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Mucoadhesive microspheres for nasal administration of an antiemetic drug, metoclopramide: in-vitro/ex-vivo studies

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Abstract

Microparticulate delivery systems designed for the nasal administration of an antiemetic drug, metoclopramide hydrochloride, were prepared. Microspheres composed of sodium alginate, chitosan hydrochloride, or both, were obtained using a spray-drying method; some batches of drug-free microparticles were prepared as a comparison. The morphology, in-vitro swelling behaviour, mucoadhesive properties and drug release from microparticles were evaluated. Ex-vivo drug permeation tests were carried out using sheep nasal mucosa; permeation test of the drug solution was peformed as comparison. During ex-vivo permeation tests, transmission electron microscopy (TEM) analyses were carried out on the nasal mucosa to study the morphological changes of epithelial cells and tight junctions, while the change in microsphere morphology was examined using photostereo microscopy (PM). Spray-dried microparticles had a mean diameter (d_{vs}) in the range of about 3–10 μm. They showed good in-vitro mucoadhesive properties. In-vitro release profiles and swelling behaviour depended on their composition: the drug release occurred in 1–3 h. Ex-vivo studies showed that drug permeation through the mucosa from microparticles based on chitosan was higher than from those consisting of alginate alone. This can be related to the penetration enhancing properties of chitosan. Complexation of chitosan with alginate led to a control of the drug release. Microscopy observation of microspheres during the permeation tests revealed that microparticles swelled and gelled, maintaining their shape. TEM analyses of the mucosa after exposure to the microparticles consisting of alginate/chitosan showed opened tight junctions. This preliminary study shows that alginate/chitosan spray-dried microspheres have promising properties for use as mucoadhesive nasal carriers of an antiemetic drug.

Introduction

Metoclopramide hydrochloride is a potent antiemetic, effective for preventing different kinds of emesis. This drug is characterized by a short half-life (3–4 h) and for this reason it is usually administered orally three or four times daily (El-Sayed et al 1995). Antiemetics play an important role in the quality of life of patients in different pathological situations, and in particular during and after chemotherapy, since they determine adequate control of the adverse effects of the medical treatment. Currently, the routes of administration of antiemetics are oral or intravenous, although patient compliance is often impaired by the difficulties associated with acute emesis or invasiveness of parenteral administration (Kraut & Fauser 2001).

In recent years the nasal route has attracted increasing attention as a suitable method for systemic drug supply (Hussain 1998; Ugwoke et al 2001). It is easy to use, painless and usually well accepted by the patients. To improve the compliance of antiemetics, nasal formulations could be considered as a possible alternative. They can be administered also to patients already emetic and with respect to the parenteral routes they are not invasive. Furthermore, the nasal route can permit the avoidance of hepatic first-pass metabolism, thus improving drug bioavailability.

The design of nasal dosage forms has to consider the anatomic and physiologic characteristics of nasal mucosa and more particularly the rapid mucociliary clearance

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that limits the time available for drug adsorption from the applied dosage form (Hussain 1998; Ugwoke et al 2001).

To overcome the rapid clearance and to facilitate the adsorption through the barrier of nasal mucosa, mucoadhesive materials (Dondeti et al 1996; Soane et al 1999; Ugwoke et al 1999a, b; Lim et al 2000) and adsorption promoters (O'Hogan et al 1990; Uchido et al 1991) are used in nasal formulations.

This study investigates the preparation of microparticulate delivery systems for the nasal administration of metoclopramide hydrochloride. Sodium alginate or chitosan hydrochloride (or both) were used as polymeric materials. These polymers are of natural origin, biocompatible and biodegradable. Alginate is an anionic polymer already employed as a matrix for the entrapment or delivery of a variety of drugs and biological molecules (Gombotz & Wee 1998). Chitosan is a cationic polymer, used in numerous pharmaceutical applications (Borchard & Junginger 2001). Chitosan has mucoadhesive properties, has no toxicity and it can be applied onto the nasal epithelium where it offers excellent enhancement of drug permeation (Illum 1999; Sinha et al 2004).

Chitosan is often cross-linked to improve the drug encapsulation and controlled release by using cross-linker agents, by complexation (Liu et al 1997; Lim et al 2000; Lucinda-Silva & Evangelista 2003; Nordby et al 2003; Sinha et al 2004).

The aim of this work was to prepare microspheres based on chitosan alone or a combination of this polymer with sodium alginate as a controlled release system using the spray-drying method; mucoadhesive properties and enhancement of nasal permeation were evaluated. The microparticles, prepared by a spray-drying technique, were characterized in terms of encapsulation efficiency, morphology, size distribution, in-vitro drug release behaviour and swelling properties. In-vitro mucoadhesive tests were performed by evaluating the amount of microspheres attached to a paper disk saturated with mucin. Ex-vivo permeation studies of the drug solution and of metoclopramide hydrochloride from microspheres across sheep nasal mucosa were carried out. Photostereo microscopy (PM) was used for the observation of the microspheres placed on the mucosa surface.

Materials and Methods

Materials

Metoclopramide hydrochloride was kindly given by AMSA (Milano, Italy); sodium alginate (Protanal LF 120L, batch 907788) and chitosan hydrochloride (Protasan UP CL 113, batch FP-110-02, MW 160 000, degree of acetylation 86%) were purchased from FMC BioPolymer AS (Drammen, Norway); silicon oil (Tegiloxan 3) was kindly given by Goldschmidth (Essen, Germany); mucin (M-2378, Type II, crude, from porcine stomach, bound sialic acid 1%, desiccated) was provided by Sigma, (St Louis, MO); ultra-pure water was prepared with the MilliQ R4 system Millipore (Milano, Italy); acetonitrile (Chromasolv), sulfuric acid 96% and $HClO_4$ 70% for analysis were obtained from Riedel-de Haen, (Milano, Italy); membrane filters (45 mm, 0.45 μm pore size) and nylon and PTFE syringe filters (13 mm, 0.45 μm porosity) were supplied by Alltech Italia Srl, (Sedriano, Milano, Italy).

All other solvents and chemicals were of analytical grade.

Preparation of microspheres

The microspheres were prepared using a spray-drying technique. Their compositions are reported in Table 1.

Microspheres consisting of metoclopramide hydrochloride and alginate or chitosan have a drug-to-polymer ratio of 1:5 chosen on the basis of the drug nasal administration; the polymer ratio used to prepare alginate/chitosan I and II (AL/CH I and II) was chosen, after several attempts to find the most suitable technological conditions to perform the spray-drying process.

The preparation of drug-loaded microspheres based on alginate or chitosan was carried out dissolving the polymer and then the drug in distilled water, under stirring and at room temperature. The total concentration of solid in solution was always kept at 1% w/v.

The microspheres consisting of both chitosan and alginate were prepared dissolving each polymer in distilled water under magnetic stirring; the drug was added to the alginate solution. The two solutions were then mixed and the suspension, obtained from the precipitation of the alginate–chitosan interaction product, was stirred at 9500 rev min^{-1} (Ultra-Turrax, T25; IKA, Germany) for 5 min and then sprayed under continuous magnetic agitation (total solid 1% w/v for AL/CH I).

The precipitation of this interaction product made the spray-drying process difficult. Thus a more diluted water suspension (0.3% w/v) was prepared to make the preparation of the feed suspension (AL/CH II microspheres) easier.

Two batches of drug-free microspheres (A and C) were prepared, as a comparison, under the conditions described above.

The microspheres were obtained by spraying the feed (solution/suspension) through the nozzle of a spray-dryer (model Mini Spray HO Pabisch; W. Pabisch S.p.A., Milano, Italy), co-current flow type, equipped with standard 0.7 mm nozzle. The process conditions were: inlet air temperature 80–90°C; outlet air temperature 52°C; spray

Table 1 Composition of spray-dried microspheres expressed as drug-to-polymer(s) weight ratio (w/w)

Microspheres	Metoclopramide hydrochloride	Alginate	Chitosan
AL	1	5	—
CH	1	—	5
AL/CH I and II	1	3	5

AL, sodium alginate; CH, chitosan hydrochloride.

pressure about 2 kg cm^{-2} and spray rate of feed about 6 mL min^{-1}. The solid microparticles were then harvested from the apparatus collector and kept under vacuum for 24 h, at room temperature.

The volume of feed sprayed for the preparation of each batch was always 200 mL. Each preparation was carried out in triplicate (s.d. within 2%)

Determination of metoclopramide content of microspheres

Accurately weighed samples of drug-loaded microparticles (15 mg) were mixed with 200 mL of distilled water until their dissolution, which occurred in about 1 h under magnetic stirring. In the case of AL/CH I and II, due to the insolubility of the microspheres, the drug was extracted with distilled water using the same procedure described above, which was sufficient to ensure complete drug recovery.

The concentration of metoclopramide hydrochloride in water was determined using a UV spectrophotometer, at a wavelength of 309 nm (Hitachi spectrophotometer U-2001; Hitachi Instruments, Tokyo, Japan). Preliminary UV scanning showed that the presence of the polymers did not interfere with the absorbance of metoclopramide hydrochloride at 309 nm.

Drug content was calculated as the detected amount of metoclopramide hydrochloride with respect to the theoretical amount of the drug used for the preparation of microspheres and expressed as a percentage. Each determination was carried out in triplicate (s.d. within 0.1%).

Size and morphology studies

The particle size and the particle size distribution of microspheres were analysed by laser diffractometry, using a Coulter LS 100Q laser size (Beckman Coulter Particle Characterization, Miami, FL). Analyses were carried out on the spray-dried microspheres dispersed in silicon oil and after sonication. The average particle size of the microspheres was expressed as the volume-surface diameter, d_{vs} (μm) (Edmundson 1967). Results are the means of triplicate experiments.

Shape and surface characteristics of the spray-dried microspheres were studied by scanning electron microscopy (SEM) (Zeiss, Germany). The samples were placed on double-sided tapes that had previously been secured on aluminium stubs and then analysed at 20 kV acceleration voltage after gold sputtering, under argon atmosphere.

In-vitro swelling studies

In-vitro swelling properties of the microspheres were determined using the Coulter laser diffraction apparatus described above and the variation of particle size versus time was evaluated. The samples were prepared by suspending the microspheres in phosphate buffer (pH 7.0) and particle size distributions and the mode values were measured, under magnetic stirring, after 0, 5, 15, 30 and 60 min.

In-vitro mucoadhesion studies

The mucoadhesive properties of drug-loaded microspheres were tested by determination of the quantity of microspheres sticking to a filter paper saturated with mucin and after applying an air load. The air jet was used to simulate breathing in and out. This is a modification of the method previously described (Conte et al 1994). It could be useful to evaluate the effect of air-flow on nasal clearance of microparticulate formulations after their administration.

Figure 1 shows the apparatus expressly designed and used for the in-vitro mucoadhesive testing of microspheres. The filter paper ($d = 2.5$ cm, $A = 4.9$ cm^2) was soaked with a mucin solution (2% w/v in distilled water) in a chamber with controlled humidity (90–100%) and temperature (25–30°C), for 10 min. The filter was then transferred to a cruet stand, 10 mg of microspheres were spread out onto the disk and a stream of air (flux = 6.37 m s^{-1}, determined by a manual thermoanemometer) was blown over the microspheres for 15 s. The amount of microspheres sticking to the filter paper after the applied air load was determined by quantifying the amount of drug in the remaining microspheres. Microparticles sticking to the disk surface were recovered by washing the filter paper with water; the volume then was adjusted to 100 mL and the amount of the drug was determined by a UV spectrophotometric analysis, at 309 nm. The in-vitro mucoadhesion behaviour of the microspheres was expressed as the percentage of microspheres remaining on the disk after exposure to the air stream. Each determination was carried out in triplicate.

In-vitro drug release tests

The in-vitro release of the drug from the microspheres was studied in phosphate buffer (pH 7.0), 400 mL, using the USP Apparatus no. 1, at 37°C and 50 rev. min^{-1} (Erweka DT 70;

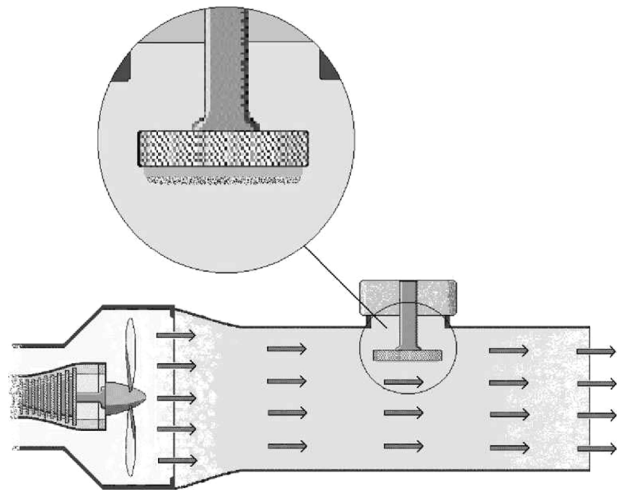

Figure 1 Apparatus used for evaluation of the in-vitro mucoadhesive properties of microspheres.

Erweka GmbH, Heusenstamm, Germany). An amount of microspheres equivalent to about 15 mg of drug was added to the medium at time zero. Samples (1 mL) were withdrawn at different time intervals up to 3 h and measured spectrophotometrically at 309 nm to evaluate amount of drug released. An equal volume of fresh medium was added after each sampling.

The dissolution rate of metoclopramide as a raw material was performed under the same conditions reported above.

Each experiment was performed in triplicate.

Ex-vivo drug permeation studies

HPLC determination of metoclopramide

HPLC methods were set up for the determination of the drug in ex-vivo permeation studies in the acceptor buffer.

The following instruments were used: Hewlett–Packard 1050 series quaternary pump, variable-wavelength UV–Vis spectrophotometer detector and auto sampler. The peak areas determined with a 3390 integrator were used for quantification (Hewlett-Packard, Waldbronn, Germany).

With reference to El-Gindy (2003), the chromatographic separation was performed using an ODS LiChrosorb 5 μm (Merck 64271 Darmstadt, Germany), 250 mm, 4.2 mm i.d. stationary phase, preceded by a Nucleosil RP-18, 5 μm guard column (Alltech, Milano, Italy). Ten microlitres of calibration standards or solution of permeate or extract samples were directly injected onto the column and eluted with a solution of acetonitrile–0.01 N sulfuric acid (60:40). The flow rate was 1.5 mL min^{-1}, at room temperature. Detection was carried out by monitoring the absorbance signals at 273 nm (0.1 AUFS). The elution period was 10 min. At the end of each working day, the column was washed with the zero-time solvent mixture. Mobile phases were filtered by a 0.45-μm pore size nylon and cellulose acetate membrane filter and degassed before their use.

Tissue preparation

Nasal mucosa of two Sardinian sheep was obtained from the local slaughter house. The time from slaughter to removal of the nose was 5 min maximum. The turbinates were fully exposed by a longitudinal incision through the nose. The turbinate mucosa was carefully removed from the underlying bone by cutting with haemostatic forceps and pulling the mucosa off. The excised tissue was stored directly on ice during transportation to the laboratory. The experiments were carried out within 3 h of procurement of the mucosa. One turbinate was used for each formulation.

Drug permeation studies

Drug solution and metoclopramide hydrochloride-loaded microspheres were tested for the permeation characteristics of drug across the sheep mucosa.

Within 60 min of removal, the mucosa was cut and mounted on the bottom of a cylindrical plastic support consisting of a tube (height 1.91 cm, diameter = 2.28 cm) connected to a drive shaft of the dissolution apparatus (Erweka DT 70; Erweka GmbH, Heusenstamm, Germany) as shown in Figure 2. The mucosa was clamped to the support by a plastic ring and then microspheres were uniformly spread out on the surface of the mucosa. The system was then inserted into the vessel containing phosphate buffer, keeping the mucosa in contact just with the surface of the liquid. Working conditions were: 100 mL of phosphate buffer pH 7.0, 37°C, 50 rev. min^{-1}.

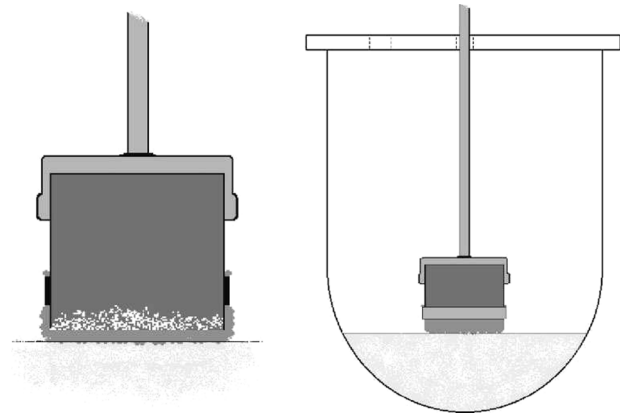

Figure 2 Modified dissolution apparatus used for evaluation of the ex-vivo permeation of metoclopramide hydrochloride from microspheres.

Samples (0.5 mL) were withdrawn at different intervals and the amount of drug permeated was measured by HPLC and calculated by referring to the calibration curve prepared in buffer pH 7.0 (standard solutions in the range of 10–100 mg L^{-1}). An equal volume of fresh medium was added after each sampling.

Transmission electron microscopy (TEM) analysis

TEM analysis of the mucosa after the permeation test carried out using AL/CH I microspheres and of the fresh excised mucosa, as comparison, was performed to study the effect of the microspheres on the morphology of the nasal epithelial cells and of the tight junctions.

The specimens were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide for 1 h, dehydrated in acetone, embedded in epoxy resin (Agar 100) and cut with an ultramicrotome. Ultra-thin sections were stained with uranium or lead salts, mounted on grids and examined by a transmission electron microscope (TEM 109 turbo; Zeiss, Germany).

Photostereo microscopy (PM) analysis

PM observation (Zeiss MC 63; Zeiss, Germany) of microspheres on the surface of the mucosa during the permeation tests was carried out to evaluate the morphology modifications of the particles after the contact with mucosal surface.

Pictures were taken at time zero and at 15, 30, 60 and 180 min (magnification 64×).

Statistical analysis

Analysis of data was performed using the Kruskal–Wallis test (GraphPad Prism, version 2.01; GraphPad Software Incorporated), following which individual differences

between treatments were identified using a non-parametric post-hoc test (Dunn's test). In all cases $P < 0.05$ denoted significance.

Results and Discussion

Microsphere preparation and drug content determination

Spray-drying is a good technique for the preparation of chitosan and alginate microparticles. It is a one-step process, easy and rapid, as it combines drying of the feed and embedding of the drug into a one-step operation. Other techniques, such as emulsification/solvent evaporation, involve different steps. Table 2 lists production yields, actual drug contents and encapsulation efficiencies of spray-dried microparticles. Production yields (expressed as weight percentage of microspheres obtained with respect to the initial amount of drug and polymer) were always relatively low (27– 35%). As previously pointed out (Giunchedi et al 2000), these values can be justified by the low quantity of feed used for the preparation of each batch and by the structure of the spray-drier apparatus, that lacked a trap to capture the smallest and lightest particles.

AL/CH I had a yield even lower (15%); this was because of the high viscosity of the feed and of the presence of gelling masses that made the spraying process difficult. In fact, a more diluted feed led to an increase in the production yield (AL/CH II, 27%).

Encapsulation efficiencies were in the range 83–97%. Microspheres prepared using alginate or chitosan alone were able to entrap higher amounts of drug than microparticles prepared with the combination of the two polymers. Decreasing the total solid in suspension for the preparation of the feed (AL/CH II) did not influence the drug entrapment.

Size and morphological studies

As shown in Table 2, drug loaded chitosan particles were characterized by a larger size (d_{vs} about 11 μm) with respect to the corresponding drug free particles, despite being prepared using the same experimental conditions (same concentration of feed solution). The size of alginate microparticles (about 7–8 μm) was not influenced by the presence of the drug. Microspheres characterized by the combination of both polymers had a size of about 11 μm for AL/CH I and 3–5 μm for AL/CH II. This difference was due to the influence of feed concentration on particle size, as previously pointed out (Giunchedi & Conte 1995); when a more diluted feed was sprayed (AL/CH II 0.3% w/v), particles with smaller size were obtained.

In-vitro swelling studies

The in-vitro swelling properties of the microspheres were studied in phosphate buffer (pH 7.0) using a Coulter LD. The results are reported in Table 2 as a modification of the mode of the distribution at different time points such as 5 and 30 min; the data show a different behaviour of the microspheres, depending on their composition. Two phenomena are involved: swelling and thus increase in particle size, and dissolution of the microparticles and then decrease in particle size. The competition in the two processes (rate) determines the final behaviour. Chitosan microparticles (CH) swell quickly within 30 min (the mode of the distribution shifts from 42 μm to about 900 μm), but after this time their size decreases, owing to the prevalence of dissolution. Alginate microparticles (AL) swell less than chitosan particles and after 30 min their size distribution curves shift towards smaller values (mode value = 4.9 μm). Swelling of AL/CH I was rapid (within 5 min) and the particles retained this size for the duration of the test (60 min). This is probably due to the low solubility of the polyelectrolyte complex (Liu et al 1997; Gupta et al 2002).

In-vitro mucoadhesion tests

The percentage of microspheres attached to the filter paper saturated with mucin was calculated from the amount of drug (metoclopramide hydrochloride) detected in the remaining microspheres after applying the air load, compared with the original amount of drug present in the applied microspheres. The results (Table 3) show that drug-loaded

Table 2 Characteristics of spray-dried microspheres

Microspheres	Production yield (%)	Actual drug content (%)[a]	Encapsulation efficiency (%)	Particle size (d_{vs}, μm)[a]	Swelling	
					5 min[a]	30 min[a]
AL	31.2	16.17 ± 0.06	97.13	7.04 ± 1.60	34.60 ± 1.20	4.90 ± 0.14
CH	35.4	15.26 ± 0.08	91.56	10.89 ± 0.58	905.10 ± 0.35	905.10 ± 0.08
AL/CH I	15.4	9.27 ± 0.01	83.45	11.34 ± 0.54	21.70 ± 1.41	26.10 ± 0.65
AL/CH II	27.3	9.26 ± 0.02	83.37	3.55 ± 0.51	29.70 ± 2.28	34.60 ± 0.98
A	30.5	—	—	8.50 ± 0.54	nd	nd
C	32.3	—	—	4.55 ± 0.05	nd	nd

[a]Mean of three determinations (n = 3) ± s.d. AL, sodium alginate; CH, chitosan hydrochloride; A, C, drug-free microspheres; nd, not determined.

Table 3 In-vitro mucoadhesive tests: percentage of microspheres attached

Microspheres	Percentage attached
AL	78.0 ± 1.4
CH	88.0 ± 2.6
AL/CH I	81.0 ± 4.4
AL/CH II	91.0 ± 2.2

Data are means ± s.d., n = 3. AL, sodium alginate; CH, chitosan hydrochloride.

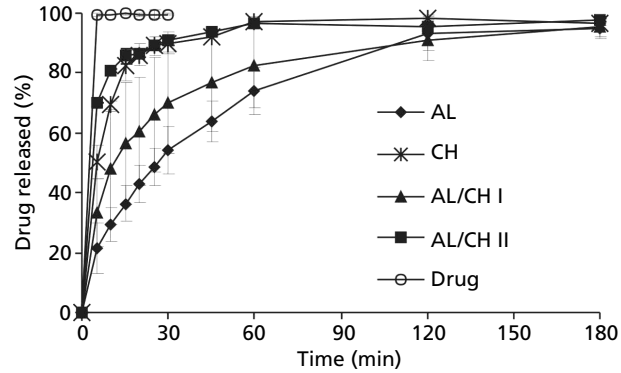

Figure 3 In-vitro metoclopramide hydrochloride release profiles from spray-dried microspheres compared with the dissolution rate of the crude drug (means ± s.d., n = 3). AL, sodium alginate; CH, chitosan hydrochloride.

microspheres were always characterized by good mucoadhesive properties. For all batches, the percentage of the originally applied mass of microspheres attached to the filter paper ranged from about 78% to 91%. The tests showed some differences in the mucoadhesive properties, which were dependent on the composition of the microparticles. Microspheres containing chitosan alone or combined with alginate were characterized by an improved in-vitro mucoadhesive behaviour with respect to the microparticles consisting of alginate alone. Mucoadhesion involves different kinds of interaction forces, such as electrostatic attraction and hydrogen bonding. In the case of chitosan-containing microspheres the electrostatic attraction could be an important factor because of the negatively charged sialic acid of the mucin and the positively charged glucosamine residues of chitosan (Hussain 1998).

The complexation of chitosan with alginate could reduce the positive charges of chitosan and lead to a decrease in mucoadhesion. However, as shown in Table 3, the AL/CH I and II formulations possessed good mucoadhesive properties. This is because mucoadhesion involves different processes, such as ionic interactions, hydrogen bonds and interpenetration of polymer chains (Illum 1999).

Comparison of the two batches with the same composition but different particle size, showed that AL/CH II (smaller size) has better adhesive properties than AL/CH I (higher size). This is probably because microspheres with a lower size have a broader surface area than the higher-size microparticles, and this may result in an improved interaction with the mucin layer.

In-vitro drug release tests

Figure 3 shows the results of the in-vitro drug release tests of the microspheres in phosphate buffer (pH 7.0) compared with the dissolution behaviour of metoclopramide hydrochloride raw material (pure drug). The method described in the USP was used for the in-vitro characterization of the release behaviour of the microparticles and not to obtain results comparable with the in-vivo situation of nasal administration. Results showed that drug alone dissolved very quickly, being freely soluble in water. All spray-dried microspheres controlled metoclopramide hydrochloride release, but while about 100% of released drug was reached from chitosan and AL/CH II microparticles, within 1 h, alginate and AL/CH I microparticles were characterized by a prolonged release over 3 h. In the case of alginate particles, this behaviour can be explained by the gelation of alginate and also by a possible interaction between alginate, anionic polymer, with metoclopramide hydrochloride, basic drug. The difference in the metoclopramide hydrochloride release rate from AL/CH I and AL/CH II microspheres (same composition) might be a consequence of their different particle sizes – the highest size corresponds to the more extended drug release.

Ex-vivo drug permeation and related studies

Drug solution and the microspheres were tested for their ex-vivo drug permeation characteristics across sheep nasal mucosa (Reardon et al 1993) using a modified dissolution apparatus (Figure 2). For a preliminary study of a nasal formulation the ex-vivo model here described presents certain advantages: it is performed using an official apparatus simply modified and the test can be performed rapidly, with simple analytical procedure, because compared with an in-vivo study the presence of plasma proteins is avoided (Wadell et al 1999).

Metoclopramide hydrochloride showed a more rapid permeation profile across nasal mucosa compared with the formulation containing alginate ($P < 0.05$) (Figure 4). However, no statistically significant differences ($P > 0.05$) were found among permeation profiles of the drug from the solution and the metoclopramide hydrochloride from chitosan and AL/CH I microspheres. The encapsulation of the drug in microspheres does not modify the chance of the drug to permeate the nasal mucosa. Furthermore, the swelling behaviour and the mucoadhesive effect of these formulations increase the residential time of the drug in the nasal cavity, protecting the metoclopramide from the mucociliary clearance due to the rapid turnover of the mucus.

Microspheres based on chitosan alone (CH) or in combination with alginate (AL/CH I and II) were characterized by a significantly higher drug permeation across nasal mucosa with respect to microspheres based on alginate

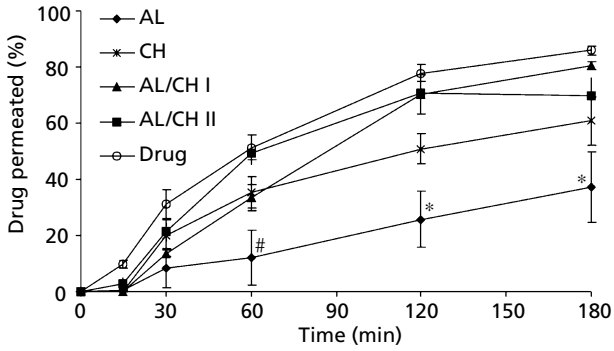

Figure 4 Ex-vivo metoclopramide hydrochloride permeation from microspheres through sheep nasal mucosa in comparison with the drug powder (means ± s.d., n = 4). AL, sodium alginate; CH, chitosan hydrochloride. Dunn's test: #significantly different from the AL and the AL/CH II formulations and metoclopramide hydrochloride; $*P < 0.05$ compared with the AL formulation and metoclopramide hydrochloride.

alone (AL). The amount of metoclopramide hydrochloride permeated through mucosa and collected in the acceptor buffer at the end of the test from chitosan microspheres is about two-fold higher than the amount of drug permeated from alginate microparticles (about 60% vs 30%). Even higher values were obtained from the microparticles AL/CH I and II (70–80%). The differences between microparticles without chitosan (lower permeation values) and microparticles containing chitosan (higher values) can be related to the known penetration-enhancing properties of this polymer across mucosal barriers (Illum 2003).

Conclusions

The spray-drying technique offers numerous advantages compared with other methods of microencapsulation. Spray-dried microspheres are characterized by high encapsulation efficiency but relatively low production yields. Microparticles based on a combination of chitosan and alginate swell quickly in the phosphate buffer, maintain their increased size and show good in-vitro mucoadhesive properties. Moreover formulations containing both polymers show the best drug permeation behaviour across ex-vivo mucosa that could be due to the effect on the widening of tight junctions. These preliminary results show that microspheres based on a combination of the two polymers may be proposed as a mucoadhesive nasal delivery system for the administration of metoclopramide.

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