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Evaluation of mucoadhesive properties of hyaluronic acid benzyl esters

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Abstract

Mucoadhesive properties of hyaluronic acid (HA) benzyl esters were evaluated. The vertical tensile force of detachment of polymer films from porcine gastric mucosal surface was measured with a microdisplacement transducer. The HA esters, namely, HYAFF 11 p25 (25% benzyl ester, 75% sodium salt), HYAFF 11 p50 (50% benzyl ester, 50% sodium salt), HYAFF 11 p75 (75% benzyl ester, 25% sodium salt) and HYAFF 11 (100% benzyl ester), were compared to glass (negative control) and to two positive controls, polycarbophil (PC) and hydroxypropylmethylcellulose (HPMC). Measurements were made for polymer films hydrated in a pH 1.2 medium, in Tyrode solution (pH 7.8) and for unhydrated films. When hydrated at pH 1.2, the more hydrophilic polymers (HPMC, HYAFF 11 p25, HYAFF 11 p50 and PC) showed significantly greater adhesion (2720–4192 dyn/cm^2) than the negative control (1980 dyn/cm^2). When hydrated at pH 7.8, only HPMC (3801 dyn/cm^2) and HYAFF 11 p25 (3198 dyn/cm^2) showed greater adhesion than the negative control. PC adhesion was significantly lower than at pH 1.2. The ionized state of PC, as well as mucus glycoprotein residues at this pH (7.8), may cause charge repulsion and reduced adhesion. As expected, adhesion of HPMC which is nonionic, was unaffected by pH of the hydration medium. Adhesion of HYAFF 11 p25, and HYAFF 11 p50 was not significantly different at the higher pH. The adhesion of dry films was greater (4203–6388 dyn/cm^2) than that of hydrated films. Adhesion of the dry films may be a result of wetting of the films and dehydration of the mucous layer by the films. The study of initial contact time showed a time dependent increase in force of adhesion after an initial equilibration time. The results indicate potential applicability of the hydrophilic HA esters as mucoadhesive polymers.

Key words: Hyaluronic acid benzyl ester; Gastric mucosa; Tensile force measurement; Mucoadhesion

1. Introduction

Mucoadhesive drug delivery systems are expected to play an increasingly important role in the targeted delivery of a variety of drugs including novel peptide and protein molecules. The importance of such systems lies in the variety of routes of administration and flexibility in the choice of physical systems offered by the mucosal surfaces in the ocular, oral, nasal, gastrointestinal, vaginal and rectal regions of the human body (Duchêne et al., 1988; Jiménez-Castellanos et al.,

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1993). The effectiveness of a mucoadhesive formulation would be determined to the greatest extent by the nature of the polymer composition used. Diverse classes of polymers have been investigated for their potential use in mucoadhesive systems. These include synthetic polymers such as polycarbophil (Ch'ng et al., 1985), hydroxypropylmethylcellulose (Lejoyeux et al., 1989) and methacrylate derivatives (Leung et al., 1990) as well as naturally occurring polymers such as chitosan (Lehr et al., 1992a). Saettone et al. (1989, 1991) studied hyaluronic acid as a mucoadhesive vehicle for ocular drug delivery systems and observed increased residence time of various ophthalmic drugs in the eye. Such studies would seem to provide a basis for testing hyaluronic acid derivatives as potential mucoadhesive materials; these materials are under investigation in our laboratories for controlled release applications. In addition to any possible mucoadhesion, systems based on hyaluronic acid would provide the added advantage of being both biocompatible and biodegradable.

Various methods have been described in the literature for the measurement of mucoadhesive properties of polymers (Park et al., 1990). Most methods involve the use of purified mucin dispersions (Smart et al., 1984) or mammalian gastrointestinal tissue (Park et al., 1985) as substrates to measure a force of adhesion. A method based on tensile force measurement (Lehr et al., 1990) was used as the basis for the mucoadhesion test system described here. The objective of the study was to identify a suitable in vitro method to assess the mucoadhesive properties of a series of hyaluronic acid benzyl esters and compare them with known mucoadhesive polymers.

2. Materials and methods

2.1. Materials

Benzyl esters of hyaluronic acid, namely, HYAFF 11 p25 (25% benzyl ester and 75% sodium salt), HYAFF 11 p50 (50% benzyl ester and 50% sodium salt), HYAFF 11 p75 (75% benzyl ester and 25% sodium salt) and HYAFF 11 (100% benzyl ester), were provided by Fidia S.p.A. (Italy). The general structure of these esters is depicted in Fig. 1. Sodium hyaluronate, hydroxypropylmethyl cellulose and glucose were purchased from Sigma Chemical Co. (St. Louis, MO). Polycarbophil (Noveon AA1) was a gift from BF Goodrich Co. (Cleveland, OH). Calcium chloride, potassium chloride, monobasic sodium phosphate, sodium bicarbonate, sodium chloride and hydrochloric acid were purchased from Fisher Scientific (Fair Lawn, NJ). All reagents were of analytical grade. Fresh pig stomachs were procured from Stinson's Meat Processing Plant (Ottawa, KS).

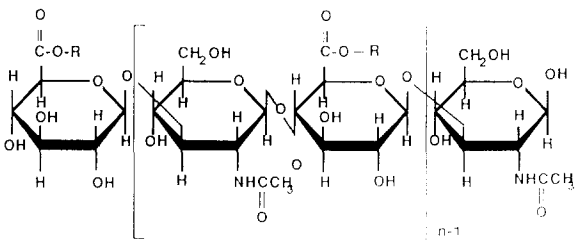

Fig. 1. General structure of hyaluronic acid benzyl esters. 'R' represents benzyl ester or sodium salt.

2.2. Instrumentation

The force detection system (Fig. 2) consists of a microdisplacement force transducer (A) (NARCO Bio-Systems® Myograph F-60) with a hook attachment, capable of detecting force in

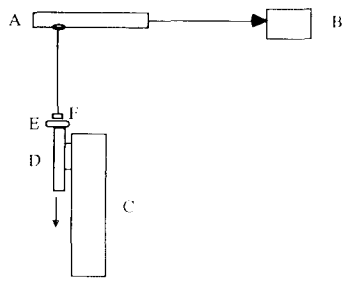

Fig. 2. Diagram of the apparatus used for measurement of mucoadhesive properties of polymer films. (A) Microdisplacement force transducer, (B) peak-capture reading meter, (C) infusion pump, (D) infusion pump impeller, (E) tissue holder and (F) sample holder.

terms of weight (g) exerted on it. The transducer is linked to a peak-capture reading meter (B) (Instrumentation Design Laboratory, The University of Kansas) capable of recording the force exerted on the transducer hook and capturing the peak force for any given measurement.

The tissue holder (E) consists of a 35 mm disposable polystyrene Petri dish which can be inserted into a clamp attached to the impeller (D) of a constant rate infusion pump (C). The pump is placed on its end so that in operation, the impeller moves the tissue holder vertically, downward and away from the transducer. Each measurement uses a new Petri dish.

The test film is stuck with Super glue® onto the flat surface of a phenolic resin testube cap (F) (15 mm diameter). A silk thread harness with a loop at the end is used to hang this cap from the transducer hook, and the cap placed on the tissue sample so that the film surface is in contact with the mucosal surface. After a predetermined contact time, the infusion pump is turned on and the impeller and the tissue holder move down until the contact between the film and tissue is broken. The force required for this event is displayed in 'grams' on the meter and can be converted into force units as follows:

Force of adhesion

$$= [\text{corrected reading (g)} \times \text{acceleration due to gravity (cm/s}^2)] / \text{surface area of the film (cm}^2)$$

Force of adhesion (dyn/cm^2)

$$= [\text{corrected reading (g)} \times 980.665] / 1.767$$

where corrected reading (g) = meter reading (g) − cap weight (1.6 g).

2.3. Tissue harvesting procedure

The test tissue selected was the fundus gland region of the porcine stomach (Sisson et al., 1961). The advantages of this tissue are the large number of samples that can be obtained from a single stomach and the thickness and sturdiness of the mucosal layer.

The stomach was collected in the slaughterhouse just as the animal was gutted, its contents were emptied, and the stomach inverted and thoroughly washed with cold normal saline containing glucose (1% w/v). It was then placed in cold oxygenated Tyrode solution and transported to the laboratory. The fundus region of the stomach was isolated and placed in fresh cold oxygenated Tyrode solution and the muscular layers stripped off using forceps and scissors. The remaining tissue, consisting of the mucosal layer and a thin layer of connective tissue, was cut into pieces approx. 1 inch^2 in area, placed in a container of fresh oxygenated Tyrode solution and stored at 4°C until use, which was always within 48 h of harvesting.

2.4. Film fabrication

Test films were prepared by dissolving 200 mg of the respective polymers in various combinations of HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) and water, poured into 60 mm glass Petri dishes. The solvent was allowed to evaporate completely in an airflow hood and the dry film peeled off and stored until use. Polycarbophil films were cast directly onto the sample holder (cap) from an aqueous-methanolic dispersion and allowed to dry overnight.

2.5. Mucoadhesion measurements

A piece of the test film was stuck to the cap surface, the Super glue® allowed to dry and the film trimmed to the edge of the cap. The caps were then placed in a preselected medium and allowed to hydrate for 1 h. A piece of mucosal tissue was taken from the Tyrode solution, the excess mucus shaken off from the surface and it was stuck on the bottom of the Petri dish with Super glue®. The Petri dish was fixed into the clamp on the infusion pump impeller. The cap harness was strung onto the transducer hook and the film surface gently placed on the mucosal surface for a contact time of 3 min. The pump was then turned on and the contact between the film and tissue allowed to break. The reading on

the meter was noted and used to calculate the force of adhesion.

Force of adhesion was measured for the hyaluronic acid esters (HYAFF 11, HYAFF 11 p75, HYAFF 11 p50 and HYAFF 11 p25), polycarbophil (PC), hydroxypropylmethylcellulose (HPMC), and sodium hyaluronate (HA). The films were tested in the unhydrated state and after hydration in an acidic medium (pH 1.2) and in Tyrode solution identical to that used for the tissue storage. Experiments were also performed to determine the effect of contact time between the film and tissue on the force of adhesion of selected polymers hydrated under acidic conditions.

2.6. Data analysis

Adhesion forces measured with different polymers were compared by ANOVA using the STATVIEW 4.0® software (Abacus Concepts, Berkeley, CA), applying the Fisher PLSD test at 95% confidence level.

3. Results and discussion

3.1. Effect of hydration on the force of adhesion

In order to determine the effect of hydration as well as the nature of the hydration medium on the force of adhesion, measurements were made using films hydrated in an acidic medium and in a neutral medium, as well as unhydrated films. The forces measured are summarized in Fig. 3. The hyaluronate benzyl esters were compared with a glass film as a negative control. Sodium hyaluronate (Na-HY), HPMC and polycarbophil (PC) were chosen as positive controls since they have been studied and established to have mucoadhesive properties (Saettone et al., 1989; Jiménez-Castellanos et al., 1993).

Upon hydration of polymer films in an acidic medium (Fig. 3), HPMC, PC, HYAFF 11 p25, HYAFF 11 p50 and HYAFF 11 p75 all showed significantly greater adhesion (2720–4192 dyn/cm^2) than the glass control (1979 dyn/cm^2) whereas the Na-HY and HYAFF 11 films did not. In this group, HPMC and PC showed greater adhesion than all other polymers. However, no significant differences were seen among the hyaluronate esters that showed significant adhesion. The relative forces of adhesion show a dependence on the hydrophilicity of the respective polymers, with the more hydrophilic polymers being more adhesive. The ionic nature of the polymers might also play a role in the adhesion since the hyaluronate esters and PC are expected to exist largely in an unionized state when hydrated at pH 1.2. The degree of esterification of the hyaluronic acid might affect the ionic character and the hydrophilicity of the polymer backbone in a similar fashion. These effects would be consistent with mechanisms based on hydration and swelling of polymers followed by interpenetration of polymer chains with those of the mucin-glycoprotein. On the other hand, Na-HY while being very hydrophilic is also very water soluble and the poor adhesion seen with its films might be due to a partial or complete breakdown or dissolution of the films themselves.

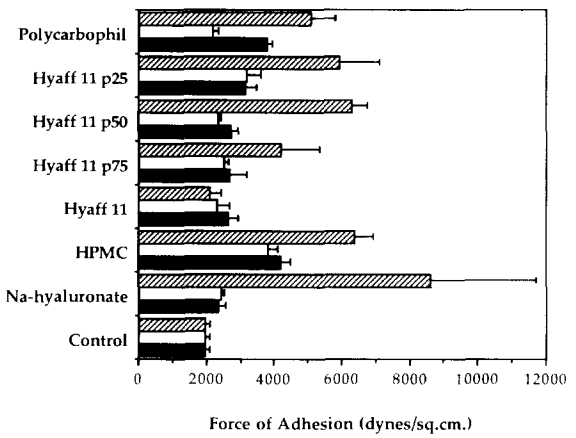

Fig. 3. Force of adhesion (dyn/cm^2) measured for polymer films, when unhydrated (hatched bars), hydrated at pH 1.2 (filled bars) and hydrated at pH 7.8 (blank bars). Means ± SE are shown (n = 3–6).

Upon hydration of the films at pH 7.8, adhesion of PC (2195 dyn/cm^2) was significantly reduced relative to that at pH 1.2 (3772 dyn/cm^2) and was not significantly different from the negative control. A similar effect was observed by Ch'ng et al. (1985). Above a pH of 7, PC with a

pK_a of approx. 4.75 would be almost fully ionized. Also groups such as the sialic acid residues ($pK_a = 2.6$) on the mucus glycoprotein backbone would be fully ionized. This could lead to a mutual charge repulsion upon mucus-PC contact. On the other hand, forces measured with HPMC, HYAFF 11 p25 and HYAFF 11 p50 films hydrated at pH 7.8 were not significantly different relative to those at pH 1.2. Adhesion of the more hydrophilic hyaluronate esters thus appears to be relatively independent of pH. Adhesion from the HYAFF 11 p75 and HYAFF 11 films was not significantly different from the negative control. Adhesion of Na-HY films was again relatively low and unchanged from that at pH 1.2. Visual observation of the film indicated at least partial solubilization of the film in the aqueous medium. Hence, no conclusions could be made regarding the mucoadhesive properties of Na-HY with respect to pH of hydration. However, a general observation can be made that the mucoadhesive properties of the hyaluronate esters depend on their hydrophilicity and state of hydration, with a general trend toward greater adhesion with greater hydrophilicity and hydration. This is consistent with theories proposed earlier to explain mucoadhesion on the basis of hydration and swelling of polymers followed by chain interpenetration with a hydrated mucus layer (Ponchel et al., 1987; Leung et al., 1990; Jabbari et al., 1993). A more hydrophilic polymer is expected to have a higher degree of swelling and chain mobility which in turn facilitates chain interpenetration and mucoadhesion.

Adhesion forces measured for unhydrated films (4203–6388 dyn/cm^2) were significantly greater than those for hydrated films. In almost every case the films were observed to have adherent globules of mucus following these measurements, indicating at least partial cohesive failure within the mucus layer. This is also an indication of the very high strength of mucoadhesive interaction of the unhydrated films. There were no significant differences in adhesion among PC, HPMC, HYAFF 11 p25, HYAFF 11 p50 and Na-HY. Also, unlike the hydrated states, Na-HY showed a very significant degree of adhesion when dry. This indicates that with an intact film, the hydrophilicity of Na-HY plays an important role in its mucoadhesion. The strong adhesion from the hydrophilic polymers relative to the hydrated films and the relatively weak adhesion from the more hydrophobic polymers HYAFF 11 and HYAFF 11 p75 indicate that the wettability of the films by the water in the mucus layer and the resulting dehydration of the mucus layer may have played an important role in promoting mucoadhesion. Similar results have been reported by Mortazavi and Smart (1993) for the dehydration of mucous gels by mucoadhesive polyacrylic acid formulations and the effects of such dehydration in strengthening mucoadhesive bonds.

The negative control, namely glass coated caps, resulted in a force much lower than any of the test polymers indicating that the system was capable of measuring polymer interactions with the mucous layer. However, the magnitude of the force obtained from the glass films indicates that an interaction does occur, even at the inert glass surface. This interaction may be attributed to wetting and surface tension effects between the glass and the mucous layer on the tissue and is supported by visual observation of the mucous layer during the measurements. This also supports in part our explanation of the high adhesion observed from unhydrated polymer films. The net force of adhesion measured for the test polymers would be expected to have a surface or interfacial

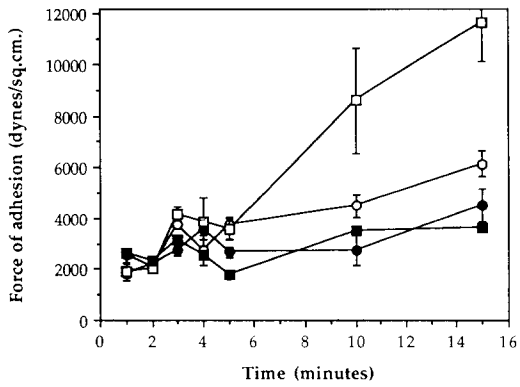

Fig. 4. Effect of contact time between the polymer film and mucosal layer on adhesion of polymer films hydrated at pH 1.2. Polycarbophil (○), HYAFF 11 p50 (●), HPMC (□) and HYAFF 11 p25 (■).

tension component. Interfacial interactions between polycarbophil and pig intestinal mucosa have been studied and found to play an important role in mucoadhesion (Lehr et al., 1992b). While the contribution from this component would play a major role in adhesion of dry films, it may be less significant when the polymer is hydrated prior to contact with the mucosal surface. Numerous versions of this 'wetting theory' proposed to explain mucoadhesive phenomena, have been documented in detail by Jiménez-Castellanos et al. (1993). The net force of adhesion would thus depend on the relative magnitudes of the interfacial and interpenetration interactions as determined by the hydration state of the polymer.

3.2. Effect of contact time on the force of adhesion

On the basis of results from the study of hydration states, HPMC, PC, HYAFF 11 p25 and HYAFF 11 p50 were selected to study the effect of the initial contact time between the film and the mucosal surface on the force of adhesion. These polymers yielded consistently high forces of adhesion under the different conditions of hydration. For the purpose of comparison, all the films were hydrated in a pH 1.2 medium. Contact times of 1, 2, 3, 4, 5, 10 and 15 min were studied; the results are depicted in Fig. 4. For all the polymers, there were few significant inter- or intra-polymer differences in the force of adhesion when the contact time was varied from 1 to 5 min. However, the forces following contact times of 10 and 15 min were significantly greater than with the shorter contact times. There seems to be an initial equilibration period in the development of mucoadhesive interactions. While the equilibration may not be exactly restricted to the first 5 min of contact, the trend observed in our study might suggest the possibility of two phases in the development of mucoadhesive bonds. Leung et al. (1990) observed an increase in adhesion with time of contact up to a maximum of 4 min. Their results were explained on the basis of an increase in the interpenetration between the polymer chains and the mucin network with time. The results from the contact time study further support the hypothesis that the initial phase may depend on wetting or interfacial interactions. In addition, the apparent later phase represented by the contact time data at 10 and 15 min might reflect polymer hydration and chain interpenetration as seen by Leung et al. (1990).

In conclusion, the hyaluronate benzyl esters appeared to adhere to gastric mucosal tissue under the current experimental conditions. The mucoadhesive properties were related to the hydrophilicity of the polymers, but were independent of the pH of hydration for the pH values studied. Unhydrated polymer films showed significantly stronger adhesion due to dehydration of the mucus and the resulting strong interfacial interactions. The study of contact time showed that mucoadhesion may be a kinetic process with initial interfacial interactions followed by a more extensive polymer chain interaction. The relative contributions of these two processes may depend on the initial extent of hydration of the polymer. The hyaluronate benzyl esters show potential applicability in systems for delivery of drugs across mucosal surfaces in the body.

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