European Journal of Pharmaceutical Sciences 121 (2018) 236–242

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Intestinal permeability determinants of norfloxacin in Ussing chamber model

Cassiana Mendes[a,b,*], Gabriela C. Meirelles[a], Marcos A.S. Silva[b], Gilles Ponchel[a]

[a] CNRS UMR 8612, Université de Paris Sud XI, Faculté de Pharmacie, 5 rue J.B. Clément, 92296 Châtenay-Malabry, France
[b] Post graduation Program in Pharmaceutical Sciences, Quality Control Laboratory, Universidade Federal de Santa Catarina, J/K 207, 88040-900 Florianópolis, SC, Brazil

ARTICLE INFO

Chemical compounds studied in this article:
Budesonide (PubChem CID: 5281004)
Cyclosporine (PubChem CID: 5284373)
Levofloxacin (PubChem CID: 149096)
Lisinopril (PubChem CID: 5362119)
MK-571 (PubChem CID: 5281888)
Norfloxacin (PubChem CID: 4539)
Novobiocin (PubChem CID: 54675769)
Quinidine (PubChem CID: 441074)
Rhodamine 123 (PubChem CID: 65217)
Verapamil (PubChem CID: 2520)

Keywords:
Efflux
Intestinal permeability
Norfloxacin
Transporter
Uptake
Ussing chamber

ABSTRACT

Recently, many efforts are taken to identify the intestinal uptake and efflux transporters since they are responsible for the absorption of many drugs as their interactions. Norfloxacin (NFX) is a fluoroquinolone that presents low solubility and low permeability, and as a consequence, low bioavailability. In this context, the aim of this study is evaluate for the first time the intestinal permeability mechanisms of NFX by Ussing chamber model. The low permeation of NFX at low temperature, where the efflux pumps are not active, reveals that NFX permeation is transporter-dependent. The permeation study at different level of intestine demonstrated that NFX passage is in the decrescent order: ileum > jejunum > duodenum > colon, probably attributed to transporters that are expressed differently along the intestinal tract. NFX intestinal flow was evaluated in the presence of many inhibitors and substrates to identify the uptake and efflux transporters implicate in NFX permeability mechanism. It could be observed that BCRP and MRPs are involved in the NFX efflux and PEPT1, PMAT and OCT in the NFX uptake transport. Furthermore, this work revealed that NFX has itself an affinity for OCTN and OATP, demonstrating that NFX could inhibit these transporters and influence the absorption of other drugs. The updated description of NFX intestinal permeability factors could contribute to the development of rational pharmaceutical formulations that could circumvent the efflux problems and consequently improve NFX biopharmaceutical properties and avoid drug-drug interactions.

1. Introduction

Oral route is the most simple, safe and convenient route of administration. The absorption of drugs that are taken orally will depend of its bioavailability. Over the past few years, much research has been focused in the importance of intestinal drug transporters as one of the determinants of pharmacokinetics. Uptake and efflux transporters identified in the intestine plays a critical role in the membrane permeability and may influence the oral absorption of drug together with passive diffusion, paracellular transport and intestinal metabolism. ATP-dependent transporters localized in the enterocytes determine oral bioavailability, intestinal efflux and drug-drug interactions (Amidon et al., 1995; Estudante et al., 2013; Kim, 2006; Lennernäs, 2014, 2007; Rozehnal et al., 2012).

A vast number of drugs have the transport facilitate or hamper by drug intestinal transporters. The expression and function of these intestinal uptake or efflux transporters markedly affect the drug safety and efficacy. Inhibition or induction of intestinal efflux transporters of the ABC family such as P-glycoprotein (P-gp or multidrug resistance gene 1), breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 1/2 (MRP1/MRP2), or inhibition of uptake transporters like organic anion transporting polypeptides (OATP) or organic cation transporters (OCT) will result in intestinal drug absorption and consequently oral bioavailability. The knowledge of human intestinal transporters are limited although the main market drugs are orally administered, besides some studies focus on a limited number of proteins such as ABCB1 (P-gp) and ABCC22 (MRP2) (Hermann et al., 2018; Müller et al., 2018).

In this context, a method that could mimetics the *in vivo* conditions most closely will be useful to predict the absorption rate of drugs. Ussing chamber is an *ex vivo* method, recommended by Food and Drug Administration (FDA), that provides good prediction of permeability, metabolism and transporters interaction (FDA, 2000; Sjöberg et al., 2013). This technique could evaluate different regions of gastrointestinal tract using integral tissues from rats, for instance, considering the effect of drug-drug interactions. Usually, parallel artificial

* Corresponding author at: Laboratory of Quality Control, Department of Pharmaceutical Sciences, Federal University of Santa Catarina, J/K 207, 88040-900 Florianópolis, SC, Brazil.
E-mail address: cassi_ana@yahoo.com.br (C. Mendes).

https://doi.org/10.1016/j.ejps.2018.05.030
Received 29 January 2018; Received in revised form 24 May 2018; Accepted 31 May 2018
Available online 31 May 2018
0928-0987/

membrane permeability (PAMPA) and cultural cell lines like Caco2 are used to permeability studies; however these systems lack the morphological and physiological features of intestine, mainly permeability involving transporter-mediated process (Gotoh et al., 2005; Kerns et al., 2004).

Norfloxacin (NFX) is a fluoroquinolone antibiotic with a broad activity spectrum, which presents low solubility and low permeability, belonging to class IV of Biopharmaceutical Classification System (Amidon et al., 1995; Breda et al., 2009). The usual dose is 400 mg (12/12 h) and only about 30–40% is absorbed. There are reports in the literature that describes higher absorption of fluoroquinolones in duodenum and relates the low bioavailability of these classes to the intestinal efflux (Alvarez et al., 2008; Merino et al., 2006; Nix and Schentag, 1988). Therefore, the aim of this study is to evaluate the intestinal permeability mechanisms of NFX by Ussing chamber model. According to the recent literature review, there is no study that explains the determinants of intestinal permeability of NFX. Ussing chamber model was used to obtain useful information regarding the contribution of all the relevant intestinal drug uptake and efflux transporters in NFX absorption.

2. Experimental section

2.1. Materials

Budesonide, Cyclosporine, Lisinopril, Levofloxacin, MK-571, Norfloxacin, Novobiocin, Quinidine, Pyruvate, Rhodamine 123 and Verapamil were obtained from Sigma Aldrich (St. Louis, USA). All other analytical reagents were of analytical grade.

2.2. Animals

This study was carried out with male Wistar rats weighing 210–250 g (Janvier Lab, Paris, France). The animals were housed in a temperature (22 ± 2 °C) and light- (12 h light/dark cycles) controlled room, with free access to water and food, submitted to fasted state 12 h before the experiment. The studies were approved by ethical committee of University of Paris-Sud in accordance with European legislation on animal experiments.

2.3. Ussing chamber experiments

Different intestinal segments of sacrificed rats were excised, washed with cold physiological saline solution and visually examined to discard sections containing Payer's patches. The tissue was mounted in the Ussing chambers (intestinal surface of 1 cm^2) bathed with Ringer's Krebs Bicarbonate solution at pH 7.4, with mucosal side facing the donor compartment and serosal side facing the receptor compartment. The system was maintained at 37 °C and continuously oxygenated with O_2/CO_2 (95/5%). NFX (150 µM) was placed in the donor chamber and 500 µL were recovered from donor side and replaced with the same volume of fresh medium each 30 min until 180 min. Samples of donor side were also recovered to verify any change in NFX concentration. In the experiments with inhibitors, the system (donor and receptor chamber) was equilibrated only with inhibitor solution, after 30 min the medium of the donor chamber was replaced by NFX solution containing inhibitor and the experiment was carried out by 180 min. Transporters inhibitors were used to investigate which transporter is involved with the passage or inhibition of NFX. Budesonide (100 µM), Cyclosporine (10 µM), Novobiocin (5 µM), Lisinopril (100 µM), Levofloxacin (100 µM), MK-571 (50 µM), Quinidine (100 µM), Rhodamine 123 (100 µM) and Verapamil (100 µM) were used as inhibitors and Pyruvate (10,000 µM) as substrate competitor. All samples were analyzed by high performance liquid chromatography (HPLC) validated method as described below. Tissue viability was assessed during the experiments by continuously recording the transmucosal potential difference. If tissue damage was suspected, the experiment was discarded. The experiment was also realized at 4 °C to evaluate NFX permeation where efflux pumps are not active. The apparent permeability coefficient (P_{app}) was calculated using:

$$P_{app} = dQ/dt \times 1/AC_0 \quad (1)$$

where dQ/dt is the flux of NFX from the mucosal to the serosal side of the mucosa, C_0 is the initial concentration of NFX in the donor compartment and A is the area of the membrane. The values of P_{app} were calculated between 30 and 180 min after addition of NFX in all experiments, in order to standardize the calculations (Bravo-Osuna et al., 2008).

2.4. High performance liquid chromatography

The chromatographic analysis of NFX was performed in a Waters 515 pump, a Waters 717 plus autosampler (Milford, MA, USA) and UV detector Waters 486 set at 270 nm. The chromatographic system was equipped with a Phenomenex® (Torrance, CA, USA) C18 reversed-phase column (150 × 4.6 mm; 5 mm particle size) conditioned at 40 °C. The column eluted in isocratic mode using a mobile phase consisting of phosphate buffer (0.04 M, pH 3.0) and acetonitrile (84:16 v/v) at a flow rate of 1.0 mL/min and injection volume of 20 µL (Oliveira et al., 2009).

To analyze verapamil samples, the mobile phase was constituted of acetonitrile water (45:55 v/v) and the pH was adjusted to 2.8 with phosphoric acid (85%) at a flow rate of 1.2 mL/min and injection volume of 20 µL. The chromatographic system was constituted of CLC-ODS (6.0 × 150 mm) Shim-Pack reverse phased at room temperature and the chromatograms were recorded at 230 nm (Sultana et al., 2011).

2.5. Statistical analysis

Results are expressed as mean ± standard deviation of three replicates. The graphs were drawn and the statistical analysis was performed using GraphPad Prism, version 6.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. NFX intestinal passage study

The Ussing chamber experiment was realized with NFX at 4 °C mucosal-to-serosal, at 37 °C mucosal-to-serosal and serosal-to-mucosal and the results are expressed as percentage of NFX that permeates in Fig. 1. The permeability values are expressed in Table 1.

Fig. 1 and Table 1 shows that NFX quantity that permeates at 4 °C in the mucosal-to-serosal direction during 180 min is very low, approximately zero. NFX permeation at different portions of rat intestine was evaluated. The results are expressed in Fig. 2.

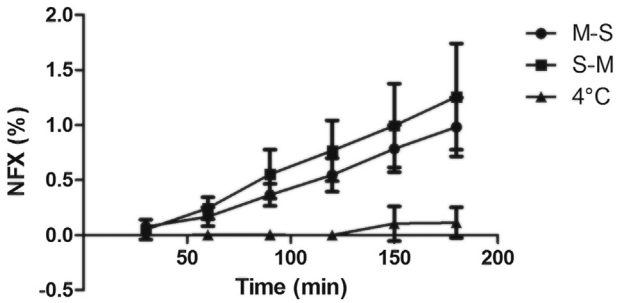

Fig. 1. NFX quantity (%) that permeates during 180 min at 37 °C mucosal-to-serosal and serosal-to-mucosal and at 4 °C.