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Study of the intestinal permeability of levofloxacin in coperfusion in rats

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1. Introduction

Levofloxacin (Levo) is a drug with an oral bioavailability near 100 % [1, 2]. According to the Biopharmaceutics Classification System [3] it belongs to the class of high permeability. For this reason, the possibility of using Levo as a model drug of high permeability in comparative studies with new substances could be considered.

However, in previous studies performed by our group using the in situ single-pass intestinal perfusion technique (SPIP) in rats, very little difference was observed between the concentration of Levo at the entrance and exit of the intestinal segment and thus increasing the variability of the results. For this reason, in order to study the case of intestinal permeability of Levo in more depth, a study of coperfusion of Levo with the high permeability standard metoprolol (Meto) and the low permeability standard acyclovir (Acyclo) [3] was performed.

2. Material and methods

2.1. Analytical Technique

The quantification of the compounds in the samples was performed by HPLC on a reversed phase column HypersilTM Elite C18 (150 mm x 4.6 mm i.d., 5 µm, ThermoFisher Scientific), with a mobile phase flux of 1 mL/min. The other conditions are listed in Table 1.

Table 1. HPLC Analytical conditions. FA aqueous phase; FO organic phase; AcN Acetonitrile; MeOH; Rt= retention time.

	Levo	Meto	Acyclo
FA	Water pH 1.5	Water pH 1.5	Water
pH 4			
FO	AcNc	AcNc	MeOHd
FA/FO	80/20	80/20	88/12
λ (nm)	223	223	254
Rt (min)	3.3 ± 0.3	6 ± 0.2	3.03 ± 0.02
Inj. vol (µL)	10	20	20

2.2. Rat Intestinal Perfusions

All animal experiments were conducted using protocols approved by the ethical committee of University of Barcelona (trial no. CEEA 124/16) and Generalitat de Catalunya (no. 6435).

The compounds were assayed at the highest dose strength in 250 mL perfusion solution [4] (3 mg/mL, 0.4 mg/mL and 1.6 mg/mL for levo, meto and acyclo, respectively). Sprague-Dawley rats (280 ± 30 g) were administered by i.p. injection with sodium pentobarbital (60 mg/kg BW). Briefly, once under general anaesthesia, a laparotomy was performed and a segment of the duodenum (ca. 10 cm) was isolated by inserting two glass

cannulas (o.d. 4 mm, i.d. 3 mm) at the proximal and distal end of the segment. After rinsing the intestinal segment properly, the experiment started by delivering the perfusion solution containing the drugs (Table 2) and phenol red (0.1 mg/mL) at a flow rate of 0.20 mL/min to the intestinal segment. The outflow perfusate was collected at 5 min intervals for 60 min. At the end of the experiment, the rat was euthanized and the length of the intestinal segment was measured. Samples were centrifuged (9000 rpm for 10 min), and the supernatant was stored at -20 ºC until its analysis by HPLC.

Table 2. Study design in the SPIP experiments.

Rats	Perfusion solution
1 to 4	Levo + Meto
5 to 8	Levo + Acyclo

2.3. Data Analysis and Statistics

Effective permeability coefficients (Peff) and percentage of absorption of Levo were calculated at steady state conditions according to equations 1 and 2 respectively, after correcting the outlet concentration following the phenol red method [5].

$$P_{\text{eff}} = \frac{-\emptyset_{\text{in}}}{2\pi RL} \times Ln\ \frac{C_{\text{out.cor}}}{C_{\text{in}}} \qquad (1)$$

$$\%fa = \left[1 - \left(\frac{C_{\text{out.cor}}}{C_{\text{in}}}\right) * 100\right] \qquad (2)$$

Where:

Φ is the perfusion solution flow rate (0.2 mL/min), Cin and Cout are the respective inlet and corrected outlet concentrations, R is radius of the intestinal segment (set to 0.2 cm) and L is the length of the perfused intestinal segment. Cout concentrations were corrected by multiplying by the factor CPRin/CPRout where: CPRin is the phenol red concentration in the inlet buffer solution and CPRout is the phenol red concentration at the specific time interval.

The stability of the tested compounds in the inlet perfusion solutions at 37ºC was assessed for 60 min. The mean parameters of Levo (n=4) were compared with the corresponding standard (n=4) by means of a paired-samples T test after testing the normal distribution of the data (Shapiro-Wilk test) and using SPSS Statistics vs. 17.0 (α= 0.05).

3. Results and discussion

The results obtained are shown in Table 3.

As depicted in Table 3, Levo showed a similar permeability to that of Metol and higher than that of Acyclo, confirming its high permeability. These results are in agreement with those obtained by Volpe et al. in Caco 2 cells [3], where Levo showed an apparent permeability coefficient (Papp) similar to that of the metoprolol. However, it is not advisable to use it as a high permeability standard, since -as demonstrated in the study of Volpe et al.- it is a substrate of p-glycoprotein [6].

Table 3. Permeability coefficients and percentage of drug absorbed in the different SPIP experiments. a, b = differences with acyclovir (p=0.001).

	Drug	P_{eff} (cm/s)	% abs
Rats 1-4	Levofloxacin	$9.66 \pm 3.62 \cdot 10^{-6}$	5.05±2.29
	Metoprolol	$9.31 \pm 3.04 \cdot 10^{-6}$	5.13±2.26
Rats 5-8	Levofloxacin	$1.185 \pm 0.284 \cdot 10^{-5a}$	7.22 ± 1.23^{b}
	Acyclovir	$2.126 \pm 1.486 \cdot 10^{-6}$	1.30±0.72

4. Conclusions

Levo is a high permeability drug demonstrated in the in situ intestinal coperfusion study in rats. Coperfusion with a high and/or low permeability standard is recommended when intending to study the permeability of a new product.

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