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Report

Mucoadhesion of Hydroxypropylmethacrylate Nanoparticles to Rat Intestinal Ileal Segments *in Vitro*

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The purpose of this study was to evaluate the adhesion of HPMA nanoparticles to mucus using a perfused rat ileum test system. Radiolabeled nanoparticles were prepared and deposited onto rat ileal segments *in vitro*. The segments were perfused and the perfusate was collected in fractions and assayed for radioactivity. Between 10 and 50% of the radioactivity was eliminated over the first 120-sec perfusion, whereas the remaining activity was firmly attached to the ileum. Among the variables tested, the time interval between nanoparticle deposition and perfusion played the major role, indicating that the mucus–nanoparticle interaction is likely to result from the diffusion of polymers into the mucus and of mucin into the polymeric matrix.

KEY WORDS: nanoparticles; bioadhesion; mucoadhesion; controlled release.

INTRODUCTION

Several orally administered drugs such as digoxin and diphenylhydantoin show a limited bioavailability because their absorption from the gastrointestinal tract occurs only through a very small window, generally located in the duodenum. Since the transient passage of the alimentary bolus through the duodenum is rapid, poor absorption results, requiring frequent dosing. Conventional controlled-release formulations offer no solution to this problem since they also exhibit a short transit time through the duodenum. For these reasons, it has been proposed that these drugs should be included in a controlled-release formulation characterized by a prolonged residence time in the upper gastrointestinal tract (1). Among the systems able to achieve such a goal, the mucoadhesive polymers are promising (2).

Polyhydroxypropylmethacrylate (HPMA) nanoparticles were developed initially as a drug-targeting system for the improvement of the therapeutic index of cytostatic drugs (3). Nanoparticles are easily prepared (4) and show a high specific surface area and a good capacity for adsorbing drugs (5). We report the results of an *in vitro* study of the bioadhesive properties of nanoparticles as a function of different variables including contact time between the particles and the mucus and the density, osmolarity, and flow rate of the perfusion medium.

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METHODS

HPMA nanoparticles were obtained by addition of the monomer (Aldrich Chemical Co., Milwaukee, WI) at 5% to an aqueous solution of poloxamer 188 0.001% (Pluronic F-68, BASF Canada, Inc., Montreal, Quebec, Canada) maintained at 90°C. A chemical initiator of radical polymerization was then added at 0.1% (potassium persulfate, Aldrich Chemical Co, Milwaukee, WI), and the reaction was carried out over 1 hr, after which the temperature was allowed to come back to normal.

In order to label the particles tritiated estradiol (2,4,6,7-^{3}H-estradiol, 3.77 TBq/mmol, Amersham International, Oakville, Ontario, Canada) freeze-dried and redissolved in dioxane, was added to the different polymerization media prior to nanoparticle formation at a concentration of 10 MBq/ml. The efficiency and irreversibility of the entrapment of this radiolabel were checked as follows. Samples of nanoparticles were diluted 10^2- to 10^6-fold in both 5% glucose and 0.9% NaCl and left for 24 hr at 37°C. These samples and aliquots of undiluted nanoparticle preparations were then centrifuged at 50,000*g* for 2 hr, the radioactivity was determined in the supernatant, and the sediment was dissolved in dimethylformamide or tetrahydrofurane. The percentage of free drug ranged from less than 1 to 5%, respectively, for the least and the most diluted suspensions.

Particle size was determined using a N4SD (Coulter Electronics, Ltd, Hialeah, FL), and the zeta potential of the particles was measured with a Malvern Zetasizer 2C instrument, equipped with a tubular cell of about 2.6-mm i.d.

Mucoadhesion of the nanoparticles was evaluated using a technique similar to that of Ho and Chan (6). Segments of male Sprague–Dawley rat ileum 8 cm long were removed under diethyl ether anesthesia and cut lengthwise. The seg-

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ments were secured on a silanized neutral glass tube (0.75-in. i.d., cut lengthwise) and inclined at an angle of 12°. The intestine was first rinsed by 2-min perfusion with a physiological solution (0.9% NaCl or 5% glucose). Perfusion was then interrupted and a sample of nanoparticles (5 or 10 μl) was deposited on the upper part of the ileum segment. The perfusion was restarted at 0 or 10 min following the application of nanoparticles and fractions of the perfusate were collected every 60 or 120 sec. A constant perfusion flow of 0.5 or 0.25 ml/min was obtained using an HPLC pump (Model 302, Gilson, Villiers-le-Bel, France). The whole system and the perfusion solution were maintained at 37°C throughout the experiment.

The radioactivity present in the fractions was determined in a beta counter (RackBecta, LKB Wallac, Turku, Finland) after the addition of 10 ml scintillation cocktail (Scintiverse, Fisher Scientific, Fair Lawn, NJ). The counter uses an external source as a reference, and an efficiency-vs-channel ratio curve was determined before the experiments. Radioactivity in the fractions was expressed as a function of the initial dose calculated from triplicate samples taken out before transfer to the intestinal segment. In order to check the recovery, the radioactivity remaining in the intestinal segments was evaluated after dissolution in 2 ml Soluene-100 (Packard Instruments Co., Downers Grove, IL) and reaction with 0.2 ml oxygen peroxide. A control experiment was performed using silanized glass instead of mucus. Under these conditions all preparations of nanoparticles were recovered entirely in the first fraction (corresponding to a 60-sec perfusion). All experiments were done in triplicate.

RESULTS AND DISCUSSION

The diameter of the nanoparticles was 250 ± 65 nm and their charge was negative, with a zeta potential of approximately −50 mV.

Figure 1 shows the cumulative elution of radioactivity obtained with a 0.25-ml/min perfusion. The variables were volume of nanoparticles suspension deposited on the mucus and time interval between deposition and beginning of the perfusion. The deposited volume showed very little influence on the elution profile. On the contrary, allowing a 10-min interval before the perfusion dramatically increased the adhesion of the particles to the mucus. Furthermore, it is obvious that most of the particles eliminated by the perfusion were recovered in the early fractions, whereas the fractions collected after 6 min of perfusion contained a very low amount of radioactivity. This shows that the fraction of nanoparticles which were not carried away from the intestine at the beginning of the perfusion had a relatively strong interaction with the mucus since they resisted further perfusion. This is also confirmed by the fact that when the perfusion flow was doubled (Fig. 2), the elution profiles of the particles were not significantly modified. The stronger interaction when a 10-min interval was allotted before perfusion indicates either that the particle–mucus interaction was much stronger in this case or that a larger number of particles were adhering. Bioadhesion of a polymeric device to mucus is effected by the development of interactions between the two surfaces. Different types of bonding can contribute to that interaction such as Van der Waals forces, hydrogen bondings, or ionic interactions. At the molecular

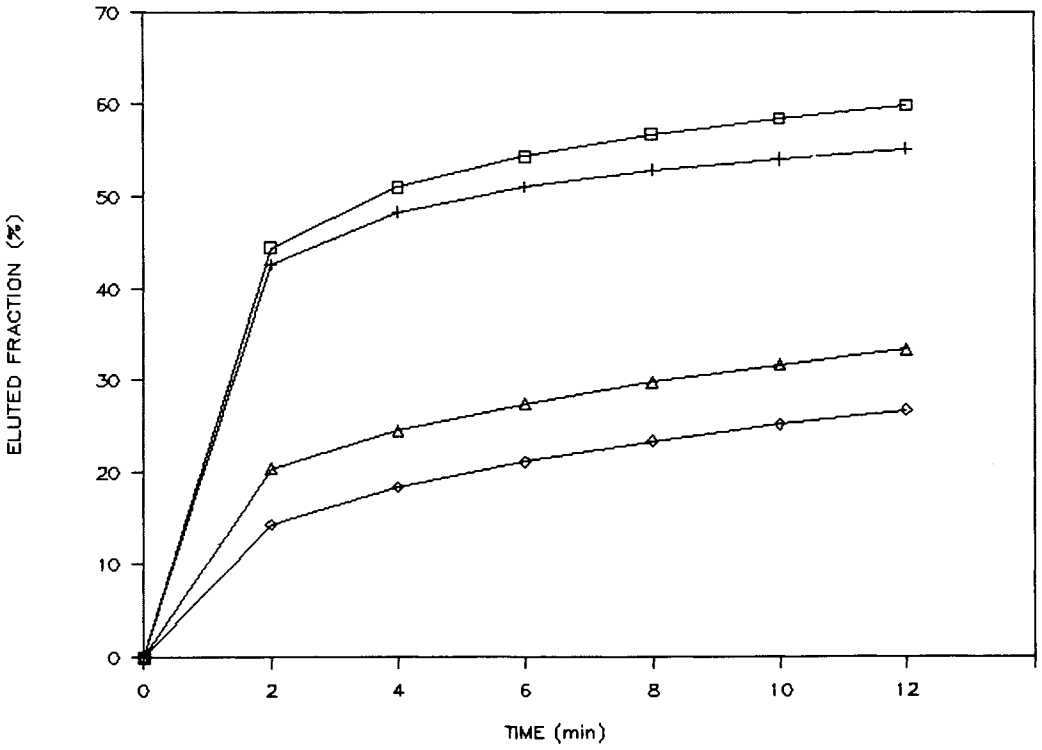

Fig. 1. Elution profiles of 5 or 10 μl of a radiolabeled polyhydroxypropylmethacrylate nanoparticle suspension deposited on a rat ileum (8 cm) at 37°C and perfused 0 or 10 min after deposition by a 5% glucose solution at a flow rate of 0.25 ml/min. (□) 5 μl, 0 min; (+) 10 μl, 0 min; (△) 5 μl, 10 min; (◇) 10 μl, 10 min.

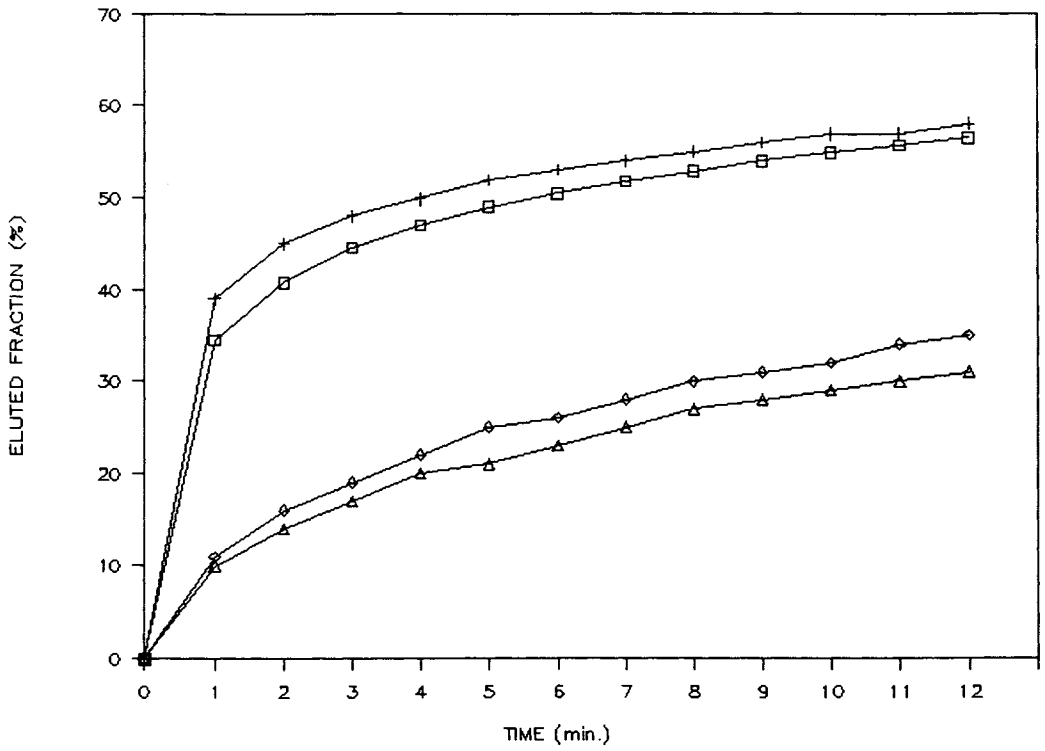

Fig. 2. Elution profiles of 5 or 10 μl of a radiolabeled polyhydroxypropylmethacrylate nanoparticle suspension deposited on a rat ileum (8 cm) at 37°C and perfused 0 or 10 min after deposition by a 5% glucose solution at a flow rate of 0.5 ml/min. (□) 5 μl, 0 min; (+) 10 μl, 0 min; (◇) 5 μl, 10 min; (△) 10 μl, 10 min.

level, the preliminary step leading to these interactions seems to be best described by the so-called "interpenetration theory" (7,8), based on the diffusion of the polymers into the mucus and of mucin into the polymeric network. Since diffusion is a time-dependent process the reported observation that nanoparticles show a higher adhesion to mucus after 10 min of contact than immediately after deposition appears consistent with this theory.

When similar experiments were repeated using a 0.9% NaCl solution as the perfusion medium and a flow rate of 0.5 ml/min, some differences appeared in the elution profiles as shown in Fig. 3. A much smaller percentage of the particles was eluted in the early fractions but significant amounts were still recovered in the later fractions. After 12 min the total eluted percentage was practically identical to what was observed with the 5% glucose perfusion. This phenomenon was observed in all cases irrespective of the time interval allotted to the particles between the application on the intestine and the beginning of the perfusion. As noted earlier, it is likely that particles left in contact with the mucus developed bonds by the so-called "interpenetration" mechanism. Initially the more loosely bound particles are eliminated rapidly, whereas the others remain on the mucus and develop increasingly strong bonds, preventing them from elution by further perfusion. However, the 5% glucose solution shows a higher density than the 0.9% NaCl solution and should, therefore, take out the loosely bound particles more efficiently as predicted by the equations describing particles detachment in a liquid flow (9). This may be the reason why the elution of the particles is faster when the glucose solution was used as compared to the NaCl solution.

The effect of hyper- and hypoosmolarity were evaluated by repeating the former experiment using a 0.1 and a 10% NaCl solution at a 0.5-ml/min flow and with a 0-min interval between nanoparticle application on the mucus and the beginning of perfusion. These experiments are reported in Fig. 4 together with the 0.9% NaCl perfusion under similar conditions of flow and time interval. Surprisingly, increasing the sodium chloride concentration resulted in lower elution profiles, although the density of the perfusion medium was higher. Furthermore, this observation suggests that the interaction between the particles and the mucus are probably not of electrostatic nature. Indeed, in this case increasing ionic strength should have resulted in faster elution profiles due to a more efficient disruption of the ionic bonds. It is therefore proposed that the main effect of the sodium chloride concentration on the elution profiles lies in the modification of the rheological properties of the mucus because of the low or high osmolarity of the perfusion liquid.

CONCLUSION

HPMA nanoparticles show a high level of adhesion to the intestinal mucosa *in vitro*. This adhesion seemingly results from an interpenetration between the polymeric and the mucus networks. It is obvious that the adhesive bonds develop relatively quickly, since even when particles are submitted to a perfusion immediately after application onto the mucosa, more than 50% of the initial dose remains on the intestine. Allotting a 10-min interval before the perfusion is started increases the adhering portion to about 90% of the initial dose, consistent with the "interpenetration" theory.

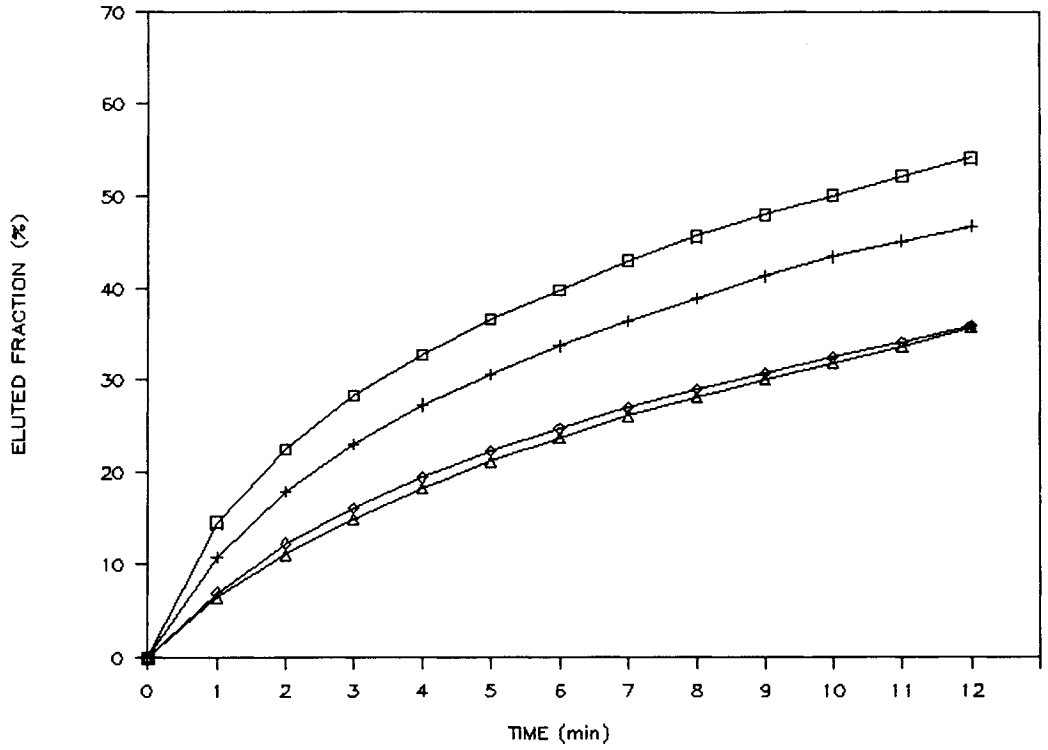

Fig. 3. Elution profiles of 5 or 10 μl of a radiolabeled polyhydroxypropylmethacrylate nanoparticle suspension deposited on a rat ileum (8 cm) at 37°C and perfused 0 or 10 min after deposition by a 0.9% NaCl solution at a flow rate of 0.5 ml/min. (□) 5 μl, 0 min; (+) 10 μl, 0 min; (◇) 5 μl, 10 min; (△) 10 μl, 10 min.

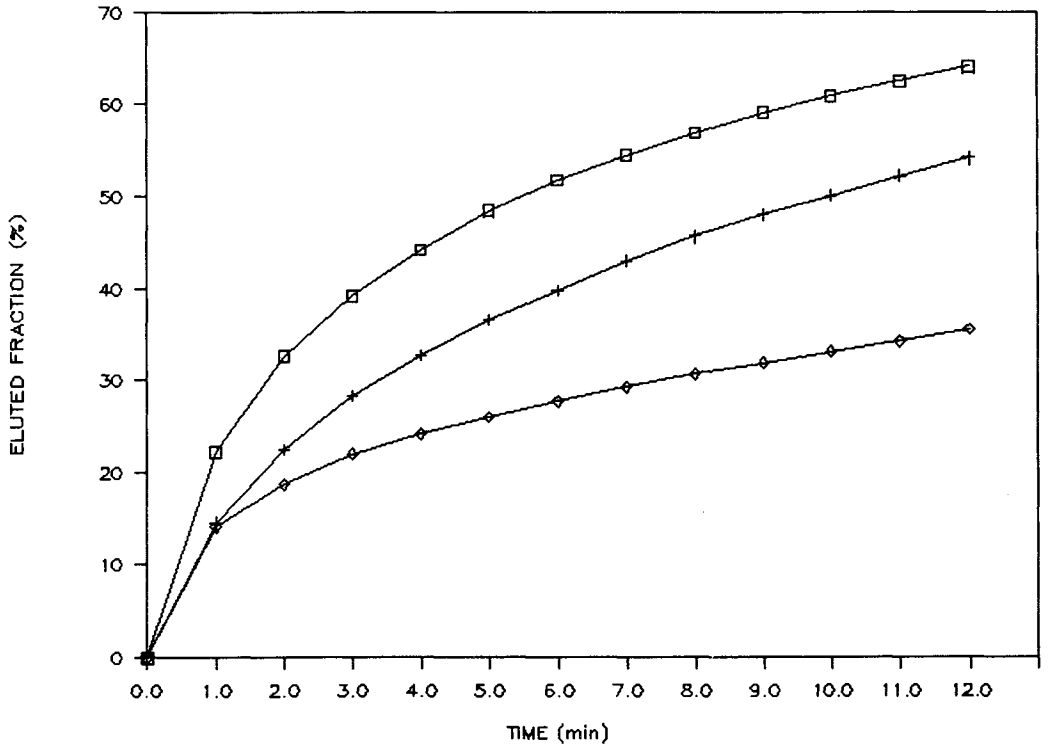

Fig. 4. Elution profile of a 5-μl radiolabeled polyhydroxypropylmethacrylate nanoparticle suspension deposited on a rat ileum (8 cm) at 37°C and perfused immediately by a 0.1% (□), 0.9% (+), or 10% (◇) NaCl solution at a flow rate of 0.5 ml/min.

For these reasons HPMA nanoparticles may be considered good candidates for the mucoadhesive delivery of drugs. Moreover, their low biodegradability in comparison with, i.e., cyanoacrylate nanoparticles (10), would in this case be less of a drawback, as compared to the use as an injectable drug-targeting system. Indeed, a large accumulation of nanoparticles in reticuloendothelial cells is hardly expected from peroral, ocular, nasal, rectal, or vaginal applications.

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