ELSEVIER

International Journal of Pharmaceutics 159 (1997) 127–130

international journal of pharmaceutics

Note

Sparfloxacin binds to rabbit intestinal brush-border membrane vesicles by ionic interactions

Estelle Cormet, Jean-François Huneau *, Daniel Tomé

INRA, Unité de Nutrition Humaine et de Physiologie Intestinale, Institut National Agronomique Paris-Grignon, 16 rue Claude Bernard, 75231 Paris, France

Received 22 July 1997; received in revised form 13 August 1997; accepted 15 August 1997

Abstract

The intestinal transport and binding of sparfloxacin has been investigated using rabbit intestinal brush-border membrane vesicles. The apparent uptake of sparfloxacin by these vesicles was very rapid. The plateau was reached after 20 min in NaCl-free buffer with a membrane binding of this drug accounting for about two-thirds of the apparent accumulation. Addition of NaCl did not affect the transport of this fluoroquinolone into the intravesicular space. However, the binding of sparfloxacin to membrane vesicles was sharply reduced by NaCl, suggesting that the binding of sparfloxacin to epithelial cells involves ionic interactions. © 1997 Elsevier Science B.V.

Keywords: Binding; Brush-border membrane vesicles; Fluoroquinolone; Ionic strength; Sparfloxacin

Sparfloxacin is a difluoroquinolone with enhanced activity against gram-positive pathogens in comparison with other quinolones such as ciprofloxacin and ofloxacin (Nakamura et al., 1990). This drug is rapidly absorbed after oral administration and exhibits both an excellent oral bioavailability (near 80%) and good penetration into most body fluids and tissues (Montay et al., 1994). Previous studies have shown that the uptake of fluoroquinolones by polymorphonuclear leukocytes as well as by other eucaryotic cells and bacteria resulted in a high intracellular-to-extracellular ratio of these drugs (Carlier et al., 1990; Furet et al., 1992; Garcia et al., 1992; Cormet et al., 1997). We have previously shown that sparfloxacin passively diffuses across the brush-border of the human intestinal epithelial cell-line Caco-2 (Cormet et al., 1997). In that model, the intracellular concentration of sparfloxacin after 60

* Corresponding author. Tel.: + 33 1 44081828; fax: + 33 1 44081825.

PII S0378-5173(97)00270-6

min incubation was more than 10-fold higher than the extracellular concentration, suggesting a high level of binding of the drug to cell membranes. The present study was designed to examine the mechanisms of sparfloxacin binding to epithelial cells using rabbit intestinal brush-border membrane vesicles (BBMV). Specifically, advantage was taken of BBMV to discriminate between binding and intracellular accumulation since zero intravesicular volume can be achieved by incubating BBMV in an hyperosmolar medium. Furthermore, we have addressed the involvement of ionic interactions in the binding of sparfloxacin since fluoroquinolones are zwitterionic compounds at physiological pH with both basic and acidic functional groups.

Sparfloxacin was kindly provided by Dr. Lecoeur (Rhône Poulenc–Rorer, Antony, France); [^{14}C]sparfloxacin (31.5 mCi/mg) was custom synthesized by Daïnippon Pharmaceutical Laboratory (Osaka, Japan). All other reagents were from Sigma (La Verpillière, France). Male New Zealand white rabbits weighing 2.5–3.0 kg were killed by intravenous pentobarbital sodium injection. The ileum was rapidly removed and rinsed with cold saline. The intestinal mucosa was gently scraped off with glass slide and vesicles were prepared by $CaCl_2$ precipitation as previously described (Kessler et al., 1978). Uptake studies were carried out at 25°C using the rapid-filtration technique (Hopfer et al., 1973). In a standard assay, the uptake was initiated by the addition of 20 μl (approximately 350 μg protein) of membrane vesicle suspension to 80 μl of the experimental buffer containing 0.15 mM [^{14}C]sparfloxacin (7.4 kBq). The exact conditions for each experiment are given in the legends of the figures. The uptake reaction was stopped by dilution of an incubation sample with 1 ml of ice-cold stop buffer (10 mM HEPES–Tris (pH 7.5), 150 mM NaCl and 0.15 mM unlabeled sparfloxacin), followed by rapid filtration through a membrane filter (Millipore HAWP, 0.45 μm). The filters were washed once with 4 ml of the ice-cold stop buffer and the amount of sparfloxacin trapped was measured by liquid scintillation counting. The equilibrium accumulation of 10 μM D-glucose was used to determine the available intravesicular space (Brachet et al., 1994). Protein concentration was assayed by the bicinchoninic acid method, using bovine serum albumin as a standard (Cormet et al., 1997) and results are expressed as pmol sparfloxacin per mg protein.

The time course of [^{14}C]sparfloxacin apparent uptake in BBMV under isoosmotic conditions ($osM_{out} = osM_{in}$) was measured at 25°C and pH 7.5, for incubation times ranging from 2 to 20 min (Fig. 1). In Na^+-free conditions, the uptake of sparfloxacin was very fast and reached a plateau after 20 min. At that time, the amount of sparfloxacin trapped by BBMV was approximately 170 pmol/mg protein. The osmotically reactive space in the vesicles was 0.355 μl/mg protein, leading to an apparent intravesicular-to-extravesicular concentration ratio of 3 for sparfloxacin. The time course of sparfloxacin accumulation was also measured in the presence of 40 mM NaCl. In these conditions, the plateau was

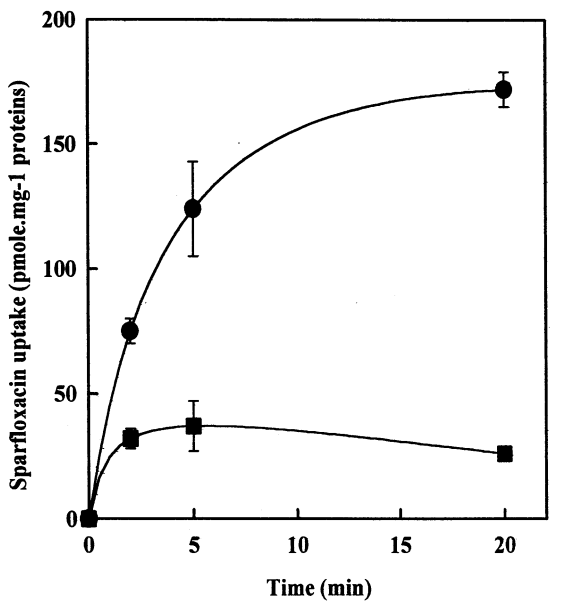

Fig. 1. Effect of NaCl on sparfloxacin apparent uptake by rabbit intestinal BBMV. Vesicles were prepared in a 300 mM mannitol and 10 mM HEPES–Tris (pH 7.5) buffer. The uptake of 0.15 mM [^{14}C]sparfloxacin was measured in a 10-mM HEPES–Tris (pH 7.5) buffer containing either 300 mM mannitol (●) or 40 mM NaCl and 220 mM mannitol (■). Results are means ± S.D. of six measurements with two different preparations of BBMV.

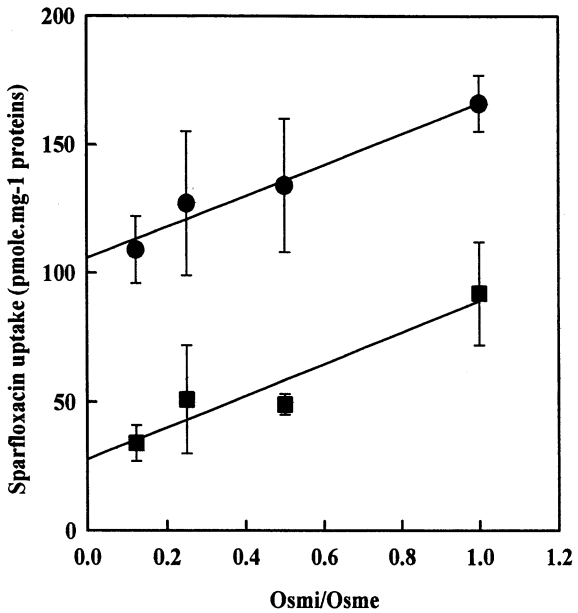

Fig. 2. Effect of increasing medium osmolarity on the uptake of sparfloxacin by rabbit intestinal BBMV. Membranes were suspended in 100 mM mannitol and 10 mM HEPES–Tris (pH 7.5). The uptake of 0.15 mM [^{14}C]sparfloxacin was measured after 2 min incubation in a 10-mM HEPES–Tris (pH 7.5) buffer containing 0 (●) or 40 mM NaCl (■), and different amounts of mannitol were added in order to achieve a final osmolarity of 100, 200, 400 and 800 mosmol. Results are means ± S.D. of $n = 6$ measurements with two different preparations of BBMV.

reached after 5 min of incubation and the overall apparent uptake of the drug was strongly reduced (-85%), when compared with medium without NaCl. These results strongly suggest a major contribution of membrane binding in the apparent uptake of sparfloxacin by intestinal BBMV in the absence of NaCl. However, using such experimental procedures did not permit discrimination between decreased binding and decreased transport of sparfloxacin.

To further evaluate the relative contributions of transport and binding in the apparent sparfloxacin uptake by intestinal BBMV, and the influence of ionic strength on these two components, the apparent accumulation of 0.15 mM [^{14}C]sparfloxacin was measured after 2 min of incubation, in Na^+-free and 40 mM NaCl-containing transport medium of increasing osmolarity (100–800 milliosmoles (mosmol)) (Fig. 2). The binding of sparfloxacin to intestinal brush-border membranes was estimated by extrapolation of our results to an infinite extravesicular osmolarity, i.e. to an intravesicular volume of zero, and was 106 and 28 pmol/mg protein, in the absence and the presence of NaCl, respectively. Thus, it appears that sparfloxacin binding to intestinal brush-border membrane vesicles is strongly affected by the ionic strength of the transport buffer. In contrast, the slope of the regression curve obtained in the absence and in the presence of 40 mM NaCl were similar (60 versus 61 pmol/mg protein, for NaCl-free and NaCl-containing buffer, respectively), indicating that the transport of sparfloxacin across the apical membrane was unaffected by NaCl. After 2 min of incubation, the contribution of membrane binding to the apparent uptake of sparfloxacin by intestinal BBMV was about 63% in the absence of NaCl, and was reduced to 31% with 40 mM NaCl.

Several investigators have reported that fluoroquinolone can bind to plasma membranes of both bacteria and eukariotic cells (Bedard and Bryan, 1989; Furet et al., 1992). Here, we provide strong evidence for sparfloxacin binding to intestinal brush-border membranes. This binding is reduced when the ionic strength is increased by the addition of NaCl to the incubation buffer, suggesting the involvement of ionic interactions. Since the pK_a values of the carboxylic and piperazinyl amino groups are 6.23 and 8.57, respectively, about 88% of sparfloxacin is recovered in zwitterionic form at pH 7.5 (Furet et al., 1992). Sparfloxacin may therefore interact with both cations, such as ethanolamine and choline moieties, and anions, such as anionic phospholipids and sialic acids. The latter have been shown to be involved in the binding of quaternary ammonium compounds such as propantheline and methochlorpromazine to intestinal brush-border membranes (Saitoh et al., 1989) and might interact with the piperazinyl amino group of sparfloxacin. Further studies will be needed to ascertain the roles of phospholipids and sialic acids in sparfloxacin binding to intestinal brush-border membranes. It also seems from our results that sparfloxacin binding does not affect the transport

of this drug, since the 4-fold reduction of apparent uptake observed with 40 mM NaCl was related only to a reduction of sparfloxacin binding. Sparfloxacin is one of the most hydrophobic fluoroquinolones (Zabinski et al., 1995) and may readily diffuse across the enterocyte membrane leaflets. Binding to yet unknown moieties of brush-border membrane does not seem to promote this diffusion.

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