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Ionic-diffusion Potential-dependent Transport of a New Quinolone, Sparfloxacin, Across Rat Intestinal Brush-border Membrane

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Abstract

The mechanism of uptake of sparfloxacin, a new quinolone, by intestinal brush-border membrane vesicles was investigated to clarify whether there is a common transport process for new quinolones mediated by the diffusion potential across the intestinal membrane bilayer.

Sparfloxacin was taken up pH-dependently by rat intestinal brush-border membrane vesicles, behaviour analogous to that of organic cations including enoxacin and ciprofloxacin. Transient overshooting uptake of this quinolone was observed in the presence of an outward H^+ gradient. Momentary dissipation of the H^+ gradient by addition of carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone did not affect the uptake of sparfloxacin, and a marked but incomplete reduction in the H^+-sensitive overshooting uptake of sparfloxacin was apparent in the voltage-clamped brush-border membrane vesicles. Furthermore, a valinomycin-induced K^+-diffusion potential (interior negative) and an inward Cl^--diffusion potential stimulated the initial uptake of sparfloxacin at pH 5·5. Sparfloxacin uptake was inhibited by tetracaine and imipramine. The inhibitory effect of these cations correlated well with changes in membrane surface charges induced by the presence of tetracaine or imipramine.

These results indicate that sparfloxacin transport across the brush-border membrane depends upon the inside-negative ionic diffusion potential, that the H^+- or K^+-diffusion-potential-dependent uptake of sparfloxacin by intestinal brush-border membrane vesicles is affected by the membrane surface potential and that inhibition of sparfloxacin uptake originates from changes in the membrane surface potential caused by the organic cations.

Orally administered new quinolones are efficacious in the therapy of bacterial infections. Animal and clinical investigations have shown that after oral administration the new quinolones are readily absorbed through the wall of the intestine with a bioavailability of approximately 90% (Lode et al 1987; Bergeron 1989). A saturable Michaelis–Menten process for ofloxacin after rat intestinal recirculation (Prieto et al 1988) and inhibition by dipeptides of sparfloxacin absorption from rat jejunal loops (Yamaguchi et al 1991) have also been reported.

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However, we have previously reported that the uptake of new quinolones such as enoxacin and ciprofloxacin by rat intestinal brush-border membrane vesicles is dependent upon the interior negative diffusion potential (Iseki et al 1992; Hirano et al 1994, 1995). As no competition was observed between glycylglycine and enoxacin, this system seems to be different from dipeptide carrier-mediated transport. Furthermore, a recent study (Cormet et al 1997) has provided strong evidence of passive absorption of sparfloxacin across intestinal epithelial cells. As mentioned above, the mechanism underlying the intestinal absorption of the new quinolones is still largely unclear.

The stimulative uptake of organic cations by an outwardly directed H^+ gradient is a consequence of

electrophoretic mobility driven by the H^+-diffusion potential, and these cations are mutual inhibitors (Sugawara et al 1992, 1995; Iseki et al 1994). The inhibitory effects of these cations can be correlated with changes in the membrane surface potential. Moreover, our previous study (Iseki et al 1992) revealed that the extent of uptake of the cationic form (at pH 5·5) of enoxacin is higher than that of the zwitterionic form (at pH 7·5) in rat intestinal brush-border membrane vesicles. Because sparfloxacin has a pK_a similar to that of enoxacin (Figure 1), it is likely that the cationic form is present at a higher concentration than the zwitterionic form in the microintestinal media, the pH of which is known to be low (pH 5·5–6·6) (Lucas et al 1976, 1980; Högerle & Winne 1983).

The objective of this study was to define a common role of membrane potential-dependent transport mechanisms in the intestinal absorption of some new quinolones, organic cation-like compounds.

Materials and Methods

Chemicals

Sparfloxacin (Figure 1) and enoxacin were kindly donated by Dainippon Pharmaceutical (Osaka, Japan). Ciprofloxacin, imipramine, tetracaine, valinomycin and carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone were purchased from Sigma (St Louis, MO). 8-Anilino-1-naphthalenesulphonate magnesium was obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest grade available and used without further purification.

Preparation of rat intestinal brush-border membrane vesicles

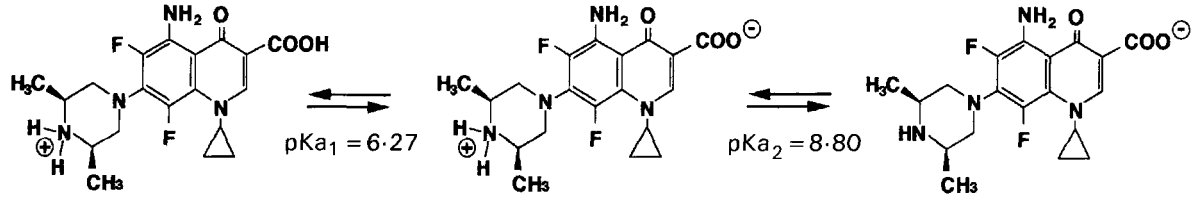
Experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. Brush-border membrane vesicles were prepared from the rat jejunum (male Wistar, 170–220 g) by a $CaCl_2$ precipitation method (Kesseler et al 1978) as described previously (Iseki et al 1992). The membrane vesicles were usually pre-loaded in the buffer used for the uptake studies. The composition of each buffer is given in the figure captions.

Uptake experiments

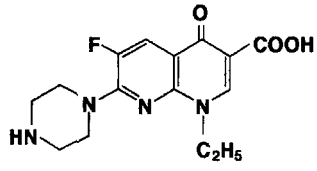
The uptake study was performed by a rapid-filtration technique using a Millipore filter (HAWP, 0·45 μm, 25 mm diam.) which was pretreated with 0·3% polyethylenimine to prevent non-specific adsorption by the filter, as described previously (Iseki et al 1992). As a blank, a membrane vesicle-free incubation medium was handled in an identical manner. All measurements were performed in triplicate, at least, with freshly prepared membranes. The results are expressed as the mean ± s.e.m.

Monitoring of membrane surface changes

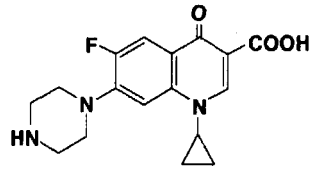
Changes in the surface potential of brush-border membrane vesicles were monitored by measuring changes in the fluorescence intensity of 8-anilino-1-naphthalenesulphonate magnesium. This method has been widely used to measure the membrane surface potential (Aiuchi et al 1977; Slavik 1982; Ohyashiki et al 1989), as described previously (Sugawara et al 1995). After addition of the solu-



Sparfloxacin



Enoxacin $pKa_1 = 6·2$, $pKa_2 = 8·8$



Ciprofloxacin $pKa_1 = 6·5$, $pKa_2 = 8·9$

Figure 1. Chemical structures of the new quinolones tested, and the ionization of sparfloxacin.

tion of 8-anilino-1-naphthalenesulphonate magnesium to the suspension of brush-border membrane vesicles, the mixture was incubated at 25°C for 30 min, and the fluorescence intensity was measured at 25°C.

Analyses

The HPLC system used for assay of sparfloxacin consisted of an Hitachi (Tokyo, Japan) L-6000 system equipped with an Hitachi L-4000 UV detector with the wavelength set at 286 nm. Separation was achieved on a reversed-phase column (Inertsil ODS, 5 μm; 25 cm × 4 mm i.d.; GL Sciences, Tokyo, Japan). The mobile phase was 30 : 70 (%v/v) acetonitrile–0·05 M KH_2PO_4 containing 7 mM 1-octanesulphonic acid (the final pH was adjusted to 3·8 by addition of HCl). The flow-rate was 0·7 mL min^{-1} at 50°C. Protein levels were determined by the method of Lowry et al (1951) with bovine serum albumin as a standard. The differences between two means were evaluated statistically by use of Student's *t*-test.

Results

Effect of pH on the uptake of sparfloxacin

The role of an inside-negative membrane potential on sparfloxacin uptake by brush-border membrane vesicles was investigated in the presence of an outward H^+ gradient. Table 1 shows that sparfloxacin uptake was rapid in the presence of an outward H^+ gradient and an early overshoot phenomenon was apparent. An inward H^+ gradient had no effect on sparfloxacin uptake, and the equilibrium uptake value was in agreement with that at pH $5{\cdot}5_{(outside = inside)}$.

The uptake of sparfloxacin was found to be dependent on the pH of the medium. As shown in Table 1, sparfloxacin uptake at pH $5{\cdot}5_{(out = in)}$ was significantly higher than that at pH $7{\cdot}5_{(outside = inside)}$. However, the impact of the outward H^+ gradient on the initial uptake of sparfloxacin was greater than that of the pH of the medium, suggesting that an outward H^+ gradient stimulates not only binding to the membrane surface but also the transport of sparfloxacin into the intra-vesicular space.

The role of an inside-negative membrane potential on sparfloxacin uptake

To determine whether the greater uptake of sparfloxacin is because of the ionic-diffusion potential produced by the different H^+ concentrations on each side of the membrane, we examined uptake of sparfloxacin in the presence of an outward H^+ gradient under experimental conditions in which the gradient was effectively dissipated. Momentary dissipation of the H^+ gradient by addition of carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone, a protonophore, did not affect the uptake of sparfloxacin (Table 2). Also, as shown in Figure 2, a marked but not complete decrease in the overshooting uptake of sparfloxacin was observed

Table 1. Effects of H^+ gradient and medium pH on sparfloxacin uptake by intestinal brush-border membrane vesicles.

Conditions	Uptake (pmol (mg protein)$^{-1}$)
pH $7{\cdot}5_{(outside)}$/pH $5{\cdot}5_{(inside)}$	
30 s	403·20 ± 24·82*
1·0 min	448·34 ± 39·19*
10·0 min	170·05 ± 15·54
pH $5{\cdot}5_{(outside = inside)}$	
30 s	286·04 ± 11·18*
1·0 min	295·08 ± 19·66*
10·0 min	295·08 ± 19·66*
pH $5{\cdot}5_{(outside)}$/pH $7{\cdot}5_{(inside)}$	
30 s	256·99 ± 19·98*
1·0 min	272·08 ± 24·00*
10·0 min	343·03 ± 37·11*
pH $7{\cdot}5_{(outside = inside)}$	
30 s	110·99 ± 17·98
1·0 min	133·08 ± 10·66
10·0 min	101·03 ± 8·27

Membrane vesicles were pre-loaded with 100 mM potassium gluconate, 100 mM D-mannitol and either 20 mM MES/Tris (pH 5·5) or 20 mM HEPES/Tris (pH 7·5). Transport solution was 60 μM sparfloxacin containing 100 mM potassium gluconate, 100 mM D-mannitol and either 20 mM MES/Tris (pH 5·5) or 20 mM HEPES/Tris (pH 7·5). Data are means ± s.e.m. of results from three to five determinations. *$P < 0{\cdot}001$, significantly different from the uptake at pH 7·5 without H^+ gradient.

Table 2. Effect of carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone on sparfloxacin uptake by intestinal brush-border membrane vesicles in the presence of an outward H^+ gradient.

Conditions	Uptake (pmol (mg protein)$^{-1}$)
15-s uptake	
Without carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	494·20 ± 34·12
With carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	442·34 ± 68·10
30-s uptake	
Without carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	357·04 ± 22·18
With carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	319·08 ± 66·60
2-min uptake	
Without carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	196·99 ± 22·98
With carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	196·22 ± 19·00
30-min uptake	
Without carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	92·32 ± 4·98
With carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone	46·08 ± 29·66

Membrane vesicles were pre-loaded with 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM MES/Tris (pH 5·5). Transport solution was 60 μM sparfloxacin containing 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM HEPES/Tris (pH 7·5) with or without 50 mM carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone. Data are means ± s.e.m. of results from three to seven determinations.

for the voltage-clamped membrane vesicles, despite the presence of an outward H^+ gradient. Under this voltage-clamped condition (Miyamoto et al 1988; Iseki et al 1993; Takahashi et al 1993), K^+ was present in equimolar concentrations both inside and outside the vesicles, and a K^+ ionophore, valinomycin, was added to the vesicle suspension beforehand. Therefore, all the ionic diffusion potentials were instantly compensated by the movement of K^+.

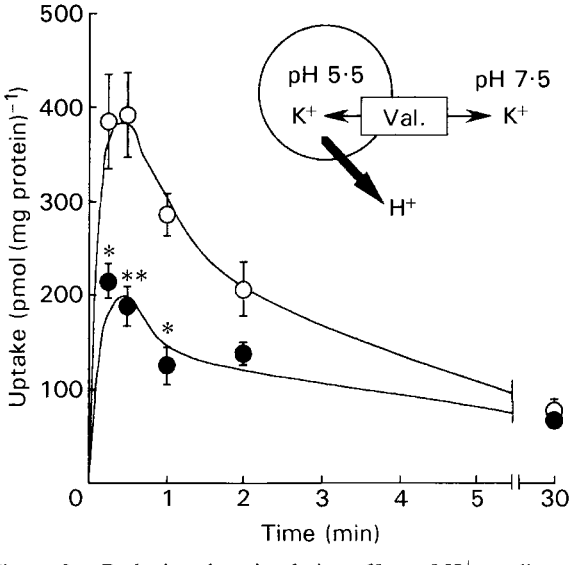


Figure 2. Reducing the stimulative effect of H^+ gradient on uptake of sparfloxacin by voltage-clamped brush-border membrane vesicles. Membrane vesicles (20 μL) were incubated in potassium gluconate (100 mM), D-mannitol (100 mM) and MES/Tris buffer (20 mM; pH 5·5) with (●) or without (○) valinomycin (7 μg (mg protein)$^{-1}$). The transport study was performed by adding 100 μL potassium gluconate (100 mM), D-mannitol (100 mM), and 20 mM MES/Tris buffer (pH 7·5) containing 60 μM sparfloxacin. Each point represents the mean ± s.e.m. of results from five or six determinations. *$P < 0·05$, **$P < 0·01$, significantly different from the value for uptake without valinomycin.

To confirm that this stimulatory effect on uptake of quinolone is not peculiar to H^+ movement, further evidence of stimulation by an intra-vesicular negative ionic diffusion potential was gained by determining the effects on sparfloxacin uptake of an inwardly directed Cl^- gradient and a valinomycin-induced K^+-diffusion potential (interior negative). A valinomycin-induced K^+-diffusion potential had a stimulative effect on the initial uptake (15 s) at pH 5·5, whereas there was no effect on the uptake at pH 7·5 (Table 3). In the presence of an inward H^+ gradient (pH $5·5_{(outside)}/7·5_{(inside)}$), the inside-negative K^+-diffusion potential seemed to be partially overwhelmed by the H^+ influx, although there was a small tendency to stimulate the uptake. Moreover, the initial uptake of sparfloxacin was increased also by an inward Cl^- gradient at a pH of 5·5. A series of these results suggests that sparfloxacin is transported electrophoretically by the inside-negative trans-membrane electrical potential similarly to enoxacin (Iseki et al 1992; Hirano et al 1994).

Inhibition of sparfloxacin uptake by the presence of other quinolones

Table 4 shows the inhibitory effects of other new quinolones, enoxacin and ciprofloxacin, on the uptake of sparfloxacin at pH 5·5. The uptake of enoxacin by brush-border membrane vesicles decreased in the presence of both quinolones, and equilibrated uptakes were also reduced.

As shown in Figure 3, the uptake of sparfloxacin into the osmoreactive space of brush-border membrane vesicles was substantially inhibited by the

Table 3. Effect of valinomycin-induced K^+-diffusion potential (inside negative) and an inwardly directed Cl^- gradient on the initial (15-s) uptake of sparfloxacin.

Conditions	Uptake (pmol (mg protein)$^{-1}$)
K^+-diffusion potential effect (inside negative)	
pH $7{\cdot}5_{(outside = inside)}$	
Control	86·52 ± 19·01
With valinomycin	100·90 ± 20·33
pH $5{\cdot}5_{(outside)}$/pH $7{\cdot}5_{(inside)}$	
Control	266·04 ± 59·31
With valinomycin	333·09 ± 40·08
pH $5{\cdot}5_{(outside = inside)}$	
Control	425·33 ± 22·10
With valinomycin	557·85 ± 59·00*
Cl^--diffusion potential effect (inside negative)	
pH $5{\cdot}5_{(outside = inside)}$	
Control	339·00 ± 38·20
With valinomycin	556·62 ± 52·10*

Membrane vesicles were equilibrated with 100 mM potassium gluconate, 100 mM D-mannitol and either 20 mM HEPES/Tris (pH 7·5; pH $7{\cdot}5_{(inside)}/5{\cdot}5_{(outside)}$; pH $7{\cdot}5_{(inside = outside)}$) or MES/Tris (pH 5·5; pH $5{\cdot}5_{(inside = outside)}$). To generate an interior negative K^+-diffusion potential, uptake studies were performed by diluting the membrane vesicle suspension with the sparfloxacin solution (60 μM) containing 100 mM D-mannitol, 100 mM potassium gluconate, and either 20 mM HEPES/Tris (pH 7·5) or 20 mM MES/Tris (pH 5·5) with/without valinomycin (7 μg (mg brush-border membrane protein)$^{-1}$). For the Cl^--gradient experiment, a drug solution containing 100 mM KCl was used instead of potassium gluconate. *$P < 0{\cdot}05$, significantly different from the uptake without valinomycin or Cl^- gradient.

presence of the new quinolone ciprofloxacin even at pH 5·5, and was almost equal to that at pH 7·5.

Effect of organic cations on the uptake of sparfloxacin and surface potential of brush-border membrane vesicles

The effects of tetracaine and imipramine on the initial uptake of sparfloxacin were investigated. As shown in Table 5, the initial uptake of sparfloxacin was inhibited by tetracaine and imipramine. The inhibitory effects were concentration-dependent, and inhibition by imipramine was stronger than by tetracaine. The uptake of sparfloxacin was well correlated with the relative surface potential changes produced by the presence of tetracaine and imipramine (Figure 4), and the effect was common to both cations.

Discussion

Evidence for the stimulative uptake of new quinolones by an interior negative ionic diffusion

Table 4. Inhibitory effects of ciprofloxacin and enoxacin on the uptake of sparfloxacin by rat intestine brush-border membrane vesicles.

Conditions	Uptake (pmol (mg protein)$^{-1}$)	Inhibition (%)
Initial uptake (15 s)		
Control (sparfloxacin only)	246·70 ± 13·08	0
With enoxacin (0·5 mM)	177·13 ± 15·78‡	28·20
With enoxacin (1·0 mM)	163·07 ± 4·93‡	33·90
With ciprofloxacin (1·0 mM)	159·86 ± 27·63*	35·90
With ciprofloxacin (5·0 mM)	148·14 ± 10·11†	39·95
Equilibrium uptake (30 min)		
Control (sparfloxacin only)	418·66 ± 12·14	0
With enoxacin (0·5 mM)	341·63 ± 12·14*	18·40
With enoxacin (1·0 mM)	332·00 ± 69·99†	20·70
With ciprofloxacin (1·0 mM)	362·98 ± 79·55	13·30
With ciprofloxacin (5·0 mM)	275·52 ± 33·49*	34·19

Brush-border membrane vesicles were pre-loaded with 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM MES/Tris (pH 5·5) at 25°C. Uptake was measured in the absence of a pH gradient, pH 5·5 for both outside and inside the vesicles, at 50 μM sparfloxacin with and without other new quinolones. Data are means ± s.e.m. of results from three or four measurements with different vesicle preparations. *$P < 0{\cdot}05$, †$P < 0{\cdot}01$, ‡$P < 0{\cdot}001$, significantly different from the uptake without valinomycin or Cl^- gradient.

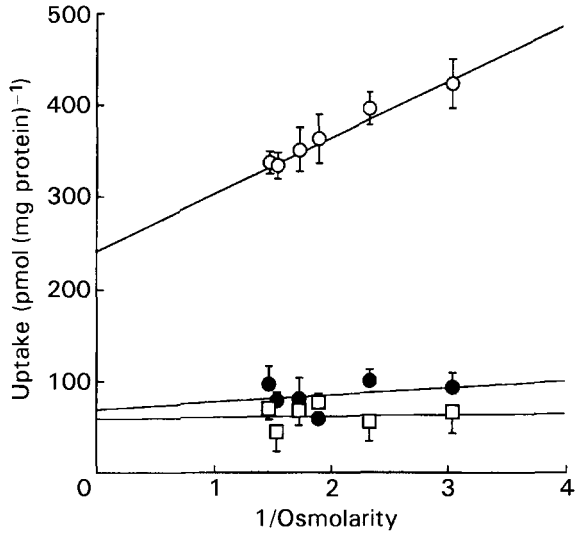


Figure 3. Inhibitory effect of ciprofloxacin on uptake of sparfloxacin by rat intestinal brush-border membrane vesicles as a function of the osmolarity of the medium. Membrane vesicles were suspended with potassium gluconate (100 mM), D-mannitol (100 mM), and either MES/Tris buffer (20 mM; pH 5·5) (●, ○) or HEPES/Tris buffer (20 mM; pH 7·5) (□). Measurement of uptake was performed with a buffer containing sparfloxacin (50 μM), D-cellobiose (0–350 mM), potassium gluconate (100 mM) and MES/Tris buffer (20 mM; pH 5·5) with (●) or without (○) 5·0 mM ciprofloxacin at 25°C for 60 min. A buffer containing sparfloxacin (50 μM), D-cellobiose (0–350 mM), potassium gluconate (100 mM) and HEPES/Tris (20 mM; pH 7·5) was used for uptake at pH 7·5 (□). Each point represents the mean ± s.e.m. of three measurements.

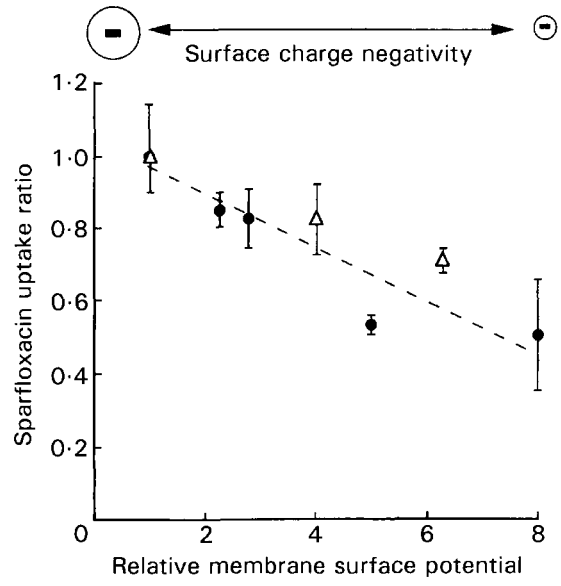


Figure 4. Correlation between the relative membrane surface potential and the initial (15 s) uptake of sparfloxacin by brush-border membrane vesicles in the presence of tetracaine (●) or imipramine (△). Vesicles (20 μL) suspended with MES/Tris (20 mM; pH 5·5) buffer containing D-mannitol (100 mM) and potassium gluconate (100 mM) were incubated in HEPES/Tris buffer (20 mM; pH 7·5; 100 μL) containing sparfloxacin (50 μM), D-mannitol (100 mM), potassium gluconate (100 mM) and different concentrations of either tetracaine or imipramine. The final concentration of external potassium gluconate was 16·7 mM. Each point represents the mean ± s.e.m. of three to six measurements.

potential was obtained by determining the effect of an outward H^+ gradient on the uptake of sparfloxacin by intestinal brush-border membrane vesicles. This result is in agreement with the effects of both the valinomycin-induced K^+-diffusion potential and the carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone-induced H^+-diffusion potential (inside negative) on the uptake of organic cations (Sugawara et al 1992; Iseki et al 1994). We have already reported that this per-

Table 5. Inhibitory effects of tetracaine and imipramine on the uptake of sparfloxacin by rat intestine brush-border membrane vesicles.

Conditions	Uptake (pmol (mg protein)$^{-1}$)	Inhibition (%)
With tetracaine		
0 mM (control)	302·66 ± 47·82	0·0
1·0 mM	257·74 ± 21·19	14·84
2·0 mM	251·75 ± 27·54	16·82
4·0 mM	165·58 ± 10·29*	45·29
8·0 mM	110·80 ± 11·20*	63·39
With imipramine		
0 mM (control)	288·23 ± 30·54	0·0
0·5 mM	240·99 ± 29·98	16·39
1·0 mM	209·08 ± 10·66*	27·47
2·0 mM	203·03 ± 47·27*	29·56

Brush-border membrane vesicles were incubated in a buffer composed of 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM HEPES/Tris (pH 7·5) at 25°C. Uptake was measured in the absence of an outward H^+ gradient at 50 μM sparfloxacin with and without tetracaine or imipramine. The incubation time was 15 s. Data are means ± s.e.m. of results from three or four measurements with different vesicle preparations. *$P < 0·05$, significantly different from control result.

meation system was considered to be composed of two continuous phases, initial binding of substrates to the membrane surface and ionic-diffusion potential-dependent transport across the brush-border membrane (Hirano et al 1995). On the other hand, uptake of the organic cation tryptamine by the brush-border membrane vesicles was found to be affected by changes in membrane surface potential monitored by 8-anilino-1-naphthalene-sulphonate magnesium (Sugawara et al 1995). In this study, it was evident that changes in the membrane surface potential induced by imipramine and tetracaine also have the same effect on the uptake of sparfloxacin by the brush-border membrane vesicles.

The results obtained from this and our previous studies indicate that the apparent uptakes of new quinolones at pH 5·5 by brush-border membrane vesicles are mutually inhibited, and that ciprofloxacin markedly reduced even the transport of sparfloxacin into the intra-vesicular space of brush-border membrane vesicles. These results provide evidence that new quinolones such as enoxacin and ciprofloxacin, which also permeated as a result of an outward H^+-diffusion potential (inside-negative) across the intestinal membrane, inhibit not only the binding of the derivative, sparfloxacin, to the membrane surface but also its subsequent uptake into the intra-vesicular space.

The stimulative effect of an outward H^+ gradient on sparfloxacin uptake did not vanish completely in the voltage-clamped brush-border membrane vesicles. Previous investigation revealed that guanidine is transported via an H^+/antiport system, which recognizes endogenous organic cations such as 5-hydroxytryptamine, dopamine and polyamines in the intestinal brush-border membrane (Miyamoto et al 1988). Similar observations have also been reported for secretion of celiprolol (a β-blocker) by a pH-sensitive transporter across intestinal Caco-2 cell layers in man (Karlsson et al 1993). Recently, Griffiths et al (1993, 1994) reported an active secretion system for fluoroquinolones by intestinal epithelial Caco-2 cell layers in man. They suggested that the substrate specificity of this secretion system is relatively tight; this might explain the variable oral bioavailability of their compounds.

In contrast, the most recent study by Cormet et al (1997) revealed that the initial uptake of sparfloxacin across the apical membrane of Caco-2 monolayers is not saturable and resists the effect of metabolic inhibitor (depletion of ATP storage or ouabain), indicating that the uptake is energy-independent. Their results from kinetic and competition experiments showed that the transport of sparfloxacin across the brush-border membrane occurs by both passive diffusion and by non-specific binding. Therefore, the incomplete disappearance of the H^+ gradient dependent stimulative uptake by the voltage-clamped brush-border membrane vesicles might be a consequence of a high intra- or extra-vesicular binding of the quinolone, although the presence of a secretory H^+/antiport system cannot be completely ruled out.

Finally, the results of the current study showed that the transport of the new quinolone sparfloxacin is driven by the membrane potential difference in the intestinal brush-border membrane, suggesting that it was shared with the organic cations. The binding of substrates to the brush-border membrane might be an important component in this mechanism.

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