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Mucoadhesive and penetration enhancement properties of three grades of hyaluronic acid using porcine buccal and vaginal tissue, Caco-2 cell lines, and rat jejunum

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Abstract

The influence of the molecular weight on mucoadhesive and penetration enhancement properties of three grades of hyaluronic acid (1878, 693 and 202 kDa) has been evaluated. The mucoadhesive properties were investigated using buccal and vaginal porcine mucosa by means of a tensile stress method and using rat jejunum by means of an inclined plane method. The mucoadhesive performances observed using animal tissues were compared with the mucoadhesive properties observed using submaxillary or gastric mucin dispersions. The penetration enhancement properties were investigated using porcine buccal epithelium membrane or vaginal tissue and a cell monolayer (Caco-2 cell line). Chitosan hydrochloride, already described as a penetration enhancer towards buccal and vaginal mucosae and Caco-2 cell monolayers, was used as reference. Aciclovir (acyclovir), a poorly soluble and absorbable drug, commonly used in the treatment of *Herpes simplex* virus (type I and II), was used as the model drug. Unlike chitosan hydrochloride, which does not show any mucoadhesive potential at pH close to neutrality (buccal and intestinal), all hyaluronic acid grades show mucoadhesive properties in all the environments considered (buccal, vaginal and intestinal). In all cases, a decrease in molecular weight of hyaluronic acid produced an increase in the mucoadhesive performance. The hyaluronic acid with the lowest molecular weight (202 kD) exhibited the best penetration enhancement properties, that, depending on the substrate under consideration, was either comparable with or even better than chitosan hydrochloride. Therefore, this grade would be the most promising for buccal, vaginal and intestinal delivery of aciclovir.

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Introduction

Hyaluronic acid is a naturally occurring linear glycosaminoglycan, biocompatible, non-immunogenic and biodegradable by host enzymatic degradation as well as the other glycosaminoglycans. Hyaluronic acid is widely distributed in the extracellular matrix of connective tissues. It is present in synovial fluid and in the aqueous and vitreous humour of the eye, where it acts as a lubricant and/or shock absorbing fluid. It is proposed that hyaluronic acid, the predominant glycosaminoglycan found in wounds, performs a regulatory as well as a structural function in tissue reconstruction following injury. Several lines of evidence have suggested that hyaluronic acid is able to modulate fibroblast proliferation and inflammatory response and presents good tolerability profiles (Goa & Benfield 1994).

Hyaluronic acid is characterized by good mucoadhesive properties in the presence of rat small intestine, even better than that of carbomer (carbopol) (Pritchard et al 1996), and hyaluronic acid prolonged formulation permanence in the conjunctiva (Saettone et al 1989). In particular, the abundance of COOH groups promotes the adhesion through hydrogen bond formation with biological substrates (Pritchard et al 1996). Some authors observed an increase in pilocarpine and tropicamide in the presence of hyaluronic acid after ophthalmic administration (Saettone at al 1989, 1991). The increase in residence time of the formulation on the absorption mucosa, due to

hyaluronic acid mucoadhesive properties, produced an intimate contact between drug and mucosa and could be the reason for the increase in drug bioavailability.

Moreover, hyaluronic acid has been demonstrated to possess penetration enhancement properties toward nasal mucosa. Some authors demonstrated that hyaluronic acid could enhance the absorption of insulin (Rydén & Edman 1992) and of vasopressin or its analogue (Morimoto et al 1991) after nasal administration.

Chitosan, a high molecular weight cationic polysaccharide, has gained increasing interest in the pharmaceutical field due to its good biocompatibility, low toxicity and biodegradability (Ravi Kumar 2000). For its good mucoadhesive properties (Lehr et al 1992; Henriksen et al 1996), chitosan can be used in mucosal site-specific systems (Sinswat & Tengamnuay 2003). Moreover chitosan has been shown to be a potential penetration enhancer towards intestinal, nasal, buccal and vaginal mucosa (Artursson et al 1994; Dodane et al 1999; Senel et al 2000; Rossi et al 2003a, b; Sinswat & Tengamnuay 2003; Sandri et al 2004). However, the use of chitosan as a penetration enhancer is impaired by its low solubility at pH above 6 (for example at the physiological pH of the buccal cavity) and incompatibility with anionic drugs.

To develop therapeutic mucoadhesive systems for the transmucosal administration of drugs, it would be interesting to study the mucoadhesive and absorption enhancement properties of different hyaluronic acid grades that might be able to overcome the disadvantages of chitosan, combining its tissue repairing action. Given this premise, the aim of the study was to evaluate the influence of molecular weight on the mucoadhesive and penetration enhancement properties of hyaluronic acid. The mucoadhesive properties were investigated using buccal and vaginal porcine mucosa, by means of the tensile stress method, and rat jejunum, by means of the inclined plane method. The mucoadhesive performances observed using animal tissues were compared with the mucoadhesive properties observed using submaxillary or gastric mucin dispersions. The penetration enhancement properties of aciclovir, an antiviral drug poorly soluble and absorbable (belonging to class IV of the Biopharmaceutical Classification System (Lindenberg & Dressman 2004)), were investigated using different types of epithelia: porcine buccal epithelium membrane, vaginal tissue, and a cell monolayer (Caco-2 cell line). In particular buccal and vaginal mucosae, characterized by a pluristratified epithelium lacking in tight junctions, and Caco-2 cell monolayers, endowed by tight junctions, were considered.

Chitosan hydrochloride, already described as a penetration enhancer towards buccal and vaginal mucosae and Caco-2 cell monolayers, was used as reference.

Materials and Methods

Sample preparation

Three grades of hyaluronic acid sodium salts (HA Bio-tech, HA1, MW 1878 kDa; Hyalectin, HA2, MW 693 kDa; Hyalastine, HA3, MW 202 kDa) (kindly donated by Fidia SpA, Italy) were hydrated in pH 6.4 phosphate buffer (USP 25). Chitosan hydrochloride (Seacure Cl313, Pronova, Norway) (viscosity of a 1% w/w water solution: 296 mPa s at 25°C; DA: 16%) was hydrated in distilled water instead of pH 6.4 buffer, due to the poor solubility properties at pH close to neutrality, to simulate the buccal environment. All the polymers were hydrated in pH 5.0 acetate buffer (BP 2002), to mimic the vaginal environment. The polymer solutions were prepared at a concentration equal to 4% w/w by gentle stirring at room temperature. Aciclovir (Sintopharm, Italy) was suspended in each polymer solution at 5% w/w concentration. Aciclovir was used instead of the more soluble aciclovir sodium salt because of the pH range of stability of the sodium salt. In fact the sodium salt was stable in an environment characterized by pH value higher than its pK_a value (9.2), not compatible with transmucosal administration. Moreover, chitosan hydrochloride was not soluble at pH values close to and higher than 7.

All the polymers were hydrated in Hank's balanced salt solution (HBSS: $CaCl_2$ anhydrous 140 mg L^{-1}, $MgCl_2$ 6 H_2O 100 mg L^{-1}, $MgSO_4$ 7 H_2O 100 mg L^{-1}, KCl 400 mg L^{-1}, KH_2PO_4 60 mg L^{-1}, $NaHCO_3$ 350 mg L^{-1}, NaCl 8000 mg L^{-1}, Na_2HPO_4 48 mg L^{-1}, D-glucose 1000 mg L^{-1}, Red Phenol 10 mg L^{-1}, Gibco BRL, NY) buffered at pH 5.5 with HCl 1 M, to assess the polymer properties in the intestinal environment. The polymer solutions were prepared at 0.4% w/v concentration. Aciclovir was dissolved into each polymer solution at 1 mM.

Particle size characterization

The samples, containing the drug suspension at 5% w/w, were characterized for aciclovir particle size and size distribution by means of a Coulter Counter (Coulter Multisizer II, Beckman Coulter, UK); each sample (with and without polymers) (10 mg) was dispersed in a prefiltered aciclovir-saturated physiologic solution (NaCl 0.9% w/v) according to the procedure of the apparatus. Each sample was dispersed by means of an ultrasound apparatus (Julabo USR8, Julabo Labortechnik GMBH, Seelbach, G) for 5 min to disperse the sample and two subsequent measures were performed for each sample using a tube with a 70-μm orifice.

Mucoadhesion measurements by means of a tensile-stress tester

The mucoadhesive properties of the polymer solutions were evaluated by means of a tensile-stress tester (Ferrari et al 1996). Porcine cheek buccal mucosa and submaxillary bovine mucin (Sigma, Milan, I) were employed as biological substrates to mimic the buccal environment. Porcine vaginal mucosa and gastric porcine mucin (Sigma, Milan, I) were used as biological substrates to simulate vaginal application.

Connective tissue from porcine cheek and vaginal mucosae (obtained from a slaughterhouse) was removed with surgical scissors and the mucosae were stored at −20°C before testing (Park & Munday 2002).

The submaxillary and gastric mucins were dispersed at a concentration of 4% (w/w) in pH 6.4 and pH 5.0 buffers, respectively. Each polymer solution (100 mg) was layered on a filter paper disc (area = 2 cm^2) and fixed on the movable carriage of the apparatus. The cheek or the vaginal mucosa was fixed, faced to the formulation, on the sample holder using cyanoacrylate glue and hydrated with pH 6.4 or 5.0 buffer (100 μL), respectively. The carriage was then moved until contact between the sample holder and the movable carriage was established. A preload of 2500 mN was applied to allow the formation of the mucoadhesive joints. After a 3-min rest, the preload was removed and the movable carriage was moved forward at a constant speed (4 mm min^{-1}) up to the complete separation of the two surfaces. The experimental conditions (preload and contact time) were chosen as the minimum force and time that allow the maximization of the mucoadhesive parameters and to obtain reproducible results. Both displacement of the movable carriage and force of detachment data were recorded and simultaneously collected on a personal computer.

The parameter work of adhesion (AUC) was calculated as the area under the force of detachment vs displacement curve by means of the trapezoidal rule (Ferrari et al 1996).

The submaxillary or gastric mucin dispersions (100 μL) were spread on a filter-paper disc (area = 2 cm^2). The mucoadhesion measurements in the presence of mucin were carried out under the same conditions used for measurements effected in the presence of mucosa. Blank measurements were performed using a filter-paper disc wetted with pH 6.4 or 5.0 acetate buffer (100 μL) instead of the biological substrate (mucosa or mucin dispersion). Such measurements aimed to evaluate the contribution of the cohesion of each sample to the mucoadhesive potential.

The normalized work of adhesion (ΔAUC/AUC) was calculated according to the following equation (Ferrari et al 1997):

$$\Delta AUC/AUC = (AUC_{bs} - AUC_{blank})/AUC_{blank}$$

where AUC_{bs} = AUC obtained in presence of the biological substrate (mucosa or mucin dispersion); AUC_{blank} = AUC obtained from blank measurements.

Such a normalization allowed the comparison of the mucoadhesive properties of the polymer solutions characterized by different consistency and consequently by different cohesive properties (Ferrari et al 1997).

Mucoadhesion measurements by means of the 'inclined plane' apparatus

The mucoadhesive properties of the polymer solutions prepared in pH 5.5 HBSS were assessed by means of the 'inclined plane' apparatus. The apparatus consisted of an inclined plane (angle of inclination = 60°, surface area = 28 cm^2) thermostated at 37°C and of an electronic microbalance (Sartorius L420P, G) connected with a personal computer (Rossi et al 2001). Porcine gastric mucin (Sigma, I) or rat jejunum were used as biological substrates. Mucin films were prepared directly on the plane: 2.5 mL 8% w/w HBSS pH 5.5 mucin dispersion was placed on the plane, which was kept horizontal at 45°C for 45 min. The polymer solutions (250 mg) were placed on the plane previously coated with the biological substrate and kept horizontal. The plane was then inclined and the amount of formulation dropped on the microbalance was recorded as a function of time.

Blank measurements were performed using the inclined plane without the biological substrate. Such measurements aimed to evaluate the contribution of the rheology of each sample to the mucoadhesive potential.

The parameter maximum amount of formulation remained on the inclined plane (A) at the end of the experiment was calculated as follows:

$$A = (S_{to} - S_t)/S_{to}$$

Where S_{to} = sample amount loaded on the inclined plane; S_t = sample amount fallen down on the microbalance at the end of the experiment.

The normalized parameter (ΔA/A) was calculated as follows:

$$\Delta A/A = (A_{bs} - A_{blank})/A_{blank}$$

where A_{bs} = A observed in the experiment effected with biological substrate; A_{blank} = A observed in the experiment effected without biological substrate.

Permeation measurements through porcine cheek epithelium

The polymer solutions, prepared in pH 6.4 buffer (buccal medium), were subjected to permeation measurement by means of Franz diffusion cells (FDC40020FF, Crown Bio Scientific Inc., New Jersey) thermostated at 37°C (Sandri et al 2004). Fresh porcine cheek mucosa was dipped for 1 min in pH 7.4 saline isotonic solution (KH_2PO_4 1.90 g L^{-1}, Na_2HPO_4 8.10 g L^{-1}, NaCl 4.11 g L^{-1}) at 70°C. The epithelium was peeled from the mucosa (Ganem-Quintanar et al 2000). The polymer solutions (100 mg) were applied on the epithelium membrane. Buffer (pH 6.4, 2 mL) was added over the sample in the donor chamber, to simulate the buccal environment, whereas pH 7.4 saline isotonic solution was used as acceptor phase (sink conditions in the acceptor phase achieved).

At fixed time intervals, 500 μL acceptor phase was withdrawn and replaced with fresh buffer. The drug was assayed by means of an HPLC method (Pramar et al 1990).

The permeation test was carried out also using 2 mL 0.25% w/w aciclovir suspension prepared in pH 6.4 buffer.

Penetration measurement into porcine vaginal mucosa

The polymer solutions prepared in pH 5.0 buffer (vaginal medium) were subjected to penetration measurements into porcine vaginal mucosa by means of a Franz diffusion cell (Sandri et al 2004). The pig vagina was isolated from the peritoneum by means of surgical scissors to obtain intact vaginal tissue. The polymer solutions (100 mg) were applied

on intact vaginal tissue, then pH 5.0 buffer (2 mL) was added in the donor chamber to mimic the vaginal environment. The acceptor chamber was filled with pH 7.4 saline isotonic solution to maintain the mucosa hydrated and thermostated. At the end of the experiment (6 h) the sample was removed from the mucosa. The mucosa was rinsed twice with pH 7.4 saline isotonic solution and stored at −20°C. Horizontal slices 40-μm thick were cut at −20°C by means of a cryostat (Leica CM1510, Leica Microsystem S.p.A., Italy) and collected in Eppendorf microtubes (five slices for each microtube) (Volpato et al 1997).

The drug that had penetrated the slices was extracted with 500 μL distilled water at 60°C for 15 min (during this time the microtubes were vortexed twice for 10 s). After the cooling, 500 μL 1 M $HClO_4$ was added to precipitate the residual proteins and the mixture was centrifuged at 5000 g for 10 min (Volpato et al 1998). The drug extracted was assayed by means of an HPLC method (Pramar et al 1990).

The permeation test was carried out also using 2 mL 0.25% w/w aciclovir suspension prepared in pH 5.0 buffer.

Release measurements

In-vitro drug release measurements were assessed in the same experimental conditions used for drug permeation and penetration measurements. The samples (100 mg) were placed on dialysis membrane (cut-off 12–14 kDa) used instead of mucosa. After 6 h the drug released was assayed by means of an HPLC method (Pramar et al 1990).

The aciclovir aqueous suspensions were subjected to the same experiment. To take into account the different release properties of the polymer solutions, drug amount permeated or penetrated was normalized with respect to the drug amount released at the same time (6 h). The value obtained was expressed as percentage (% drug permeated/released for the buccal samples and % drug penetrated/released for the vaginal samples).

Permeability studies performed by means of Caco-2 cell monolayer

The polymer solutions prepared in pH 5.5 HBSS were subjected to a permeability test across Caco-2 cell monolayers.

Caco-2 cells (TC7) (passage 37) were seeded on tissue-culture-treated polycarbonate filters (area = 1 cm^2) in Costar Snapwell 6 plates (Costar Corning, NY) at a seeding density of 2.5×10^5 cells cm^{-2}. Dulbecco's modified Eagle's medium (DMEM, pH 7.40; Gibco BRL, NY) supplemented with 1% non-essential amino acids, 10% foetal bovine serum, benzylpenicillin G (160 U mL^{-1}) and streptomycin sulfate (100 μg mL^{-1}) (Gibco BRL, NY) was used as culture medium. Cell cultures were kept at 37°C in an atmosphere of 95% air and 5% CO_2 and 95% relative humidity. Filters were used for trans-epithelial electrical resistance (TEER) measurements and transport experiments 21–23 days after seeding. The filters divided the Grass-Sweetana diffusion chambers in two compartments in which a CO_2 (5%) and O_2 (95%) gas mixture was fluxed. The polymer solutions (5 mL) were used as the donor phase and were added on the apical side of the monolayers. HBSS at pH 7.4 (5 mL) was used as the receptor phase and added on the basolateral side of the monolayers. Samples of 300 μL were withdrawn at a fixed time from the apical and the basolateral phases. The apparent permeability coefficient (P_{app}) was calculated according to the following equation (Kotzé et al 1998):

$$P_{app} = dQ/dt/(A \cdot 60 \cdot C_0)$$

Where dQ/dt = permeability rate (drug amount permeated per min); A = diffusion area of the monolayer; C_0 = initial aciclovir concentration.

During the experiments the integrity of the monolayers was assessed by means of TEER measurements at fixed time using a Millicell ERS-meter (Millipore Corp., Bedford, MA). At the end of the permeability test (after 2 h of contact with the polymer solutions) the viability of the Caco-2 cell monolayers was established by means of the Neutral Red test. The cell monolayers were grown in Costar 96 plates (Costar Corning, NY) and were incubated apically with a 0.4% w/v polymer solution in pH 5.5 HBSS containing 0.2 mg mL^{-1} Neutral Red (Sigma, Italy) for 2 h. At the end of the experiment, the monolayer was rinsed twice with phosphate-buffered saline. The monolayer was treated with an aqueous mixture of ethanol (50% w/v) and $NH_4H_2CO_3$ (5 mM) to extract the retained dye in the viable cells. The dye extracted was assayed by means of a spectrophotometric method at 450 nm. A sample without polymer was used as control (S). The parameter % viability was calculated as the ratio % between the amount of Neutral Red extracted from monolayer treated with respect to the control (S).

Statistical evaluation

Statistical differences were determined using one-way analysis of variance and post-hoc Sheffe test for multiple comparisons (Siphar, Creteil, France). Differences between groups were considered to be significant at $P < 0.05$.

Results and Discussion

Particle size characterization

Table 1 shows the statistical mean surface weighted diameter (d_{vs}), d50% and d90% for each sample. The samples containing polymers did not show a marked difference in particle size and size distribution, while slight differences were observed for the drug suspension without polymers.

Mucoadhesion properties by means of tensile-stress tester

Figure 1 shows the results of the mucoadhesion measurements, expressed by the normalized parameter $\Delta AUC/AUC$, obtained in the buccal environment (porcine buccal

Table 1 The d_{vs}, d50% and d90% values calculated for each sample

	d_{vs} (μm)	d50% (μm)	d90% (μm)
Chitosan hydrochloride	5.40 ± 0.22	6.30 ± 0.17	11.96 ± 0.58
HA1 (MW 1878 kDa)	5.26 ± 0.08	5.82 ± 0.03	10.50 ± 0.78
HA2 (MW 693 kDa)	5.16 ± 0.17	5.83 ± 0.12	10.57 ± 0.23
HA3 (MW 202 kDa)	5.31 ± 0.09	5.86 ± 0.08	10.02 ± 0.18
Suspension	6.02 ± 0.38	6.86 ± 0.01	11.51 ± 0.58

Values are mean ± s.d.; n = 4.

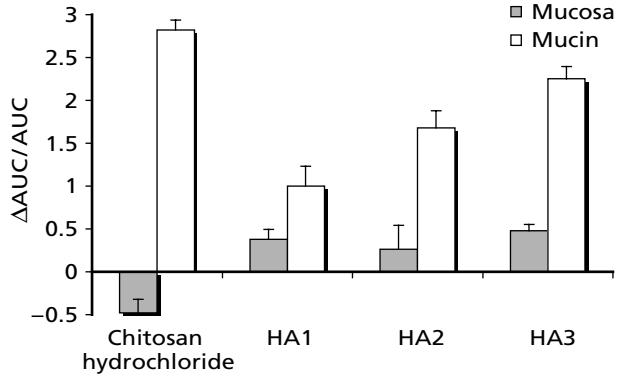

Figure 1 ΔAUC/AUC values calculated for all the polymer solutions in presence of porcine buccal mucosa and of submaxillary bovine mucin dispersion (mean values ± s.e.; n = 8).

mucosa and bovine submaxillary mucin dispersion) for all the polymers tested. In the presence of mucosa, the three grades of hyaluronic acid presented mucoadhesive properties that were superimposable, higher than those observed for chitosan hydrochloride (the reference polymer). In the presence of mucin, chitosan hydrochloride showed the best mucoadhesive potential while the three hyaluronic acid grades showed properties that increased on decreasing molecular weight.

Figure 2 shows the results of the mucoadhesive measurements, expressed by the normalized parameter ΔAUC/AUC, obtained in the vaginal environment (porcine vaginal mucosa and gastric mucin dispersion) for all the polymers tested. Among the three grades of hyaluronic acid, HA3 (MW 202 kDa) showed the best mucoadhesive properties, significantly higher than those observed for chitosan hydrochloride ($P < 0.05$).

Only in the buccal environment were significant differences observed when mucin or mucosa were used ($P < 0.05$). This could have been due to the higher buffering capability of the mucosa that caused a lower concentration of free hyaluronic acid at the mucoadhesive interface. The free hyaluronic acid is mainly responsible for the mucoadhesive joint (forming hydrogen bonds with the biological substrate). For the same reason all the hyaluronic

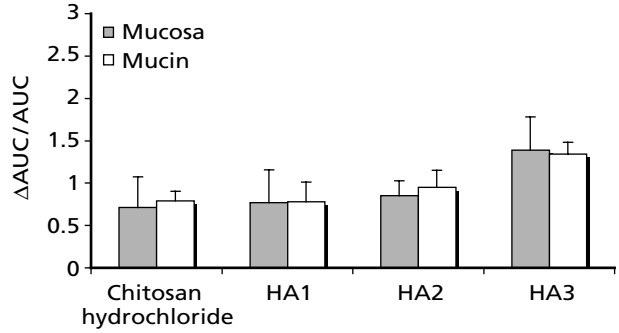

Figure 2 ΔAUC/AUC values calculated for all the polymer solutions in presence of porcine vaginal mucosa and of gastric porcine mucin dispersion (mean values ± s.e.; n = 8).

acid grades showed better mucoadhesive properties in the buccal environment.

The negative ΔAUC/AUC value observed for chitosan hydrochloride in the buccal environment was again due to the higher buffer capability of the mucosa which had an effect on chitosan hydrochloride solubility.

Mucoadhesion properties by means of 'inclined plane' apparatus

In Figure 3 the values of the normalized parameter ΔA/A, calculated for each polymer solution in the presence of the rat jejunum or porcine gastric mucin film, are reported. For hyaluronic acid solutions, the ΔA/A values, calculated in the presence of the two biological substrates, were not significantly different. The decrease in the molecular weight of hyaluronic acid produced an increase in mucoadhesive properties. Chitosan hydrochloride, the reference polymer, presented the lowest ΔA/A values ($P < 0.05$) and showed a different behaviour depending on the biological substrate. In particular in the presence of rat jejunum, the value of the mucoadhesive parameter ΔA/A was negative to indicate a poor interaction of the polymer with the biological substrate. This was probably due to the higher buffering

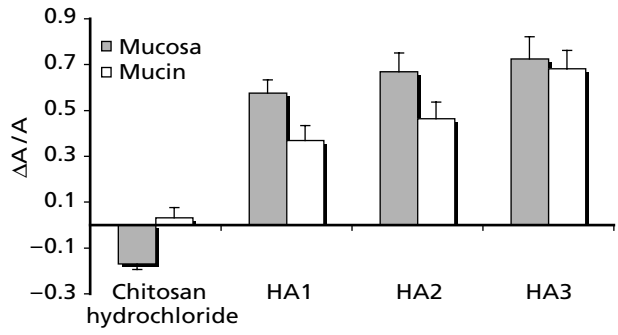

Figure 3 ΔA/A values calculated for all the polymer solutions in presence of rat jejunum and gastric porcine film (mean values ± s.e.; n = 8).

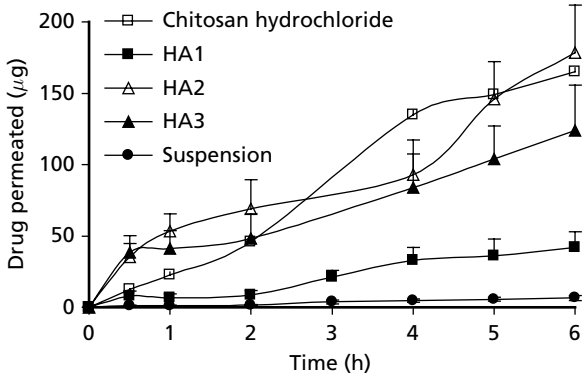

Figure 4 Drug amount (μg) permeated across buccal epithelium vs time profiles observed for all the polymer solutions and the aciclovir suspension (mean values ± s.e.; n = 6).

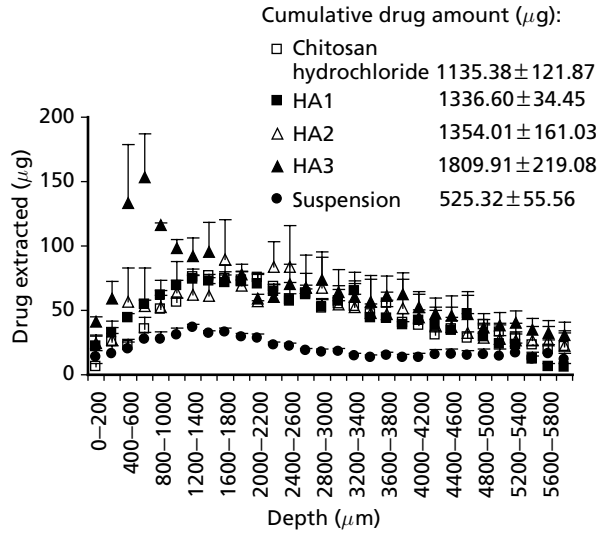

Figure 5 Drug amount (μg) penetrated into vaginal tissue vs distance from the mucosa surface (depth μm) observed for all the polymer solutions and the aciclovir suspension (mean values ± s.e.; n = 6). In the inset the cumulative drug amount (μg) in whole tissue is reported (mean values ± s.e.; n = 6).

properties of the intestinal mucosa with respect to the mucin film. Such capability would be likely to cause a chitosan hydrochloride precipitation at the polymer solution/mucosa interface, which would weaken the mucoadhesive joint.

Permeation measurements through porcine cheek epithelium

In Figure 4 the drug permeation profiles through porcine cheek epithelium obtained for all the polymer solutions and an aciclovir aqueous suspension are reported as a function of time. Chitosan hydrochloride, HA3 and HA2 showed permeation profiles not significantly different and higher than that of HA1 ($P < 0.05$). All the polymer solutions presented permeation profiles higher than that of the aciclovir suspension, indicating penetration enhancement properties ($P < 0.05$).

Penetration measurement into porcine vaginal mucosa

In Figure 5 the drug penetration profiles into porcine vaginal intact tissue obtained for all the polymer solutions and an aciclovir aqueous suspension are reported as a function of the distance from the mucosa surface (depth). All the samples displayed the same trend in drug distribution into the tissue: the drug amount increased with depth up to a maximum reached at a depth of 600–1400 μm. This behaviour was due probably to the presence of deep invaginations on the mucosal surface: the superficial slices were characterized by a lower surface area than that of the deeper slices and consequently the amount of drug penetrated was lower than that penetrated into the deeper ones. HA3 was characterized by the highest penetration profile (in the range 400–1000 μm) ($P < 0.05$). All the polymer solutions presented drug distribution profiles higher than that of the aciclovir suspension ($P < 0.05$).

The inset of Figure 5 shows the cumulative amounts of drug penetrated into the vaginal tissue. HA3 showed the highest cumulative drug amount penetrated into the intact vaginal tissue, followed by HA2 and HA1. All the hyaluronic acid grades showed cumulative values higher than chitosan hydrochloride, the HA2 and HA3 values being significantly different ($P < 0.001$). The suspension showed the lowest value ($P < 0.001$).

Permeation or penetration properties: normalized parameter

In Figure 6 the values of the normalized parameters, % of drug permeated/released (buccal environment) and % of drug penetrated/released (vaginal environment), calculated for all the polymer solutions and the aciclovir suspensions, are reported. Such normalization allows the comparison of all the polymer solutions characterized by different release properties on a homogeneous basis. In fact the normalized parameters allowed us to consider the drug that is really available at the sample/mucosa interface ready to permeate across the buccal mucosa or to penetrate into the vaginal tissue.

In the buccal environment, HA3 showed the highest value of the normalized parameter, % drug permeated/released, followed by chitosan hydrochloride and HA2 (values not significantly different) and HA1. All the polymer solutions showed values higher than the suspension ($P < 0.001$).

In the vaginal environment, HA3 showed the highest value of the normalized parameter followed by HA2, HA1 and chitosan hydrochloride. All the polymer solutions presented values higher than the aciclovir suspension

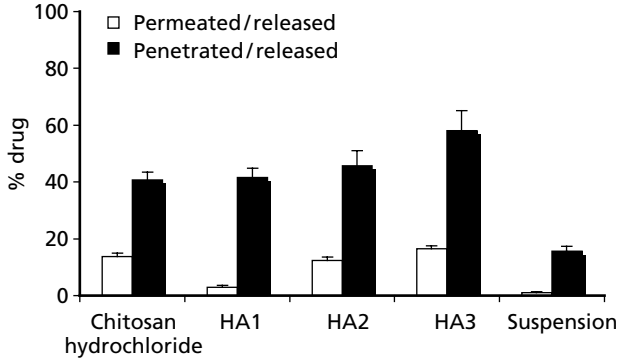

Figure 6 Values of the normalized parameter % drug permeated/released and % drug penetrated/released calculated for all the polymer solutions and the aciclovir suspensions in buccal and in vaginal environments (mean values ± s.e.; n = 6).

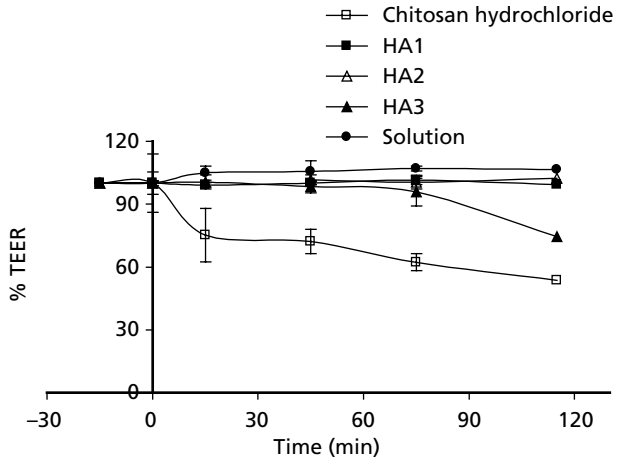

Figure 8 % TEER profiles observed for all the polymer solutions and the drug solution (mean values ± s.e.; n = 6).

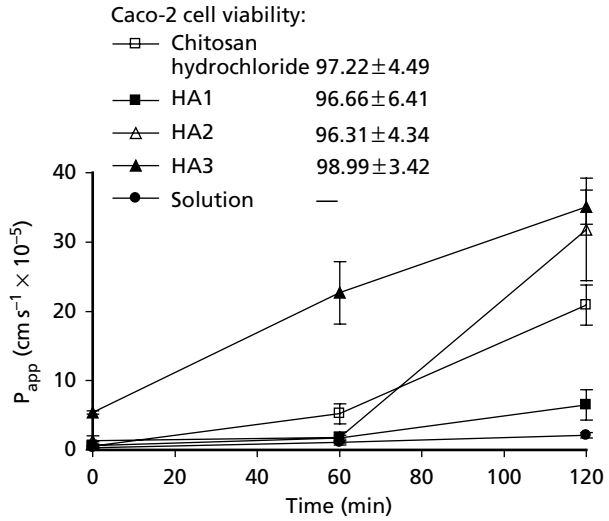

Figure 7 P_{app} (cm s^{-1}) vs time profiles for all the polymer solutions and the drug solution (mean values ± s.e.; n = 6). In the inset the Caco-2 cell viability values are reported (mean values ± s.e.; n = 6).

($P < 0.05$). Among the hyaluronic acid grades only HA3 showed penetration enhancement properties higher than those of chitosan hydrochloride ($P < 0.05$).

Permeability studies performed by means of Caco-2 cell monolayer

Figure 7 shows the P_{app} vs time profiles across the Caco-2 cell monolayers for the polymer and drug solutions. HA3 showed the highest P_{app} profile followed by HA2, chitosan hydrochloride and HA1. All three hyaluronic acid grades showed profiles higher than that of aciclovir solution ($P < 0.05$). The penetration enhancement properties were not accompanied by cellular toxicity (Figure 7 inset). This was supported by the cellular viability higher than 95% for all the polymer solutions evaluated.

Figure 8 shows the % TEER profiles observed before and during the experiment for the polymer solutions. For HA1, HA2 and the drug solution S (control), the % TEER profiles remained constant (close to 100%) during all the experiments. A significant decrease in % TEER profiles ($P < 0.001$) could be observed for HA3 and chitosan hydrochloride, which presented the lowest profiles ($P < 0.001$). The decrease in % TEER profiles was evidence that the opening of the tight junctions was due to polymer action.

Conclusions

In all the environments considered (buccal, vaginal and intestinal) a decrease in molecular weight produced an increase in mucoadhesive properties. Unlike chitosan hydrochloride, all hyaluronic acid grades were able to interact with buccal and intestinal mucosae. The results were in agreement with those obtained by Pritchard et al (1996), who investigated the mucoadhesive potential of hyaluronic acid towards the rat small intestine epithelium.

HA3 (MW 202 kDa) showed the best penetration enhancement properties towards the three biological substrates considered. HA3 mucoadhesive and penetration enhancement properties pointed to the occurrence of a deeper interaction between HA3 and the mucosal surfaces. The polymer chains probably presented an optimal length and tridimensional conformation to form the mucoadhesive joint and to interact with the epithelium cells. The intimate contact between polymer chains and epithelium cells consequently caused an improvement of the hyaluronic acid penetration enhancement properties. Such properties were even better than those observed for chitosan hydrochloride.

HA3, the low grade of hyaluronic acid, could be considered the most promising grade to enhance the aciclovir absorption through the buccal, vaginal and intestinal mucosae.

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