J. Pharm. Pharmacol. 1997, 49: 145–149
Received May 28, 1996
Accepted June 11, 1996

Absorption of Ciprofloxacin and Norfloxacin when Administered as Niosome-encapsulated Inclusion Complexes

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Abstract

An attempt has been made to design suitable niosome-encapsulated drug delivery system for ciprofloxacin and norfloxacin. Encapsulation of ciprofloxacin and norfloxacin in niosomes was investigated and the nasal and intestinal absorption of the products studied.

More than 80% of the drugs were successfully encapsulated to give products with sustained release characteristics. Encapsulation in niosomes also improved the stability of the antibacterial compounds.

Although the systemic availability of these niosome-encapsulated antibacterial compounds was not increased after nasal administration, intestinal absorption was significantly higher in comparison with that of plain inclusion complexes.

In recent years, pharmaceutical modification by inclusion complexation has been extensively developed to improve the solubility, dissolution rate, chemical stability and bioavailability of drug molecules. In this study attempts have been made to prepare niosomes of ciprofloxacin and norfloxacin inclusion complexes to improve the entrapment and release characteristics of these drugs.

Non-ionic surfactant vesicles known as niosomes are microscopic lamellar structures formed on admixture of a non-ionic surfactant, cholesterol and dicetyl phosphate with subsequent hydration in aqueous media. Niosomes are biodegradable, biocompatible, non-toxic and capable of encapsulating large quantities of material in relatively small volumes of vesicles (Khandare et al 1994).

Inclusion complexation involves entrapment of guest molecules totally or partially in the cavity of the host molecule without formation of any covalent bonds. When inclusion complexes are used, the rate of decomposition, light and gastric acid exposure are reduced. The solubility and dissolution rate of drugs are improved in β-cyclodextrin (β-CyD) complexes. β-Cyclodextrin is most widely used for complexation because of its unique cavity size and ease with which it can be obtained on an industrial scale (Aithal & Udupa 1995).

Intranasal administration of drugs has the advantage that it avoids first pass elimination, gut wall metabolism and destruction in the gastrointestinal tract. The rate and extent of absorption and the plasma concentration-time profile are comparable with those obtained after intravenous administration; the rich vasculature and highly permeable structure of the nasal membrane promotes absorption (Chein et al 1989).

The technique of Doluisio et al (1969) for studying intestinal absorption of drugs involves sampling from the intestinal lumen, determination of intrinsic uptake by eliminating environmental factors, and determination of uptake from a defined section of the small intestine.

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Materials and Methods

Ciprofloxacin hydrochloride was obtained from Cipla, Bangalore, India; norfloxacin from Dr Reddy's Laboratory, Hyderabad, India; and β-cyclodextrin from Nippo Shokuhin Kaako, Tokyo, Japan.

Preparation of β-cyclodextrin-drug complex by the neutralization method

Equimolar concentrations of norfloxacin or ciprofloxacin and β-cyclodextrin (1:1 M) were separately dissolved in 0·1 N NaOH, mixed and stirred for approximately 30 min, the pH was recorded and 0·1 N HCl was added dropwise while stirring until the pH reached 7·5, whereupon the complex precipitated. The residue was filtered, washed until free from chloride ions ($AgNO_3$test), dried at 25°C for 24 h, and stored in a desiccator.

Preparation of niosomes by the lipid layer hydration method

Niosomes were prepared by adopting the procedure of Azmin et al (1985). Surfactant and cholesterol (1:1; 75 mg of each) were dissolved in diethyl ether and the solvent was evaporated by use of a rotary flash evaporator. Phosphate-buffered saline (PBS; 8 mL) containing the drug complex was added to the dried thin film with gentle agitation. The mixture was intermittently mixed on a vortex mixer.

In-vitro release of drug-complex from niosomes into phosphate-buffered saline (Fig. 1)

After separation of the free drug-complex, the niosome preparation was transferred to a dialysis tube and subjected to dialysis with the dialysis tube immersed in a receptor compartment containing PBS (100 mL). At different time intervals for 6 h, 5-mL samples were withdrawn from the receptor compartment and the drug-complex content was determined spectrophotometrically at 272 nm. Each sample withdrawn was replaced by an equal volume of PBS.

Stability

The formulated niosomes were separated into three portions. One portion was kept at room temperature, the second at 37°C,

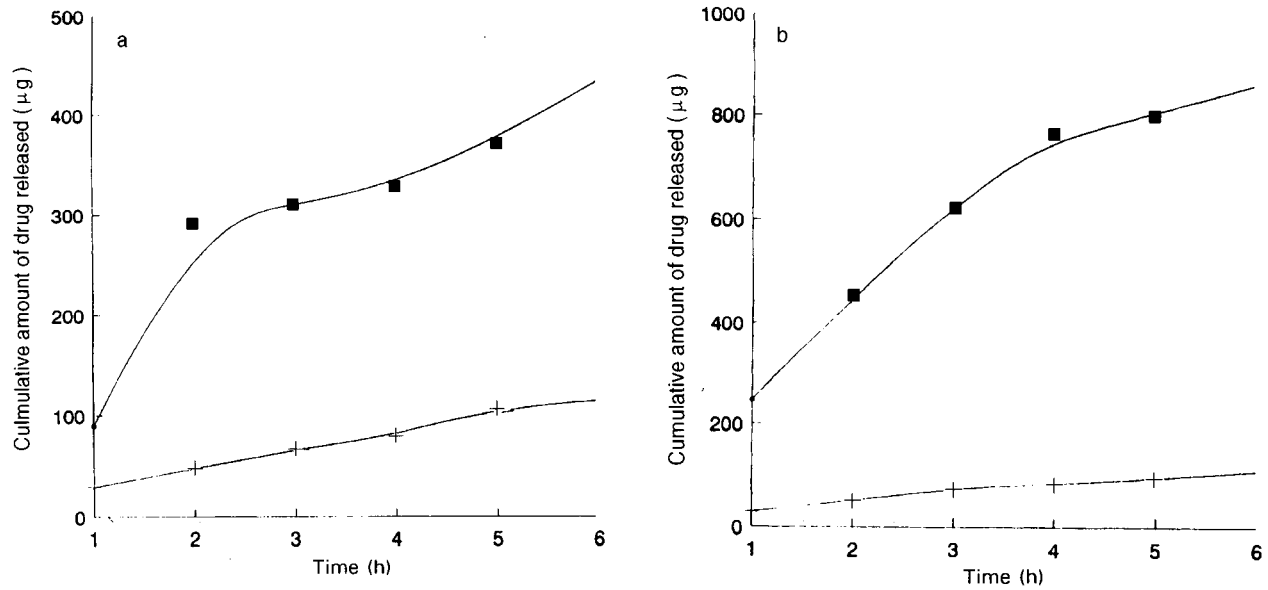

FIG. 1. In-vitro release patterns for ciprofloxacin plus β-clodextrin (a) and norfloxacin plus β-cyclodextrin (b) in phosphate-buffered saline, pH 7.4. ■ Plain drug, + niosome-encapsulated drug.

and the third at 4°C for 1 month. At 15-day intervals these portions were then evaluated for their drug content and release characteristics (Table 1).

In-situ nasal perfusion

The in-situ nasal perfusion technique demonstrated by Hussain et al (1980) was used in this study. Albino rats, 200–220 g, were anaesthetized with a 45 mg kg^{-1} dose of sodium pentobarbitone. The trachea was exposed and cannulated with polyethylene tubing. The oesophagus was then cannulated towards the posterior part of the nasal cavity with a second polyethylene tube. The nasopalantine was sealed with an adhesive agent and perfusate was introduced through a tube into the second cannula. The nasal cavity was rinsed using normal saline (5 mL). The perfusate was circulated through the nasal cavity at a flow rate of 2·0 mL min^{-1}, by use of a volumetric infusion pump, and was collected through a funnel into the reservoir for recirculation. Samples (1 mL) of the perfusate were withdrawn after 5, 10, 15, 20, 30, 45 and 60 min (Table 2).

The drug content in each sample was measured spectrophotometrically; before analysis of niosomes the lipid materials were removed by use of Triton X.

Study of the in-situ absorption drugs in rat intestine by the Doluisio technique

Healthy albino rats, 200–250 g, were selected for study of drug absorption from intestine. After abdominal incision, the intestine was cleaned by irrigation with pH 7·4 buffer and then L-shaped tubes with 3-way stop-cocks were introduced in the duodenal and ilial ends of the intestine. After removal of the buffer, drug solution (10 mL) was introduced through a syringe. Samples were collected from the intestine at 15-min intervals (Tables 3, 4 and 5).

Table 1. Efect of temperature on stability of ciprofloxacin preparations.

	Temperature	Amount of drug degraded (%) after 30 days
Ciprofloxacin plus β-cyclodextrin	4°C	16·43
	Room temp	24·99
	37°C	34·86
Niosome-encapsulated ciprofloxacin plus β-cyclodextrin	4°C	10·83
	Room temp	19·37
	37°C	35·62
Norfloxacin plus β-cyclodextrin	4°C	9·63
	Room temp	21·81
	37°C	27·69
Niosome-encapsulated norfloxacin plus β-cyclodextrin	4°C	4·27
	Room temp	22·51
	37°C	36·72

Results

Size and entrapment efficiency

The formulated niosomes were in the size range 12–36 μm, the mean diameter being 24–25 μm. For niosomes prepared from ciprofloxacin and β-CyD entrapment was 80·41%; niosomes prepared from norfloxacin and β-CyD entrapment was 91·22%.

In-vitro release studies in phosphate-buffered saline (Fig. 1)

For niosome-encapsulated (ciprofloxacin + β-CyD) 15·96% of the drug complex was released in 6 h whereas with plain (ciprofloxacin + β-CyD) 65·96% of the drug complex was released after 6 h. For niosome-encapsulated (norfloxacin + β-CyD) 11·24% of the drug complex was released in 6 h, whereas with plain (norfloxacin + β-CyD) 97·5% of the drug complex was released in 6 h.

With all the parameters studied using various tonicity conditions, non-ionic surfactant, Tween 80 and niosome encap-

Table 2. Absorption of ciprofloxacin after in-situ nasal perfusion of ciprofloxacin preparations.

Preparation	Cumulative amount absorbed (μg)	Absorption rate constant (h^{-1})	Area under the concentration–time curve (μg h mL^{-1})
Ciprofloxacin plus β-cyclodextrin			
Control	298·98	10·01	227·98
Niosome encapsulated	207·39	7·49	143·10
Isotonic medium	159·85	4·99	105·47
Hypotonic medium	165·83	5·32	83·07
Hypertonic medium	329·65	10·43	161·69
With 0·2% Tween 80	129·02	5·72	57·31
Norfloxacin plus β-cyclodextrin			
Control	329·09	9·46	254·03
Isotonic	435·63	13·03	235·96
Hypotonic	92·57	3·89	57·31
Hypertonic	211·60	6·75	81·69

Results are averages of triplicate measurements.

Table 3. In-situ intestinal absorption (μg) of ciprofloxacin from ciprofloxacin plus β-cyclodextrin.

Formulation	Time (min)				
	0	15	30	45	60
Plain ciprofloxacin	0	89·27	168·77	331·01	625·37
Control ciprofloxacin plus β-cyclodextrin	0	525·60	798·83	1024·18	1071·20
Niosome-encapsulated ciprofloxacin plus β-cyclodextrin	0	498·56	750·56	1129·44	1169·68
With Tween 80	0	578·3	655·86	745·55	964·91

Results are averages of triplicate measurements.

Table 4. In-situ intestinal absorption (μg) of norfloxacin from norfloxacin plus β-cyclodextrin.

Formulation	Time (min)				
	0	15	30	45	60
Plain norfloxacin	0	596·00	771·28	940·65	971·38
Control norfloxacin plus β-cyclodextrin	0	813·79	965·08	962·62	1203·84
Niosome-encapsulated norfloxacin plus β-cyclodextrin	0	1479·36	1581·15	2028·57	2080·70
With Tween 80	0	651·00	850·12	976·61	1090·64

Results are averages of triplicate measurements.

Table 5. In-situ intestinal absorption of ciprofloxacin and norfloxacin.

Drug	Cumulative amount absorbed (μg)	Absorption rate constant (h^{-1})	Area under the concentration–time curve (μg h mL^{-1})
Ciprofloxacin plus β-cyclodextrin			
Plain ciprofloxacin	625·37	22·9	225·3
Control	1071·20	40·54	721·05
Niosome encapsulated	1169·68	45·60	834·67
With 0·2% Tween 80	964·91	32·19	615·53
Norfloxacin plus β-cyclodextrin			
Plain norfloxacin	971·38	35·05	698·40
Control	1203·84	39·25	835·15
Niosome encapsulated	2080·70	72·31	1532·33
With 0·2% Tween 80	1090·64	38·49	755·76

sulation it was shown that the nasal absorption rate decreased up to 45 min (Table 2; Figs 2 and 3). It is, therefore, clearly evident that it is not possible to enhance the nasal absorption of these inclusion complexes by niosome encapsulation or by use of permeation enhancers, or by changing the tonicity.

The absorption of the drug was greatest from the plain solution (control).

Discussion

In-vitro release pattern in phosphate-buffered saline

The comparative release data for ciprofloxacin plus β-CyD, norfloxacin + β-CyD, both plain and niosome-encapsulated indicate that by niosome encapsulation it is possible to sustain and control the release of the drug for longer duration. This interesting finding reveals that niosome encapsulation may be very useful for designing sustained release drug delivery formulations (Fig. 1).

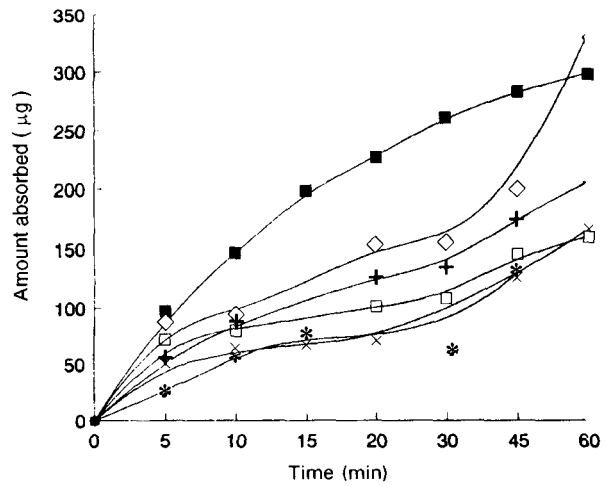

FIG. 2. In-situ nasal perfusion data for ciprofloxacin plus β-cyclodextrin. ■ Control, + niosome-encapsulated, * 2% tween 80, □ isotonic, × hypotonic, ◇ hypertonic. Perfusion rate = 2mL min^{-1}, perfusate volume = 50 mL.

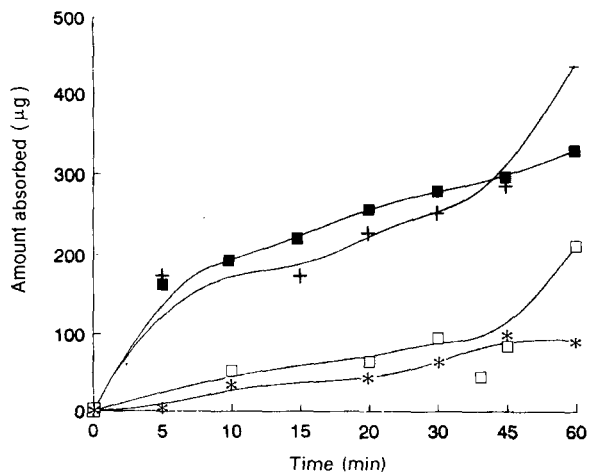

FIG. 3. In-situ nasal perfusion data for norfloxacin plus β-cyclodextrin. ■ Control, + isotonic, * hypotonic, □ hypertonic. Perfusion rate = 2 mL min^{-1}, perfusate volume = 50 mL.

Stability

Stability data clearly indicate that niosome encapsulation gives excellent protection for inclusion complexes of norfloxacin and ciprofloxacin, at least under refrigerated conditions. Thus niosome encapsulation may be very useful for improving the stability characteristics of drugs such as ciprofloxacin, norfloxacin and other antibiotics. It may be helpful for maintaining the therapeutic efficacy of these drugs for longer periods of storage. It is, however, also observed that at higher temperatures the rate of degradation is greater even in the niosome-encapsulated form. Higher degradation may be a consequence of the presence of lipid materials. e.g. cholesterol, and fatty material, e.g. span 60 (sorbitan monostearate). After storage for 1 month there was no significant change in the release pattern of the niosome-encapsulated inclusion complexes of ciprofloxacin and norfloxacin (Table 1).

In-situ intestinal absorption

It was observed that inclusion complexation of norfloxacin and ciprofloxacin with β-CyD resulted in a significant increase in

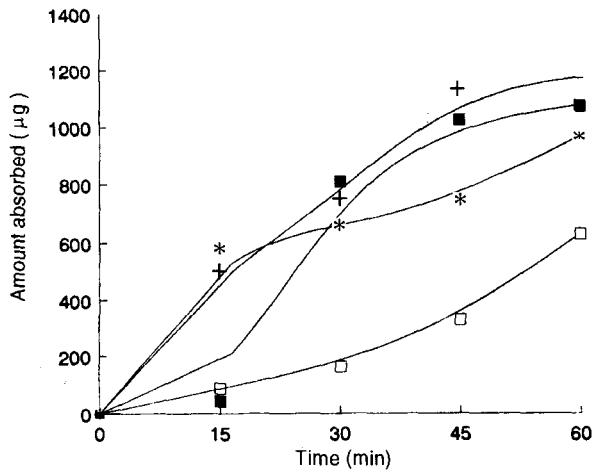

FIG. 4. In-situ intestinal absorption data for ciprofloxacin plus β-cyclodextrin. ■ Control, + niosome-encapsulated, * 2% tween 80, □ plain.

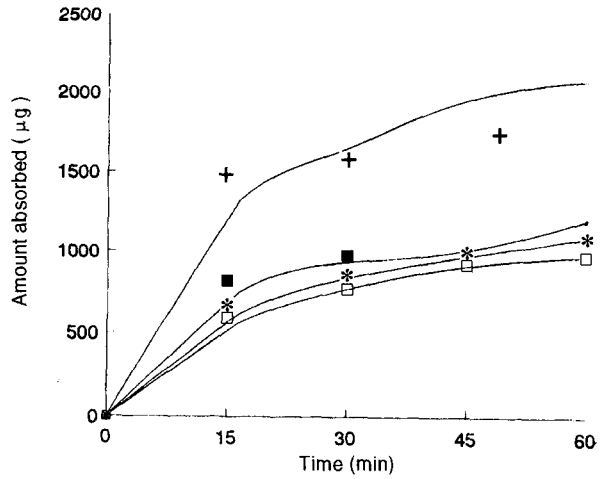

FIG. 5. In-situ intestinal absorption data for norfloxacin plus β-cyclodextrin. ■ Control, + niosome-encapsulated, * 2% tween 80, □ plain.

intestinal absorption of the drug. Although use of 0·2% tween 80 non-ionic surfactant resulted in a slight decrease in intestinal absorption of these anti-infective drugs, a significant increase in the rate of absorption of these inclusion complexes was observed after niosome encapsulation (Tables 3, 4 and 5; Figs 4 and 5) This niosome-encapsulated drug delivery system might, therefore, be very useful for enhancing the absorption of drug through the intestine after oral administration.

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