature was maintained during anesthesia by heating with a lamp. Samples were withdrawn every 5 min for 30 min. Rats were sacrificed humanely at the end of experimentation. In order to separate solid components (e.g. mucus, intestinal contents) from drug solution, samples were centrifuged 5 min at 5000 rpm (1530 g), and then quantified, as described below.

2.4. HPLC analysis of samples

The samples were analyzed by HPLC with fluorescence detection. Metoprolol (λex = 307 nm and λem = 231 nm) using a mobile phase (v/v) 20:20:60 Acetonitrile:Methanol:Phosphate buffer (pH 3); and Verapamil (λex = 320 nm and λem = 275 nm) using a mobile phase (v/v) 40:60 Acetonitrile:Phosphate buffer (pH 3). A Novapack C18 (Waters®) cartridge-type column (4 μm particle size, 3.9 × 250 mm) was used. Retention time of Metoprolol and Verapamil was around 3 min. The method was previously validated over a known concentration range of the same magnitude as samples.

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