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Research paper

Transintestinal secretion of ciprofloxacin, grepafloxacin and sparfloxacin: in vitro and in situ inhibition studies

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Abstract

The influence of the secretion process on the absorption of ciprofloxacin, grepafloxacin and sparfloxacin has been evaluated by means of inhibition studies. Two well known P-glycoprotein inhibitors (cyclosporine, verapamil), a mixed inhibitor of P-glycoprotein and the organic cation transporter OCT1 (quinidine) and a well established MRP substrate (*p*-aminohipuric acid) have been selected in order to distinguish the possible carriers implicated. An in situ rat gut perfusion model and CACO-2 permeability studies are used. Both methods suggest the involvement of several types of efflux transporters for every fluoroquinolone. The relevance of the secretory pathway depends on the intrinsic permeability of the quinolone. The in vitro model seems to be more suitable for discriminating mechanisms underlying the absorption process, while in situ studies are less sensitive to inhibition studies.

Keywords: Secretion; Fluoroquinolones; Inhibition; Gut perfusion studies; CACO-2 permeability

1. Introduction

In recent years, the importance of the intestinal efflux mediated by several carriers has been shown. This process runs counter current to the absorptive transport or diffusion of drugs, restricting the extent of oral absorption [1,2].

Many times this is the reason for the failure of predicting bioavailability from the structure [3]. A considerable effort has been made to identify the structural features that determine the interaction with this type of transporters [4], in order to avoid them in the design and development of new drugs. On the other hand, as pointed out by several authors such as Lentz [5], Doppenschmitt [6] and Stephens [7], affinity to P-glycoprotein (Pgp) observed in vitro does not always imply a reduction in the rate or extent of absorption, nor in the 'in vivo' activity since other factors such as passive permeability and gut wall metabolism will affect the overall performance of the drug.

There has been a great interest in studying possible inhibitors of the process as they would allow a better use of several drugs, mainly chemotherapeutic agents and anti-HIV drugs, whose resistance is based on the efflux at the target site by the same type of carriers [8–10].

Fluoroquinolones have been reported as a group of structures able to undergo efflux, which can explain the low bioavailability of some of them [11,12]. The aim of the study is to test the influence of efflux on the absorption of three fluoroquinolones differing in effective permeabilities while maintaining the main structural features. This would allow us to gain an insight on the characteristics of this class of drugs that lead to interaction with intestinal transporters. The knowledge could be of great value in understanding the efflux process and in the future design of new fluoroquinolones with improved bioavailability. As inhibitors two well known Pgp inhibitors, cyclosporine (cA) and verapamil (V), and a mixed inhibitor of Pgp and the organic cation transporter OCT1, quinidine (Q), and a well established MRP substrate, *p*-aminohipuric acid (pA), have been

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selected, in order to point out the possible carriers implicated.

2. Materials and methods

2.1. Compounds assayed

Ciprofloxacin (CIP) and Sparfloxacin (SPX) were kindly donated by CENAVISA (Reus, Spain), Grepafloxacin hydrochloride (GRX) was given by Glaxo–Wellcome (United Kingdom). Verapamil hydrochloride (V), *p*-aminohipuric acid (pA), quinidine (Q) and cyclosporine-A (cA) were purchased from Sigma (Barcelona, Spain).

2.2. In situ absorption studies

The study was approved by the Scientific Committee of Animal Use of the Faculty of Pharmacy and follows 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 (RDE 223/1988, BOE 67, 18-3-98: 8509-8511].

The School of Pharmacy of the University of Valencia has an authorized animal facility housing (E (1989) 13th October, 1989, BOE 250, 18-10-89, 32682-32683) which provides the animals for the experiments and ensures their welfare and health conditions before their use. Male Wistar rats weighing 200–290 g were used.

The in situ perfusion ('closed loop') rat gut preparation [13,14] adapted as previously described [15] was performed. The animals were fasted overnight before the experiment, allowing free access to water. The surgical procedure was performed under anesthesia (i.p. administration of 1 g/kg of ethyluretane solution 25% w/V). The bile duct was ligated in order to prevent enterohepatic recycling.

No regional differences in CIP and SPX absorption were detected in previous works [16,17], indicating that the relevance of the efflux system is negligible. Therefore, the solutions of these drugs were perfused into the whole intestine as representative of any part of it. On the other hand, as it was previously observed that the absorption of GPX was significantly lower in the proximal fraction (33 cm) of the intestine [16], the inhibition studies were performed only in this part of the tract to be able to point out the differences more adequately.

Test solutions were prepared in isotonic saline adjusted to pH 7.0 with 1% Sörensen phosphate buffer (V/V). Volumes of 10 ml of the test solution were perfused in the assays carried out in the whole intestine of the rat, while 5 ml were used when the experiments were performed in the proximal fraction.

CPX and GPX were perfused at a concentration 50 μM without additives and in the presence of V 100 μM, Q 100 μM, cA 20 μM or pA 20 mM. SPX was assayed in the same conditions but it could not be administered in the presence of Q and pA due to analytical interferences. The inhibitors were dissolved into the plain perfusion vehicle prior to the addition of the corresponding quinolone.

Samples of the luminal fluid were collected into eppendorfs every 5 min for a total of 30 min, and analyzed for the drug content.

As a significant reduction in the volume of the perfusate at the end of the experiments was observed, changes in volume must be taken into account to calculate the absorption rate constants. Estimation of the water reabsorption process is based on direct measurement of the remaining volume of the test solution at the beginning of the experiment (V_0) in groups of three animals and at the end (V_{30}) in every animal used. An apparent zero-order kinetics was assumed [15] to calculate the remaining volume at every sampling time (V_t). The concentration in the sample C_e was corrected as follows: $A_t = C_e V_t/V_0$, where A_t represents the remaining concentration in the gut that would exist in the absence of the water reabsorption process at time t.

The A_t values were used to calculate the apparent absorption rate constants k_a by non-linear regression versus time, using Sigma Plot 2.0 (Jandel Scientific, GmbH, Erkrath, Germany). Once the test solution is placed into the gut, the compound is adsorbed to the membrane and loaded into the enterocyte compartment. After 5 min, it can be considered that a quasi-steady-state is achieved and that the compound disappearing from lumen is appearing in the blood side. In these conditions, the disappearance from lumen can be considered as a first order process. Sampling began after 5 min in order to prevent adsorption, dilution and loading effects [13,15].

The intestinal permeability values (P_{eff}) were calculated taking into account the relationship between the absorption rate constant (k_a) and P_{eff}: $P_{eff} = (k_a R)/2$, where R is the radius of the perfused intestinal segment. The intestinal effective permeabilities (P_{eff}) of the tested compounds (means of five or six animals) were used as index of the efficiency of absorption.

2.3. In vitro permeability studies

CACO-2 cells were a gift from Dr M. Hu, Washington State University, USA. Cells were maintained routinely at 37°C in an atmosphere of O_2:CO_2 (95:5) and were grown in Dulbecco's modified Eagle's Medium powder from Sigma (Barcelona, Spain) with $NaHCO_3$ (Panreac Química, Barcelona, Spain) (3.7 g/l), HEPES (Acros Organics, Geel, Belgium) (1.3 g/l) and glucose (3.5 g/l) supplemented with 10% foetal bovine serum, 1% non-essential amino acids, 200 mM (1%) L-glutamine, 100 IU/ml Penicilin G and 100 μg/ml Streptomycin (all from Sigma, Barcelona, Spain). Medium was replaced every 2 days. Cell monolayers were prepared by seeding at a concentration of 25 cells/μl (2 ml total) onto tissue culture inserts (transwell cell

culture insert surface 4.2 cm^2, 3 μM pore size (Multiwell™ six wells, Becton Dickinson, France). Confluence was reached 7 days after seeding, and the transport experiments were conducted with the monolayers on days 12–14 post confluence. The epithelial integrity of monolayers was checked by transepithelial electrical resistance (TEER) (Millicel ERS™ device, Millipore, Scharlab, Barcelona). The TEER values obtained were around 800 Ω cm^2. The solution used in all the experiments contained Hank's balanced salt solution (HBSS) (Sigma, Barcelona, Spain) (9.8 g/l) with $NaHCO_3$ (0.37 g/l), HEPES (5.96 g/l) and glucose (3.5 g/l) as vehicle.

To measure apical-to-basolateral (P_{ab}) and basolateral-to-apical efflux (P_{ba}), the test compound was included in the apical or basolateral side, respectively. A shaker device was used to maintain a constant agitation rate (50 rpm) and temperature (37°C) throughout the experiment. At the designated times, samples of 0.2 ml were taken from the receptor compartment for analysis of contents and replaced with an equal volume of HBSS. Test quinolone and inhibitor solution concentrations were the same as those perfused 'in situ', no other additives being used.

2.4. Analysis of the samples

An original high performance liquid chromatography (HPLC) procedure was used to quantify the solute concentration in the samples. The method was carried out on a Perkin Elmer system (Perkin Elmer, Barcelona, Spain) equipped with a Novapak C18 column (Waters, Barcelona) (3.9 × 150 mm). The mobile phase consisted of mixtures of methanol (Scharlab, Barcelona, Spain), acetonitrile (Scharlab, Barcelona, Spain), and an aqueous solution consisting of 15 mM phosphate buffer, adjusted to pH 2.4 (for CIP) or pH 3.0 (for GRX and SPX) with orthophosphoric acid. The ratio of solvent volumes used were: 25/5/70 for CIP, 20/20/60 for GRX and 0/23/77 for SPX of methanol, acetonitrile and buffer, respectively. Analysis was carried out at 22°C at a flow rate of 1 ml/min.

Quantification was achieved by fluorometry, with an excitation wavelength of 285 nm and an emission wavelength of 445 nm, for CIP and GPX, while for SPX, 295 and 525 nm were selected.

The retention time of CIP was 2.40 min and the detection limit was 50 ng/ml. GRX was eluted in 3.25 min and the limit was 25 ng/ml. SPX showed a retention time of 3.4 min and the detection limit was estimated as 75 ng/ml.

Samples were centrifuged and directly injected. Every method was validated beforehand in the range of concentrations assayed.

2.5. Statistical analysis

Student's *t*-test and one way analysis of variance (ANOVA) followed by Scheffé multiple test were performed in order to detect statistical differences between permeability coefficients. All statistical analyses were performed by means of SPSS 10.0 (SPSS Inc, Chicago, USA).

3. Results and discussion

3.1. Ciprofloxacin studies

Permeability values of CIP in the different situations assayed are listed in Table 1. As demonstrated in a previous work [16], if CIP is perfused in situ at 1.5 μM, the simultaneous presence of V produces a significant increase in the permeability, implying that there is a significant contribution of the secretion by Pgp. Nevertheless, as can be observed in Table 1, increasing CIP concentration to 50 μM results in a lack of inhibitor effect. Even higher concentrations of V (2 mM, 20 times the previous) do not reverse

Table 1
Permeability values (cm/s) and standard deviation obtained in situ and in vitro

			Without additives	In the presence of inhibitors			
				V 100 μM	Q 100 μM	cA 20 μM	pA 20 mM
CIP 50 μM	Whole intestine		1.20 e-5 (2.34e-6)	1.26 e-5 (3.08e-6)	1.34 e-5 (2.61e-6)	1.30 e-5 (2.84e-6)	1.57 e-5 (2.11e-6)
	CACO-2 cell line	P_{ab}	2.99 e-6 (2.92e-7)	5.15 e-6 (2.53e-6)	4.94 e-6 (2.36e-6)	3.38 e-6 (4.96e-7)	7.20e-6 (1.22e-6)
		P_{ba}	5.95 e-6 (3.74e-7)	4.07 e-6 (5.41e-7)	3.91 e-6 (3.88e-7)	5.88 e-6 (1.10e-6)	6.41e-6 (1.53e-6)
SPX 50 μM	Whole intestine		4.38 e-5 (3.71e-6)	8.03 e-5 (7.20e-6)*		7.04 e-5 (3.48e-6)*	
	CACO-2 cell line	P_{ab}	1.49 e-5 (1.08e-6)	2.26 e-5 (9.31e-7)*		1.83 e-5 (3.92e-7)	
		P_{ba}	2.41 e-5 (2.32e-6)	2.21 e-5 (1.49e-6)		2.18 e-5 (5.15e-7)	
GPX 50 μM	Proximal fraction		3.80 e-5 (3.31e-6)	4.52 e-5 (4.04e-6)	5.08 e-5 (5.32e-6)*	4.57 e-5 (5.70e-6)	2.73e-5 (3.61e-6)*
	CACO-2 cell line	P_{ab}	1.28 e-5 (3.24e-6)	1.79 e-5 (1.18e-6)*	2.09 e-5 (1.45e-6)*	1.70 e-5 (6.97e-7)	8.02e-6 (5.11e-7)*
		P_{ba}	2.27 e-5 (1.24e-6)	2.03 e-5 (4.47e-7)	1.93 e-5 (8.32e-7)	1.81 e-5 (5.56e-7)*	2.38e-5 (1.55e-6)

Apical–basolteral permeability (P_{ab}), and basolateral–apical permeability (P_{ba}).* Denotes statistical differences respect to the permeability in the absence of inhibitor.

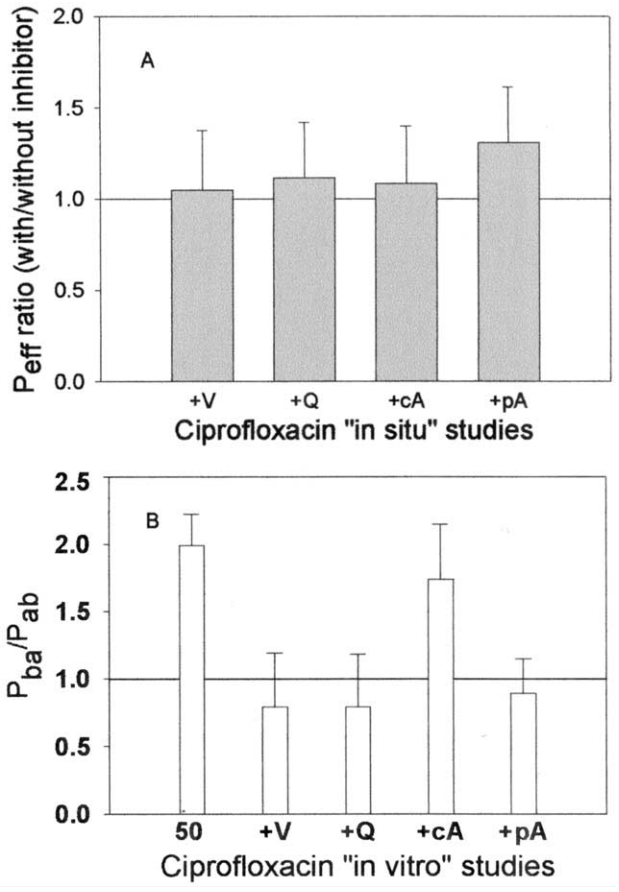

Fig. 1. (A) Ratios between permeability coefficients (P_{eff}) of ciprofloxacin (CIP) in the presence and absence of inhibitors obtained in rat intestine. (B) Ratios between basolateral–apical and apical–basolateral permeabilities of ciprofloxacin (CIP) obtained in CACO-2 cells. V (verapamil), Q (quinidine), cA (cyclosporine A) and pA (*p*-aminohipuric acid).

the effect of inhibition produced by the rise in antibiotic concentration (data not shown). The same result is obtained with Q or cA. On the other hand, CIP effective permeability changes are greater with pA, suggesting the possibility that several types of efflux carriers (Pgp, MRP, OCT1) are responsible for the secretion of the antibiotic, a fact already mentioned by Dautrey et al.[18]. In fact, more research with specific inhibitors would be needed to clearly define the nature of the carrier (i.e. Leukotriene C4 to distinguish between MRP-proteins and anion transporters).

Considering the in vitro experimental model, results also point out a possible secretion process. There is a decrease in the ratio P_{ba}/P_{ab} when an inhibitor is included in the medium (see Fig. 1), even though the change is not statistically significant. The effect of V and Q is consistent with previous findings (P_{ab} increases while P_{ba} is reduced); the effect of cA is negligible; but pA produces a significant increase in P_{ab} that is not reflected in the ratio because there is a simultaneous increase in P_{ba}. This phenomenon can be explained on the basis of an interaction with both anionic transporters MRP1 and MRP2. These results are in accordance with those obtained by Lowes and Simmons [19] in the same cell culture, but the authors concluded that CIP is not a substrate of Pgp nor of MRP2.

Comparison of the two methodologies points out the ability of cultured cells to better distinguish the underlying mechanism, probably due to a higher expression level of efflux proteins in the CACO-2 monolayers than in the rat intestine. The secretion process has little significance on the overall absorption, as observed by Doppenschmitt and Lentz [5,6] considering the low passive permeability of the quinolone although other factors such as a low affinity of the compound for the carrier could also explain this fact.

3.2. Grepafloxacin

This quinolone shows greater permeability than the parent compound, consistent with its more lipophilic character [16,20]. This would mean that passive diffusion through the membrane plays an important role in its absorption. Nevertheless, we have previously demonstrated that GRX has a concentration dependent absorption, consistent with a secretory pathway in which both Pgp and MRP2 seem to be involved [16]. In a previous study, we have shown that V produces a significant increase in the permeability coefficient when perfused at 2 mM in the presence of low concentrations of the quinolone (2.5 and 25 μM), but this effect cannot be seen if the concentration of the quinolone is raised to 50 μM and the inhibitor V is reduced to 100 μM (see Table 1), because interaction depends on affinity as well as concentration. Other inhibitors, such as Q and pA are able to modify, at least partially, the absorption of the quinolone at 50 μM, as can be observed in Table 1. It has to be emphasized that the inhibitors act in different ways, while Q significantly increases the permeability, the presence of pA produces a reduction. The permeability ratios are graphically outlined in Fig. 2A. Accordingly, it seems that the absorption process of this quinolone comprises passive diffusion and active transport in both directions, entrance and efflux. The active entrance is associated with anion transporters that can be inhibited by pA, probably belonging to the MRP family. The efflux process seems to be related to Pgp sensitive to Q. When compared to CIP results, a higher impact of secretion on GRX absorption can be derived.

Concerning in vitro experiences, the transport process of GRX is affected by the presence of inhibitors, regardless of the parameters considered: P_{ab} is significantly higher in the presence of V and Q; on the other hand, P_{ba} is significantly lower in the presence of cA. Due to this the ratio P_{ba}/P_{ab} in the presence of V, Q and cA turns out to be near one, as predicted when the secretion is inhibited completely (Fig. 2B). Once again, as seen ‘in situ’, the presence of pA produces a different effect from that of the other inhibitors, as it decreases the entrance permeability and has no effect on the exit from the enterocyte. These results are in agreement with those found by Kazumasa et al. [11] in CACO-2 cells, and confirm those found ‘in situ’. The results

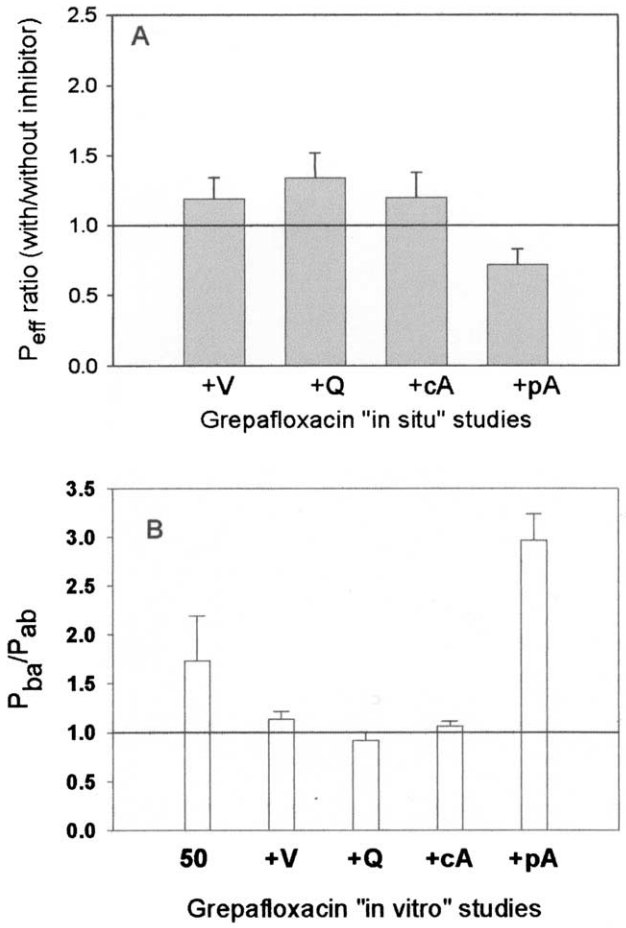

Fig. 2. (A) Ratios between permeability coefficients (P_{eff}) of grepafloxacin (GRX) in the presence and absence of inhibitors obtained in the proximal fraction of rat intestine. (B) Ratios between basolateral–apical and apical–basolateral permeabilities of GRX obtained in CACO-2 cells. V (verapamil), Q (quinidine), cA (cyclosporine A) and pA (*p*-aminohipuric acid).

from both experimental models are also in accordance with the hypothesis of Lentz and Dopenschmith about efflux being important only for intermediate permeability compounds, such as is GRX [5,6].

3.3. Sparfloxacin

Its structure presents two fluorinated atoms, which counteract the increase in molecular weight, producing an intermediate lipophilicity. There is controversy concerning the absorption of this quinolone. Cormet et al. [21–23] in different publications showed that the absorption of this drug is altered by certain Pgp inhibitors, but when trying to describe in detail the permeability as a function of the concentration administered, they conclude that it can be characterised as a passive process in the range used (1–200 μM). We have previously determined its intrinsic permeability in the whole intestine at two different concentrations, 25 and 300 μM, and no differences were detected [17]. The inhibition studies with this compound have been limited by the analysis of the samples (Q and pA peaks overlap with that of the antibiotic). The results are similar to those of GRX; a significant increase in the permeability determined in situ in the presence of both, cA and V can be seen. Results are listed in Table 1, and ratios outlined in Fig. 3A.

Results from the experiences with CACO-2 monolayers are very similar to those found 'in situ'. Two different concentrations, 50 and 300 μM, were administered and no statistical differences in permeability were seen (data not shown). Concerning the inhibitors, only in the presence of V a significant increase in P_{ab}, is evidenced, but both inhibitors assayed are able to reduce the ratio P_{ab}/P_{ba} from about two to one, consistently with the abolishment of the efflux. Ratios are represented in Fig. 3B. These results confirm the existence of an efflux system implied in the absorption of SPX. The process probably has low capacity of transport and saturates at the concentrations administered.

In summary, the results obtained in this work show the

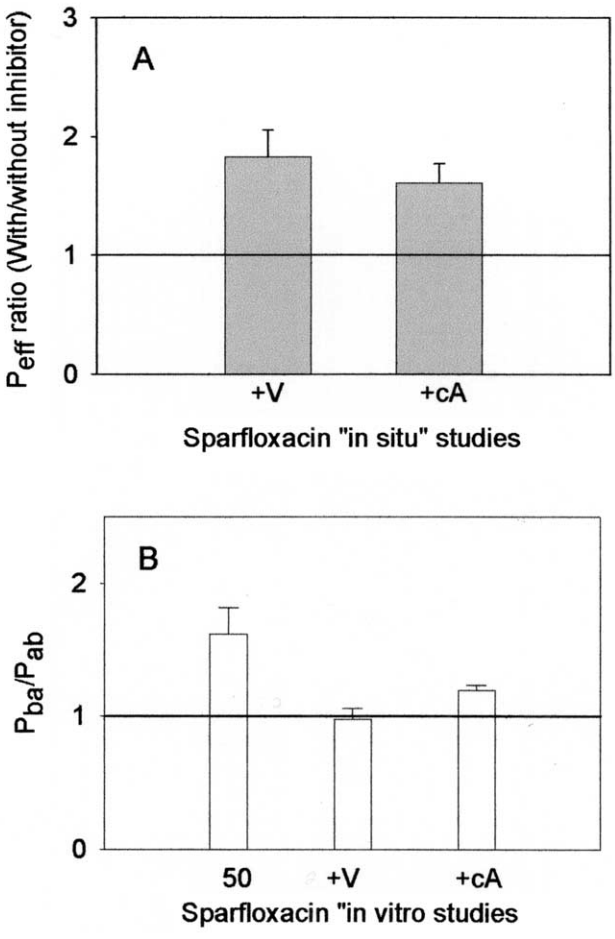

Fig. 3. (A) Ratios between permeability coefficients (P_{eff}) of sparfloxacin (SPX) in the presence and absence of inhibitors obtained in rat intestine. (B) Ratios between basolateral–apical and apical–basolateral permeabilities of SPX obtained in CACO-2 cells. V (verapamil), Q (quinidine), cA (cyclosporine A) and pA (*p*-aminohipuric acid).

possible role of efflux proteins in the ADE characteristics of fluoroquinolones. The combined approach used ('in situ' intestinal rat perfusion and 'in vitro' CACO-2 cell monolayer permeability) presents concordance of findings despite the different sensitivity of the experimental methods. The characterization of the efflux processes of quinolones could help to explain their low absorbability in some cases and also to prevent drug interactions at absorption levels. On the other hand, the quinolone affinity for secretion transporters make these substances potential MDR reversal agents and could be used as a strategy to increase chemotherapy cytotoxicity. For example, Dinofloxacin has already demonstrated its ability to increase cell sensitivity to MRP substrates such as Vincristine, Doxorubicine and Daunorubicine [24,25]. Nevertheless, it should be taken into account that the carriers are expressed not only in tumoral cells but also in normal tissues so the kinetic consequences of efflux inhibition should be checked 'in vivo'. Moreover, it is necessary to accurately determine the transporter implied in each case to reach satisfactory results.

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References

[1] H. Suzuki, Y. Sugiyama, Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine, Eur. J. Pharm. Sci. 12 (2000) 3–12.
[2] J. Hunter, H.B. Hirst, Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption, Adv. Drug Deliv. Rev. 25 (1997) 129–157.
[3] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeny, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev 23 (1997) 3–25.
[4] A. Seelig, E. Landwojtowicz, Structure-activity relationship of P-glycoprotein substrates and modifiers, Eur. J. Pharm. Sci. 12 (2000) 31–40.
[5] K.A. Lentz, J.W. Polli, S.A. Wring, J.E. Humphreys, J.E. Polli, Influence of passive permeability on apparent P-glycoprotein kinetics, Pharm. Res. 17 (2000) 1456–1460.
[6] S. Doppenschmitt, H. Spahn-Langguth, C.G. Regardh, P. Langguth, Role of P-glycoprotein-mediated secretion in absorptive drug permeability: an approach using passive membrane permeability and affinity to P-glycoprotein, J. Pharm. Sci. 88 (1999) 1067–1072.
[7] R.H. Stephens, C.A. O'Neill, A. Warhurst, G.L. Carlson, M. Rowland, G. Warghurst, Kinetic profiling of P-glycoprotein-mediated drug efflux in rat and human intestinal epithelia, J. Pharm. Exp. Ther. 296 (2001) 584–591.
[8] I. Bosch, J. Croop, P-glycoprotein multidrug resistance and cancer, Biochim. Biophys. Acta. 1288 (1996) F37–F54.
[9] S.V. Ambdudkar, S. Dey, C.A. Hrycyna, M. Ramachandra, I. Pastan, M.M. Gottesman, Biochemical, cellular, and pharmacological aspects of the multidrug transporter, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 361–398.
[10] B.J. Aungst, P-glycoprotein, secretory transport and other barriers to the oral delivery of anti-HIV drugs, Adv. Drug Deliv. Rev. 39 (1999) 105–116.
[11] N. Kazumasa, T. Ikumi, I. Natsuko, M. Hiromi, S. Yoschimichi, S. Nagao, T. Akira, Active intestinal secretion of new quinolone antimicrobials and the partial contribution of P-glycoprotein, J. Pharm. Pharmcol. 53 (2001) 699–709.
[12] H. Yamaguchi, I. Yano, Y. Hashimoto, K. Inui, Secretory mechanism of grepafloxacin and levofloxacin in the human intestinal cell line CACO-2, J. Pharmacol. Exp. Ther. 295 (2000) 360–366.
[13] J.T. Doluisio, W.G. Crouthamel, G.H. Tan, J.V. Swintosky, L.W. Dittert, Effect of membrane storage on the kinetics of drug absorption, J. Pharm. Sci. 59 (1970) 72–76.
[14] J.T. Doluisio, N.F. Billups, L.W. Dittert, E.T. Sugita, J.V. Swintosky, An in situ rat gut technique yielding realistic absorption rates, J. Pharm. Sci. 58 (1969) 1196–1200.
[15] A. Martín-Villodre, J.M. Plá-Delfina, J. Moreno, M.D. Pérez-Buendía, J. Miralles, E.F. Collado, E. Sanchez-Moyano, A. Del Pozo, Studies on the reliability of a bihyperbolic functional absorption model. I. Ring substituted anilines, J, Pharmacokin. Biopharm. 14 (1986) 615–633.
[16] Rodríguez-Ibañez, M., Sánchez-Castaño, G., Montalar-Montero, M., Bermejo, M., Garrigues, T.M., Merino, V., Kinetics of transintestinal secretion of ciprofloxacin and grepafloxacin: comparison of in situ and in vitro studies, J. Pharm. Pharmacol., submitted for publication (2002).
[17] Fernández-Teruel, C., González-Alvarez, I., Merino, V., Garrigues, T.M., Ruiz-García, A., Bermejo, M.V., Intestinal absorption of sarafloxacin and sparfloxacin: prediction of lipophilicity values and a biophysical absorption model. Poster presentation at Pfizer Drug Discovery 2001. September 2001. Sandwich, UK.
[18] S. Dautrey, K. Felice, A. Petiet, B. Lacour, C. Carbon, R. Farinotti, Active intestinal elimination of ciprofloxacin in rats: modulation by different substrates, Br. J. Pharmacol. 127 (1999) 1728–1734.
[19] S. Lowes, N.L. Simmonds, Multiple pathways for fluoroquinolone secretion by human intestinal epithelial (CACO-2) cells, Br. J. Pharmacol. 135 (2002) 1263–1275.
[20] V. Merino, J. Freixas, M.V. Bermejo, T.M. Garrigues, J. Moreno, J.M. Plá-Delfina, Biophysical models as an approach to study passive absorption in drug development: 6-fluorquinolones, J. Pharm. Sci. 84 (1995) 777–782.
[21] E. Cormet-Boyaka, J.F. Huneau, A. Mordrelle, P.N. Boyaka, C. Carbon, E. Rubinstein, D. Tome, Secretion of sparfloxacin from the human intestinal CACO-2 cell line is altered by P-glycoprotein inhibitors, Antimicrob. Agents Chemother. 42 (1998) 2607–2611.
[22] E. Cormet, J.F. Huneau, M. Bouras, C. Carbon, E. Rubinstein, D. Tome, Evidence for a passive diffusion mechanism for sparfloxacin uptake at the brush-border membrane of the human intestinal cell-line CACO-2, J. Pharm. Sci. 86 (1997) 33–36.
[23] E. Cormet, A.M. Barlier, J.F. Huneau, E. Rubinstein, C. Carbon, R. Farinotti, D. Tome, Sparfloxacin secretion across CACO-2 cells involves a multidrug resistance-like mechanism, Drugs 49 (Suppl. 2) (1995) 307–309.
[24] M.D. Norris, J. Madafiglio, J. Gilbert, G.M. Marshall, M. Haber, Reversal of multidrug resistance-associated protein-mediated drug resistance in cultured human neuroblastoma cells by the quinolone antibiotic difloxacin, Med. Pediatr. Oncol. 36 (2001) 177–180.
[25] S. Gollapudi, F. Thadepalli, C.H. Kim, S. Gupta, Difloxacin reverses multidrug resistance in HL-60/AR cells that overexpress the multidrug resistance-related protein (MRP) gene, Oncol. Res. 7 (1995) 21–25.