The Intestinal Transport Mechanism of Fluoroquinolones: Inhibitory Effect of Ciprofloxacin, an Enoxacin Derivative, on the Membrane Potential-Dependent Uptake of Enoxacin

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Purpose. To clarify the absorption-structure relationship for the fluoroquinolones from the point of view of inhibitory behavior. ***Methods.*** The inhibitory effects of ciprofloxacin on the transport process of enoxacin across the rat intestinal brush-border membrane was examined. ***Results.*** Ciprofloxacin, which has a similar structure to enoxacin, exhibited a pH-dependent interference with enoxacin absorption from rat jejunal loops. The uptake experiments using BBM vesicles showed that ciprofloxacin significantly reduced not only the initial binding of enoxacin to the membrane surface, but also the K^+- or H^+-diffusion potential-dependent transport across the membrane. Furthermore, an H^+-diffusion potential (interior negative) also exhibited a stimulative uptake of ciprofloxacin. ***Conclusions.*** These results suggest that the inhibition behavior of ciprofloxacin from the jejunal loop was closely related to the ionic diffusion potential-dependent uptake of enoxacin across the brush-border membrane.

KEY WORDS: fluoroquinolone; enoxacin; ciprofloxacin; transport; brush-border membrane (BBM), diffusion potential.

INTRODUCTION

Our previous studies demonstrated that a greater uptake of the cationic form (at pH 5.5) of enoxacin was observed by using rat intestinal brush-border membrane (BBM) vesicles (1), and that the transport of this antibacterial fluoroquinolone was stimulated by an interior negative ionic-diffusion potential (2). The pH in the vicinity of the intestinal epithelial cells is weakly acidic (3,4), and the transport of several organic cations across the intestinal BBM was found to be sensitive to an interior negative diffusion potential, but not to be carrier-mediated (5,6). Since enoxacin exists in its cationic form in the physiological media of the intestinal lumen (pH 5–6), it is likely that the diffusion potential-dependent transport mechanism of the organic cations plays an important role in the intestinal absorption process of enoxacin. However, it is still not definite whether the absorptions of all the fluoroquinolones are mediated by a common pathway or not.

The aim of the present report was to clarify the absorption-structure relationship for the fluoroquinolones by examining the inhibitory effect of ciprofloxacin on the transport of enoxacin by rat intestinal BBM vesicles. The results demonstrated that ciprofloxacin exhibited a specific interference with the diffusion potential-dependent uptake of enoxacin into the membrane vesicles.

MATERIALS AND METHODS

Materials

Enoxacin was kindly donated by Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Ciprofloxacin and valinomycin were purchased from Sigma Chemicals (St Louis, MO, USA). All other chemicals were of the highest grade available and were used without further purification.

Preparation of the BBM 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. BBM vesicles were prepared from rat small intestine (Wistar, male; 180–230 g) by a $CaCl_2$ precipitation method (7) as described previously (1,8,9). The membrane vesicles were usually preloaded in the buffer used for the uptake studies. The composition of each buffer is given in the legends of the figures.

Uptake Experiments

The uptake study was performed by a rapid filtration technique using a Millipore Filter (HAWP, 0.45 μm, 25 mm diameter) which was pre-treated with 0.3% polyethylenimine to avoid the nonspecific adsorption to the filter, as described previously (1). As a blank, a membrane vesicle-free incubation medium was handled in an identical manner.

Absorption Study Using Rat Jejunal Loops

The absorption experiment was performed at various pH values (5.0–7.5) using the *in situ* loop technique of Levine and Pelikan (10). A loop (10 cm) was prepared in the jejunum of a male Wistar rat (200–230 g). The drug was dissolved in modified Ringer's solution (11) and adjusted to the required pH in advance. Using a syringe, 1 ml of enoxacin at a concentration of 0.1 mM was injected into the loop. After 30 min, the contents of the loop were recovered as completely as possible, and the inside of the loop was washed with modified Ringer's solution to give a total volume of 5 ml. One milliliter aliquots of the samples were diluted with 0.6 ml of 0.2 M acetic acid and shaken with 2 ml of chloroform to clean up the samples. The aqueous layer was used for HPLC injection.

Analytical Procedures

The enoxacin and ciprofloxacin were analyzed by

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HPLC (Hitachi L-6000, Hitachi, Tokyo, Japan) with a reversed phase column (Inertsil ODS, 5 μm, 25 cm × 4 mm i.d.) as described in previous reports (1,2). Protein levels were determined by the method of Lowry *et al.* (12) with bovine serum albumin as a standard.

RESULTS

pH-Dependent Absorption Behavior of Enoxacin from Rat Jejunal Loop

Figure 1 shows the results of the disappearance of enoxacin from rat jejunal loops and the effect of the presence of ciprofloxacin (1.5 mM). Enoxacin absorption was found to be dependent on the pH of the medium, and it was evident that the disappearances at pH 5 were significantly greater than those at pH 6.0–7.5. The inhibition experiments at various pHs showed that the effects of ciprofloxacin on enoxacin absorption were exhibited only at pH 5 and 5.5, suggesting that the cationic form of ciprofloxacin with an undissociated carboxyl group (pK_{a1} = 6.5) and a protonated piperazinyl group (pK_{a2} = 8.5) is effective. Furthermore, ciprofloxacin inhibited the enoxacin absorption from the jejunal loop at pH 5.5 in a dose-dependent manner (Figure 2).

Effect of Ciprofloxacin on Enoxacin Uptake

In order to add further details on the absorption mechanisms, the effect of ciprofloxacin on enoxacin uptake by intestinal BBM vesicles was investigated. At pH 5.5, ciprofloxacin reduced both the initial and equilibrated uptakes of enoxacin by the BBM vesicles (Figure 3). This result suggested that the pH-dependent inhibition given in Figure 1 was due to the effect on the membrane transport across the BBM. On the other hand, the uptake of ciprofloxacin by BBMV was stimulated by an outward H^+-gradient as indicated in Figure 4. The voltage-clamped BBM vesicles exhibited a marked decrease in the overshooting uptake despite the presence of an outward H^+-gradient (Figure 4). In the voltage-clamped BBM vesicles, all the ionic diffusion potentials were instantly compensated by a rapid K^+ movement. Moreover, we also confirmed that the dissipation of the H^+-gradient by a protonophore, FCCP, made no effects on the uptake of ciprofloxacin (Figure 4).

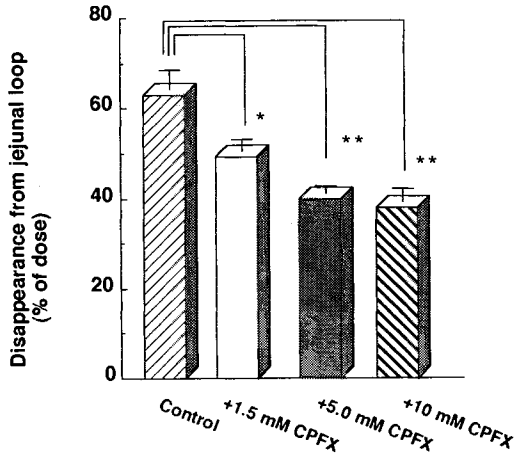

Fig. 2. Dose-dependent inhibitory effect of ciprofloxacin upon the disappearance of enoxacin (0.1 mM) from jejunal loops at pH 5.5. Each value represents the mean with SE of five observations. *: $P < 0.05$; **: $P < 0.01$.

Inhibitory Effect of Ciprofloxacin on the Uptake of Enoxacin

Figure 5 shows that the uptake of enoxacin by BBM vesicles was affected by ciprofloxacin in the presence of an interior negative K^+-diffusion potential induced by valinomycin. Enoxacin uptake increased linearly as a function of the time up to 30 sec in all the conditions regardless of the presence of the inhibitor. The presence of ciprofloxacin exhibited a strong inhibition on the K^+-diffusion potential-

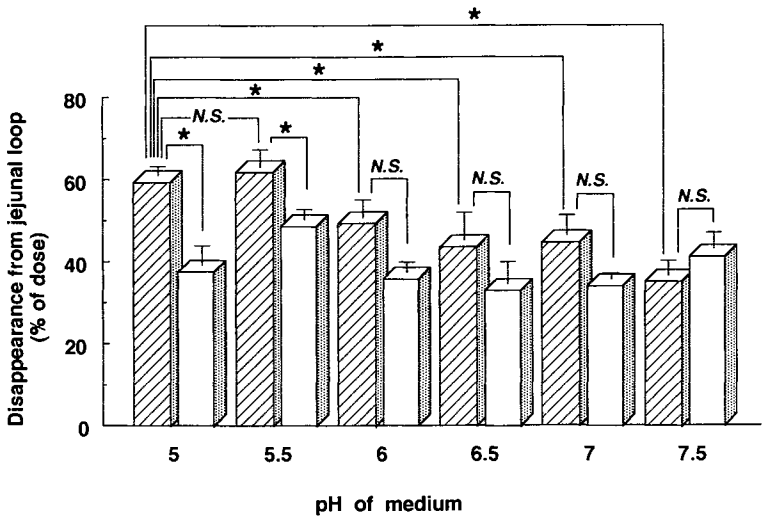

Fig. 1. pH-Dependent disappearance of enoxacin and inhibition by ciprofloxacin (1.5 mM) from rat jejunal loops. 0.1 μmole (0.1 mM, 1 ml) of enoxacin was administered into the jejunal loop. Each column represents the mean with SE of 4–7 observations. control: ▨; with ciprofloxacin: □ * ; $P < 0.05$, N.S.; not significant.

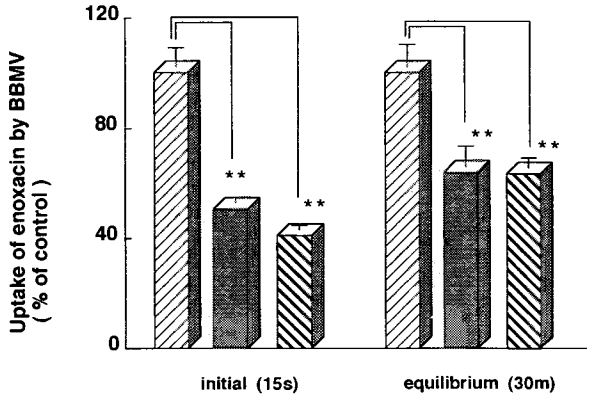

Fig. 3. Effect of ciprofloxacin on the uptake of enoxacin by the intestinal BBM vesicles at pH 5.5. Transport studies were performed in a buffer containing 0.1 mM enoxacin, 100 mM D-mannitol, 100 mM potassium gluconate and 20 mM MES-Tris (pH 5.5) in the presence of ciprofloxacin. Each column represents the mean ± SE of 5–9 measurements. The concentrations of ciprofloxacin were 0 (▨, control), 5 mM (▩) and 10 mM (▧), respectively. **: Significantly different from control, $P < 0.001$.

dependent permeation of enoxacin across the BBM. Moreover, there was also a distinct difference in the initial binding (estimated by extrapolation to time zero) of enoxacin to the membrane surface between the presence and absence of ciprofloxacin.

Inhibitory Effect of Ciprofloxacin on the an Outward H^+-Gradient Dependent Uptake of Enoxacin

In our previous reports (1,2), enoxacin was taken up into the BBM vesicles by an interior negative H^+-diffusion potential. To further clarify that the inhibitory effects described in the present study were related not only to an initial

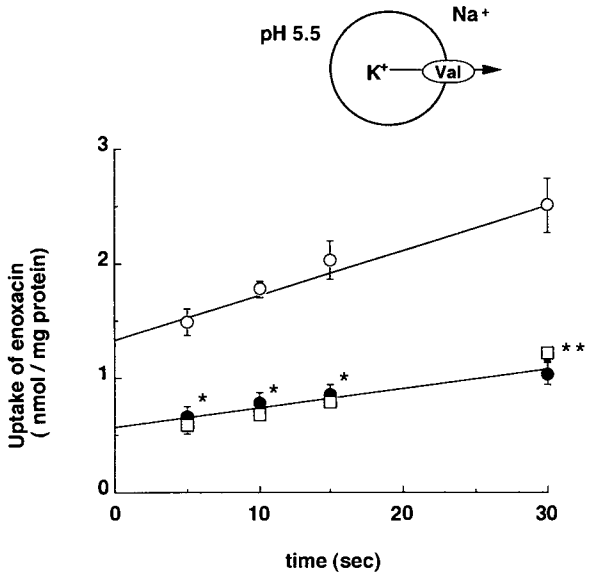

Fig. 5. Time course of enoxacin uptake regulated by a valinomycin-induced K^+-diffusion potential (interior negative) in the presence of ciprofloxacin. Membrane vesicles (20 μl) were preincubated in 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM MES-Tris buffer (pH 5.5). Uptake studies were performed by adding 100 μl of transport buffer containing 100 mM sodium gluconate, 100 mM D-mannitol, 0.12 mM enoxacin, 20 mM MES-Tris buffer (pH 5.5) and either valinomycin (●) in ethanol at a concentration of 6 μg/mg (membrane protein) or ethanol only (○). The inhibition experiments were performed in the media containing 10 mM ciprofloxacin (□) in the presence of a K^+-diffusion potential (interior-negative). Final concentration of enoxacin in the reaction mixture was 0.1 mM. Each point represents the mean ± SE of 3–6 measurements. *: $P < 0.05$; **: $P < 0.01$.

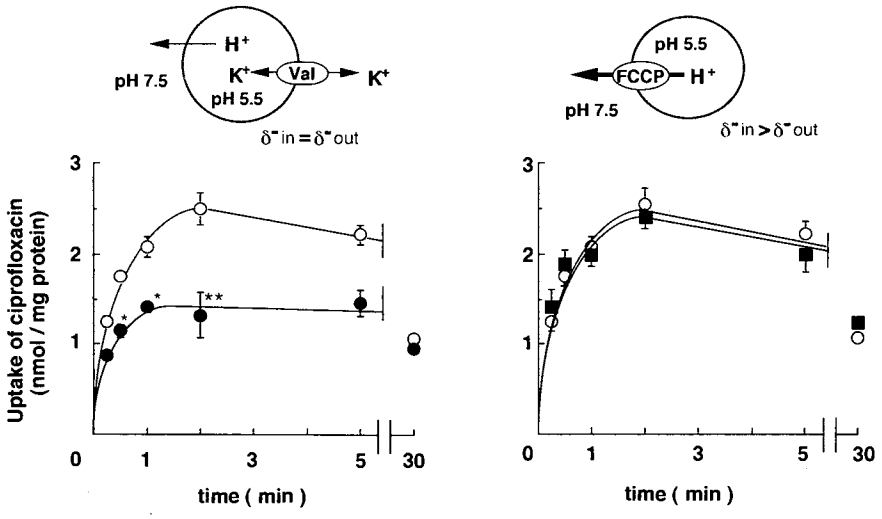

Fig. 4. Effects of the voltage-clamping by valinomycin and H^+-dissipation by FCCP on ciprofloxacin uptake into the brush-border membrane vesicles. Membrane vesicles (20 μl) were preincubated in 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM Mes-Tris buffer (pH 5.5) in the absence (○) or presence of (●) valinomycin (6 μg/mg protein). Uptake studies were performed by adding 100 μl of transport buffer containing 0.6 mM ciprofloxacin, 100 mM potassium gluconate, 100 mM D-mannitol, 20 mM Hepes-Tris buffer (pH 7.5) and either 50 μM FCCP (■) or not (○, ●). Each point represents the mean ± SE of four determinations. *: $P < 0.005$.; **: $P < 0.001$.

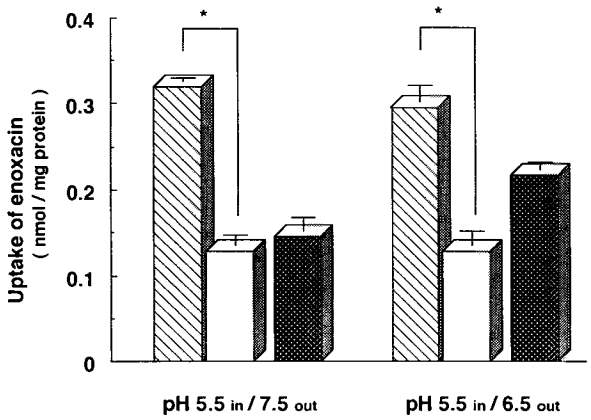

Fig. 6. Effect of ciprofloxacin on the initial (30 sec) uptake of enoxacin by the intestinal BBM vesicles in the presence of an outwardly directed H^+-gradient. Membrane vesicles were preloaded with 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM MES-Tris buffer (pH 5.5). The transport buffer (100 μl) was composed of 0.12 mM enoxacin, 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM HEPES-Tris buffer (either pH 7.5 or pH 6.5) with (□) or without (▨) ciprofloxacin (1.0 mM). The other column (▩) represents the equilibrated (30 min) uptake of enoxacin without inhibitor. The uptake values are expressed as the mean ± SE of three or four determinations. *: Significantly different from control, $P < 0.005$.

binding but also to a membrane transport, an inhibition study under the presence of an outward H^+-gradient was carried out. Ciprofloxacin significantly inhibited the H^+-gradient-induced overshooting uptake of enoxacin (Figure 6).

DISCUSSION

The results obtained from our present and previous studies (1,2) showed that the intestinal transport of enoxacin was pH sensitive and dependent upon the ionic diffusion potential (inside-negative), and that the diffusion potential-dependent uptake was inhibited by ciprofloxacin, an analogue of enoxacin. These characteristics of enoxacin transport can account for the absorption behavior of this drug from rat jejunal loops such as the pH-dependent suppression by ciprofloxacin. On the other hand, ciprofloxacin, which inhibited the enoxacin uptake, was also permeated stimulatively by an outward H^+-diffusion potential (inside-negative) into the BBM vesicles (Figure 4). These results agreed well with the uptake behavior of enoxacin (1,2), indicating that ciprofloxacin uptake depends also upon an interior negative ionic-diffusion potential across the intestinal BBM.

The substrate specificities of this membrane permeation system seem to consist of two continuous phases. The first is the binding to the surface of the BBM. At this step, enoxacin binding was inhibited by the fluoroquinolone analogue, ciprofloxacin, as shown in Figure 5. We have previously found that several organic cations interacted with the negative-charged surface of the BBM at the initial time of uptake (5,6). It was evident that this interaction was relatively specific as in a case of a carrier-mediated system despite the fact that no carrier-proteins were involved (13). Therefore, the inhibition induced by ciprofloxacin observed at pH 5.5 was considered to be based on competitive interactions between the cationic forms of fluoroquinolones with the negative charges on the BBM surface. On the other hand, the next phase is the ionic diffusion potential-dependent transport across the BBM. The experiments in this study showed that ciprofloxacin inhibited the K^+- and H^+-diffusion potential-dependent uptake of enoxacin together with the initial binding to the membrane surface (Figures 4 and 5). In previous works, we have found out that the effect of the transmembrane electrical potential difference on the uptake by the BBM vesicles contributed to the uptakes of several organic cations such as tyramine (5), tryptamine, benzyloxytryptamine (6), disopyramide (14), and the cationic form of enoxacin (1). Therefore, it is likely that the uptake processes of the two fluoroquinolones used in the present work are mediated by a common transport mechanism depending on the ionic diffusion potential across the BBM.

In conclusion, enoxacin absorption behavior agreed with its transport properties across the intestinal BBM. It is suggested that the inhibitory effect of ciprofloxacin on the absorption of enoxacin from the jejunal loop was related to the reduction of the ionic diffusion potential-dependent uptake of enoxacin observed in the BBM vesicles.

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