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In vitro bioadhesion of carbohydrate-containing *N*-(2-hydroxypropyl)methacrylamide copolymers to the GI tract of guinea pigs

Blanka Říhová, Ramesh C. Rathi, Pavla Kopečková and Jindřich Kopeček

Departments of Pharmaceutics / CCCD and Bioengineering, University of Utah, Salt Lake City, UT 84112 (USA)

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Summary

N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymers with different content of pendant saccharide moieties (galactosamine, fucosylamine, glucosamine, mannosamine) were synthesized and their bioadhesive properties to the gastrointestinal (GI) tract of female adult guinea pigs have been studied in vitro. Radioiodinated HPMA copolymers were incubated with everted sacs or enterocytes isolated from guinea pig small intestine and colon. The results obtained with everted sacs clearly indicated preferential binding of fucosylamine containing HPMA copolymers to the colonic mucus. The extent of binding increased with increasing fucosylamine content in the copolymers and with the time of incubation, and decreased with increasing molecular weight of the copolymers. The binding was partially inhibited by the presence of free fucose or glucose in the incubation medium. Fucosylamine containing HPMA copolymers also bind to isolated enterocytes and the extent of binding increases with increased fucosylamine content of HPMA copolymers. Contrary to the results obtained on everted sacs, there was no difference in binding to enterocytes isolated either from the small intestine or colon. The results obtained indicate the presence of lectin-like structures in the guinea pig GI tract.

Introduction

Polymeric bioadhesives have been proposed for the regulation of gastrointestinal (GI) transit time. Park and Robinson (1984, 1985) found that negatively charged polyelectrolytes with a high density of carboxyl groups can be used as potential bioadhesives in the GI tract. Their results indicate that the interaction between the adhesive polymer and the biological surface occurs through hydrogen bonding. Consequently, bioadhesion is favored when the carboxylic groups are not ionized. This concept may lead to partial success, however, if specific recognition systems could be found which are an integral part of human and animal physiology, the specificity and binding strength of such bioadhesive systems would have a much higher potential.

Correspondence to: J. Kopeček, Departments of Pharmaceutics/CCCD and Bioengineering, University of Utah, Salt Lake City, UT 84112, U.S.A.

Abbreviations: AIBN, 2,2′-azobisisobutyronitrile; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; FCS, fetal calf serum; GalN, galactosamine; FucN, fucosylamine; GlcN, glucosamine; ManN, mannosamine; GI, gastrointestinal; HPMA, *N*-(2-hydroxypropyl)methacrylamide; MEM, minimum essential medium (Eagle); PBS, phosphate-buffered saline; SI, small intestine.

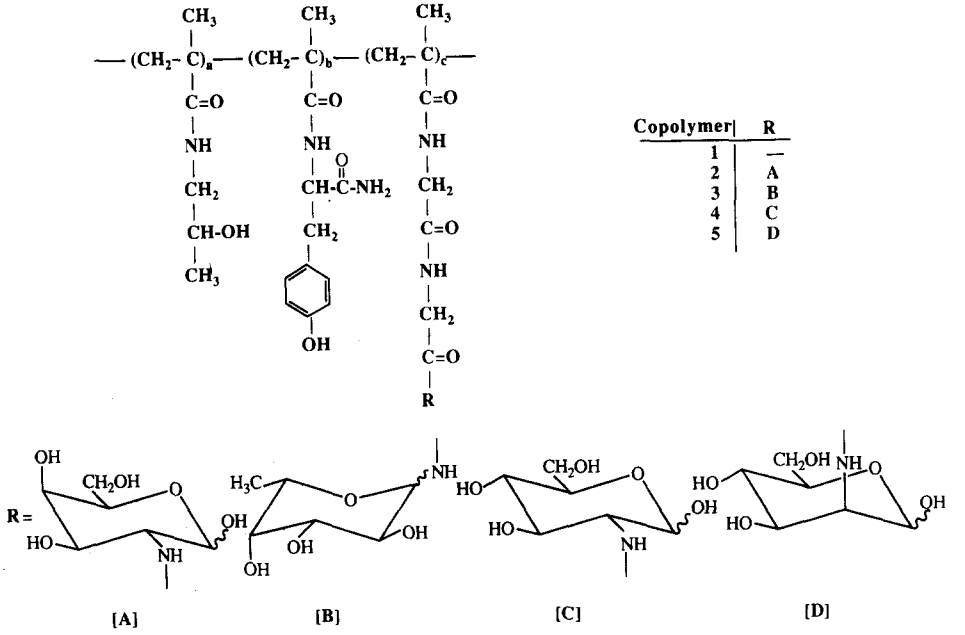
It was reported that sugar moieties can be important in the adherence to the GI tract. It appears that intestinal cells of guinea pigs produce lectin-like mucosal adhesins which in the presence of calcium cause fucose-sensitive adherence of certain invasive enteropathogens (Izhar et al., 1982; Mirelman et al., 1982; Ashkenazi, 1986). Based on these reports we have designed water-soluble *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers containing pendant side-chains terminating in saccharide moieties and shown in preliminary experiments that fucosylamine containing HPMA copolymers adhere specifically to the guinea pig colon (Rathi et al., 1991). Such copolymers could be used for prolongation of the transit time and for the development of colon-specific drug delivery systems. In the latter, drugs are bound to polymeric carriers via bonds which are susceptible to degradation by microbial enzymes in the colon (Brown et al., 1983; Kopečková and Kopeček, 1990; Grim and Kopeček, 1991). A combination of colon-specific bioadhesion with colon-specific drug delivery would increase the therapeutic efficacy of drugs for the treatment of colon disease (Kopeček et al., 1992).

In this study, HPMA copolymers containing pendant saccharide moieties (galactosamine, fucosylamine, glucosamine, mannosamine) were synthesized and their bioadhesive properties evaluated in vitro. Everted sacs and enterocytes were isolated from guinea pig small intestine and colon and incubated with radioiodinated HPMA copolymers. The relationship between the structure of saccharide containing HPMA copolymers and their bioadhesiveness was determined.

Materials and Methods

Synthesis of HPMA copolymers

Sugar containing monomers, namely, *N*-methacryloylglycylglycylgalactosamine [MA-Gly-Gly-GalN], *N*-methacryloylglycylglycylfucosylamine [MA-Gly-Gly-FucN], *N*-methacryloylglycylglycylglucosamine [MA-Gly-Gly-GlcN] and *N*-methacryloylglycylglycylmannosamine [MA-Gly-Gly-ManN] were synthesized and characterized as previously described (Rathi et al., 1991). Their copolymers (Scheme 1) with *N*-(2-hydroxypropyl) methacrylamide (**1, 2, 3a–d, 4** and **5**) were prepared by free radical copolymerization in ace-



Scheme 1.

TABLE 1

Characterization of HPMA copolymers

Copolymer	a^a (mol%)	b^a (mol%)	c^a (mol%)	M_W	M_W/M_n
1	99.0	1.0	0.0	76000	2.0
2	79.5	1.0	19.5	51000	1.5
3a	96.4	1.4	2.2	39000	1.5
3b	89.0	1.1	9.9	50000	1.7
3c	80.8	1.1	18.1	59000	1.8
3d	65.8	1.0	33.2	85000	2.1
4	80.0	0.9	19.1	60000	1.8
5	83.6	1.1	15.3	48000	1.7

[a] *a*, content of HPMA in the copolymers; *b*, content of MA-Tyr-NH_2 in the copolymers; *c*, content of MA-Gly-Gly-GalN (**2**), MA-Gly-Gly-FucN (**3a–d**), MA-Gly-Gly-GlcN (**4**) or MA-Gly-Gly-ManN (**5**). HPMA copolymer without sugar moiety (**1**). Conversion during copolymerization was 57–71%.

tone/DMSO, at 50°C for 24 h using AIBN as initiator (Kopečková and Kopeček, 1990; Rathi et al., 1991). A small amount (~ 1 mol%) of *N*-methacryloyltyrosinamide (Lee et al., 1990) was incorporated into the copolymers to permit radioiodination. Copolymers (**1–5**) used in this study are summarized in Table 1. Different molecular weight fractions (**3d**$_x$, **3d**$_y$ and **3d**$_z$) of HPMA copolymer **3d** were separated by size-exclusion chromatography using a Superose-6 column and TRIS buffer (0.05 M Tris + 0.5 M NaCl, pH 8) as eluent (Table 2).

Radioiodination

The HPMA copolymers were radioiodinated (Rathi et al., 1991) with $Na^{125}I$ using a modified chloramine T method (Moriarity and Savage, 1980). Free iodine was removed by chromatography on a Sephadex G-25 (PD-10) column using PBS as eluent. The specific radioactivity was approx. 200 μCi/mg of polymer.

TABLE 2

Characterization of molecular weight fractions of HPMA copolymer (3d)

Copolymer fraction	Content of FucN (mol%)	M_W	M_W/M_n
3d$_x$	31.5	211000	1.1
3d$_y$	30.1	86000	1.2
3d$_z$	30.0	28000	1.3

Assay for in vitro bioadhesion of carbohydrate containing HPMA copolymers to everted intestinal sacs

Non-inbred Hartley strain female guinea pigs (250–300 g) were obtained from Sasco. The animals were killed by ether narcosis. Everted sacs were prepared from small intestine, colon and occasionally from cecum. Small intestine and colon were cut into four pieces of equal length (cecum into two), thoroughly washed with prewarmed (37°C) and preoxidized (95% O_2; 5% CO_2) PBS (phosphate-buffered saline) and weighed. Each segment was sleeved onto a glass rod, everted, tied with surgical thread on both the sides and immediately immersed into prewarmed and preoxidized MEM (minimum essential medium) containing 5% FCS (fetal calf serum). The sacs were incubated under continuous stirring for 30 min (unless otherwise stated) at 37°C in 10 ml MEM with 5% FCS containing ^{125}I-labeled HPMA copolymers ($\approx$ 2 μg/ml; total radioactivity in incubation medium was 1.5–2.0 μCi). After incubation, the sacs were intensively washed and counted for radioactivity in a Packard gamma counter. Results are reported as μg of copolymer bound per g of tissue, and represent the mean ($\pm$SD) of at least five experimental values.

Preparation of intestinal epithelial cells

Enterocytes were isolated according to the modified techniques of Weiser (1973), Ashkenazi

(1986) and Izhar et al. (1982). The small intestine and colon were removed and cut into four pieces each and rinsed with prewarmed and preoxidized PBS. Segments were everted using a glass rod, cut into small pieces and immersed in PBS containing 1.5 mM EDTA and 0.5 mM dithiothreitol. No Ca^{2+} or Mg^{2+} ions were present in the immersion solution. Segments were incubated at 37°C for 10 min in a water bath with gentle shaking. After 10 min the supernatant was discarded and fresh PBS containing 1.5 mM EDTA and 0.5 mM DTT was added and incubation proceeded at 37°C for 45 min in water bath with gentle shaking. The supernatant containing suspended cells was centrifuged ($800 \times g$, 5 min) and washed twice with prewarmed and preoxidized PBS. The cell suspension was counted on a hemocytometer and the cell viability determined with a trypan blue exclusion test. The cell concentration was adjusted to 4×10^6/5 ml of MEM containing 5% FCS and 1 mM $CaCl_2$.

Assay for the in vitro bioadhesion of carbohydrate containing HPMA copolymers to isolated enterocytes from the small intestine and colon

Isolated enterocytes (4×10^6 cells in 5 ml of MEM containing 5% FCS and 1 mM $CaCl_2$) were mixed with ^{125}I-labeled HPMA copolymers and incubated in a shaking water bath for 45 min at 37°C. Afterwards, the cells were washed three times with PBS ($800 \times g$, 5 min) and counted for radioactivity.

Inhibition of binding to everted intestinal sacs and enterocytes

Everted intestine sacs or isolated enterocytes were first incubated at 37°C in prewarmed, preoxidized MEM with 5% FCS (and 1 mM $CaCl_2$ only in the case of enterocytes) containing 0.1 M fucose, 0.1 M glucose or 0.1 M mannose using gentle shaking. After 25 min radiolabeled HPMA copolymer was added and incubation further proceeded for 30 min in the case of everted sacs and 45 min in the case of isolated enterocytes. Finally, everted sacs or cells were intensively washed and determined for radioactivity using a gamma counter.

Results

Bioadhesion of ^{125}I-HPMA copolymers to everted intestinal sacs

Bioadhesion to different parts of GI tract

The bioadhesion of fucosylamine containing HPMA copolymer (**3d**) along the GI tract of guinea pigs in vitro was studied. Fig. 1 shows the binding of ^{125}I-labeled HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine to everted sacs, taken from different parts of the small intestine (starting from the pyloric sphincter segments are designated S-1–S-4), cecum (Ce-1 and Ce-2) and colon (starting from ascending colon segments are designated as C-1–C-4). As shown in Fig. 1, fucosylamine containing ^{125}I-labeled HPMA copolymer (**3d**) binds predominantly to the everted sacs taken from the colon where significant regional differences in the bioadhesivity were also detected. The binding to the colonic tissue increases from ascending to descending colon. Adhesion to the small intestine is lower

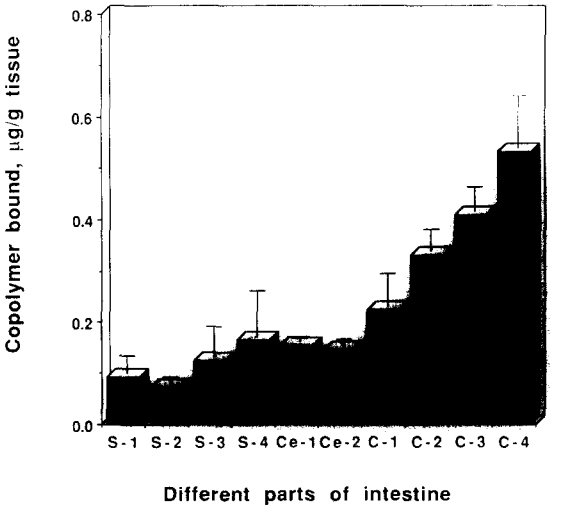


Fig. 1. Binding of ^{125}I-labeled HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine to the everted sacs from different parts of the intestine in vitro after 30 min incubation at 37°C. S-1–S-4, different parts of the small intestine starting from pyloric sphincter to ileum; Ce-1 and Ce-2 (different parts of cecum) and C-1–C-4 different parts of the colon starting from ascending to descending colon. Columns represent the mean (±SD) of at least five experimental values.

TABLE 3

Bioadhesion of fucosylamine containing ^{125}I-labeled HPMA copolymers to everted sacs from small intestine and colon (Rathi et al., 1991)

Copolymer	Copolymer bound (μg/g of tissue)	
	Small intestine	Colon
1	0.015 ± 0.005	0.026 ± 0.011
3a	0.115 ± 0.083	0.169 ± 0.067
3b	0.130 ± 0.050	0.260 ± 0.091
3c	0.082 ± 0.050	0.295 ± 0.069
3d	0.111 ± 0.052	0.401 ± 0.155

Segments were incubated with radioiodinated HPMA copolymers for 30 min at 37°C. Subsequently, the segments were intensively washed and their radioactivity was measured. The values represent the mean (± SD) of at least five experimental values. Statistical evaluation **3a, 3b, 3c** and **3d** vs **1**: SI, $p < 0.05$; colon, $p < 0.005$; colon vs SI: **3a**, no significant difference; **3b**, $p < 0.05$; **3c, 3d**, $p < 0.001$.

and relatively uniform. Binding to both parts of the cecum tissue was almost the same as to the small intestine, i.e., significantly lower when compared to the colon.

The effect of fucosylamine content on bioadhesion

The influence of fucosylamine content in HPMA copolymers (**3a–d**) on their bioadhesivity to guinea pig small intestine and to the colon is shown in Table 3. Binding of HPMA copolymers, with different content of fucosylamine (**3a–d**), to the colon increases with the increased amount of fucosylamine in the copolymers. On the other hand, binding to the small intestine does not show any clear dependence on carbohydrate content of HPMA copolymers and was considerably lower when compared to colon. HPMA copolymer (**1**) without carbohydrate moieties shows very little binding which indicates that bioadhesion is carbohydrate dependent.

Effect of carbohydrate structure on the bioadhesion of HPMA copolymers to guinea pig small intestine and colon

Experiments were performed to evaluate the possible contribution of different carbohydrates to the binding of HPMA copolymers to guinea pig intestinal surface. The bioadhesion of HPMA copolymers (**2, 3c, 4** and **5**) containing different carbohydrate moieties (galactosamine, fucosylamine, glucosamine, or mannosamine) is demonstrated in Fig. 2. These results indicate that the binding to the small intestine everted sacs is always lower than that to the colonic everted sacs and of the four different carbohydrate containing polymers studied, fucosylamine containing HPMA copolymers showed the highest colonic adhesion level which is 3–4-times higher when compared to the small intestine.

Effect of time of incubation on binding of HPMA copolymers

Time of incubation might affect the bioadhesion of carbohydrate containing HPMA copolymers. Hence, the bioadhesion of different sugar containing HPMA copolymers (**2, 3c, 4** and **5**) to colonic tissue was tested over different time periods ranging from 0.5–2 h. It was shown by Quadros et al. (1992) that in this time interval the

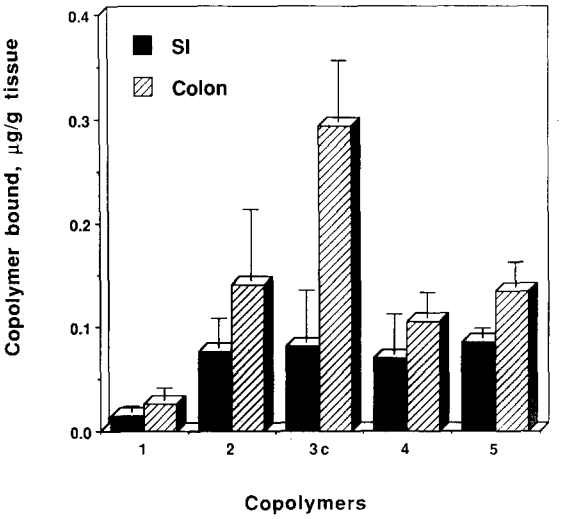


Fig. 2. Binding of different carbohydrate containing ^{125}I-labeled HPMA copolymers to the everted sacs from small intestine and colon in vitro after 30 min incubation at 37°C. **1**, control (HPMA copolymer without carbohydrate moieties); **2**, HPMA copolymer containing 19.5 mol% of galactosamine; **3c**, HPMA copolymer containing 18.1 mol% of fucosylamine; **4**, HPMA copolymer containing 19.1 mol% of glucosamine; **5**, HPMA copolymer containing 15.3 mol% of mannosamine. Statistical evaluation: **2, 3c** and **4** and **5** vs **1**: SI, $p < 0.05$; colon, $p < 0.005$; colon vs SI, **2, 4** and **5**: no significant differences; **3c**, $p < 0.001$.

colonic tissue remains viable. Results are shown in Fig. 3. Bioadhesion of all four types of polymers as well as of control increases gradually with time. Out of four different sugar containing HPMA copolymers tested the fucosylamine containing copolymer (**3c**) consistently showed the greatest colonic adhesion level at all time periods.

Effect of molecular weight on binding of HPMA copolymers

Molecular weight of the HPMA copolymer could also have an impact on bioadhesion of HPMA copolymers. Hence, different molecular weight fractions of HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine were isolated by size-exclusion chromatography (Table 2) and their bioadhesion to everted sacs of small intestine and colon was studied. The results are depicted in Fig. 4. Binding to colonic tissue increases with decreasing molecular weight of the copolymer. In contrast, binding to the small intestine did not show any clear dependence on the molecular weight. These results indicate that the diffusion of copolymers into the mucus layer contributes to the bioadhesion process.

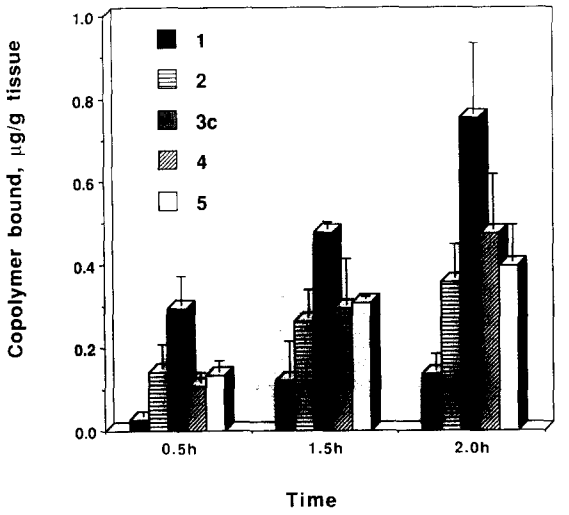


Fig. 3. Binding of different carbohydrate containing ^{125}I-labeled HPMA copolymers to the everted sacs of colon in vitro at different time periods. **1**, control; **2**, HPMA copolymer containing 19.5 mol% of galactosamine; **3c**, HPMA copolymer containing 18.1 mol% of fucosylamine; **4**, HPMA copolymer containing 19.1 mol% of glucosamine; **5**, HPMA copolymer containing 15.3 mol% of mannosamine. Statistical evaluation: **2, 3c, 4** and **5** vs 1 at different time periods. 0.5 h: **2, 3c, 4** and **5**, $p < 0.001$; 1.5 h: **3c**, $p < 0.05$; **2**, **4** and **5**, no significant differences; 2 h: **2, 3c, 4** and **5**, $p < 0.05$.

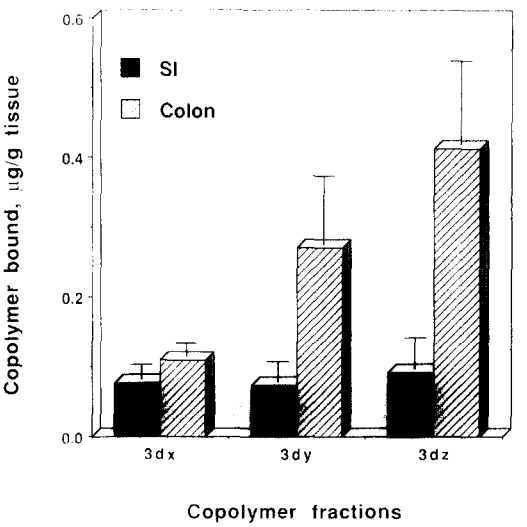


Fig. 4. Bioadhesion of different molecular weight fractions ($\mathbf{3d}_x$, $\mathbf{3d}_y$ and $\mathbf{3d}_z$ of ^{125}I-labeled HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine to the everted sacs from the small intestine and the colon in vitro after 30 min of incubation at 37°C. Statistical evaluation: colon vs SI; $\mathbf{3d}_x$, $\mathbf{3d}_y$: $p < 0.05$; $\mathbf{3d}_z$: $p < 0.001$.

Inhibition of the bioadhesion of fucosylamine containing HPMA copolymers by fucose, glucose and mannose

In order to study the inhibition of binding to everted sacs of small intestine and colon, ^{125}I-labeled HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine was used and different sugars, namely, 0.1 M fucose, 0.1 M glucose or 0.1 M mannose, were tested for their ability to inhibit the binding. The results obtained are shown in Fig. 5. Fucose and glucose inhibit the binding to colon by 60 and 50%, respectively. Mannose inhibited the binding to colon by only 28%. Inhibition of binding to small intestine was lower (10–15%) by all the three sugars studied.

*Bioadhesion of copolymer **3d** to everted sacs before and after removal of mucus*

To evaluate the possible role of mucus layer in the binding of HPMA copolymers to everted sacs from small intestine and colon, segments were

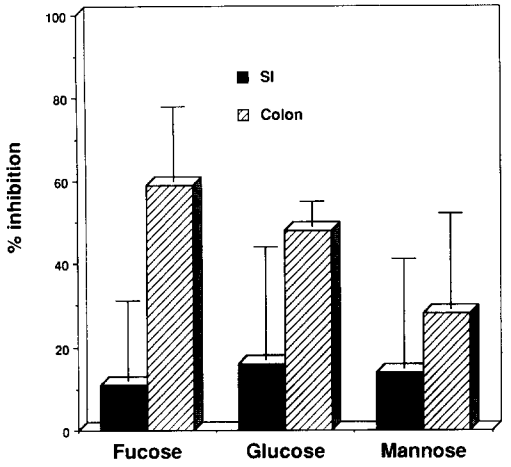


Fig. 5. Inhibition of binding of ^{125}I-labeled HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine to the everted sacs from the small intestine and the colon by 0.1 M fucose, 0.1 M glucose or 0.1 M mannose.

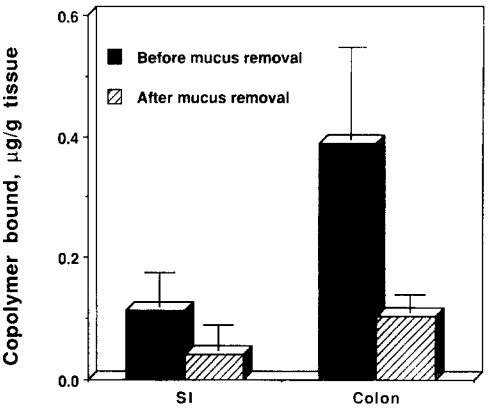


Fig. 6. Binding of ^{125}I-labeled HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine to the everted sacs from the small intestine and the colon before and after treatment with 1.5 mM EDTA and 0.5 mM DTT. See Materials and Methods for experimental details.

first incubated with copolymer **3d** followed by treatment with PBS containing 1.5 mM EDTA and 0.5 mM DTT for 10 min at 37°C so as to remove the mucus gel layer. It was observed that radioactivity associated with the colon as well as with the small intestine decreases considerably after this treatment (Fig. 6). Binding to colonic tissue was decreased by 80% and to small intestine by 60%. These results suggest that the binding of fucosylamine containing HPMA copolymers to the colonic tissue is mainly due to their attachment to the mucus layer.

Bioadhesion of fucosylamine containing HPMA copolymers to isolated enterocytes

Effect of carbohydrate content on the binding

Intestinal epithelial cells from small intestine and colonic tissue were isolated by 45 min incubation of the tissue at 37°C with chelating agent (1.5 mM EDTA) and 0.5 mM DTT. Cells were carefully washed and incubated with radioiodinated HPMA copolymers for 45 min at 37°C. The results obtained are shown in Fig. 7. Fucosylamine containing HPMA copolymers (**3a–d**) bind to isolated intestinal cells and the binding is proportional to the carbohydrate content of copolymers. In contrast to the results obtained with everted sacs, no difference in binding to enterocytes isolated from the small intestine and the colon was observed. Binding of HPMA copolymer without carbohydrate moiety (**1**) to both types of enterocytes was negligible.

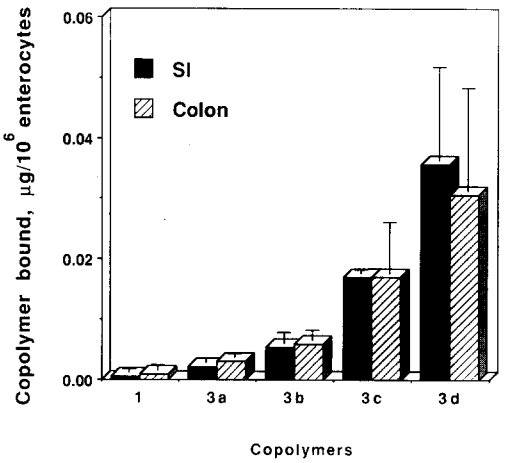


Fig. 7. Bioadhesion of ^{125}I-labeled HPMA copolymers (**1**, **3a–d**) containing different amounts of fucosylamine to enterocytes isolated from the small intestine and the colon after 45 min incubation at 37°C. Statistical evaluation: **3a**, **3b**, **3c** and **3d** vs 1: SI, $p < 0.05$; colon, $p < 0.05$; colon vs SI, no significant differences.

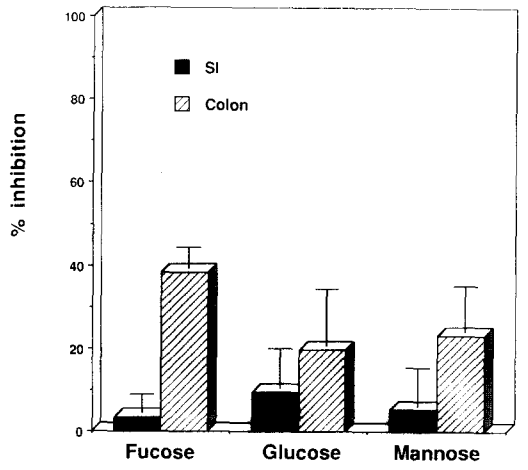


Fig. 8. Inhibition of binding of ^{125}I-labeled HPMA copolymer (**3d**) containing 33.2 mol% of fucosylamine to the enterocytes isolated from the small intestine and the colon by 0.1 M fucose, 0.1 M glucose or 0.1 M mannose.

Inhibition of bioadhesion to enterocytes by different carbohydrates

Binding of fucosylamine (33.2 mol%) containing ^{125}I-labeled HPMA copolymer (**3d**) to isolated cells was inhibited by fucose, glucose and mannose. The extent of inhibition was lower when compared to inhibition to everted sacs. The results obtained are illustrated in Fig. 8. 0.1 M fucose, 0.1 M glucose, and 0.1 M mannose inhibited the binding to the colonic enterocytes by 40, 20 and 25%, respectively. Inhibition of binding to small intestine enterocytes was only 5–15%. This suggests that binding sites of different character and/or affinity are involved in the adherence of HPMA copolymers to the mucus layer and to enterocytes.

Discussion

The term bioadhesion describes a phenomenon in which synthetic or biological macromolecules are able to adhere and be retained on a biological system for an extended period of time (Peppas and Buri, 1985). The extent of bioadhesion depends on the interfacial force which holds together the adhesive material and the biological tissue (Gu et al., 1988).

The design of the bioadhesive HPMA copolymers studied here is based on the observation that the adherence of *Shigella flexneri* to guinea pig intestinal cells is mediated by a mucosal adhesin (Izhar et al., 1982). It was found that this adherence was a Ca^{2+}-dependent fucose- and glucose-sensitive process in which the host cell provided the adhesin. Similar results were obtained with non-fimbriate entero-invasive *Escherichia coli 0124. E. coli* binding adhesin was secreted with the colonic mucus and binding was fucose and glucose specific (Ashkenazi and Mirelman, 1984; Ashkenazi, 1986).

Two in vitro approaches to test the specificity of bioadhesion of HPMA copolymers to GI tract of guinea pigs have been used. Binding of carbohydrate containing HPMA copolymers was tested using the everted sac technique and using isolated enterocytes from small intestine and colon. Four sugars (galactosamine, fucosylamine, glucosamine, mannosamine) were incorporated into HPMA copolymers and their ability to contribute to the bioadhesion of the copolymers was evaluated. Considerable differences in the bioadhesion of carbohydrate containing HPMA copolymers (**2–5**) to the everted sacs from the SI and colon (Table 3, Fig. 2) were observed. The attachment of all four types of copolymers to the small intestine was lower as compared to the colon (Fig. 2). The most striking differences were observed with the HPMA copolymer containing 33.2 mol% of fucosylamine (**3d**, Table 3). Its in vitro binding to the colon segments was 3–4-times higher than to the small intestine segments. The bioadhesion was highest to the segments taken from transverse and descending regions of the colon (Fig. 1) and proportional to the fucosylamine content of HPMA copolymers (Table 3). The reasons for these regional differences may be due to the fact that colonic mucus is distinguished from mucus in proximal intestine (Allen et al., 1984), by its greater sialylation and marked degree of sulfation (Rhodes, 1989). The ascending and descending colons are known to have different physiological functions, including ion transport (Kliger et al.,

1981) and secretagogue responsiveness (Schwartz et al., 1980). Differences in regional bacterial population have also been recognized (Allison et al., 1979). To what extent these factors are responsible for the observed local and regional variations in bioadhesion of fucosylamine containing HPMA copolymers is not clear. However, it is interesting to note that *S. flexneri* avidly adhered to colonic epithelial cells from the transverse and descending sections of the colon (Izhar et al., 1982).

The nature of the recognition system on mucus as well as on enterocytes is also unknown. There is a good reason to believe that the binding to the intact segments of colon (and in a lesser extent to small intestine) as well as to enterocytes is mediated by carbohydrate binding molecules, most probably lectins. Lectins are non-immune proteins or glycoproteins that recognize and bind the terminal sugars of glycoproteins and glycolipids. They are abundant in the seeds of leguminous plants, but their presence was also demonstrated in animal tissues including goblet cells of chicken intestine (Beyer et al., 1980). They could have specificities for one or more particular sugars. It is generally recognized that the inhibitory sugars are not always totally effective in blocking the reactions of lectins (Gonatas and Avrameas, 1973). We have observed that 50–60% of binding to colonic mucus could be specifically blocked by 0.1 M fucose or 0.1 M glucose. 0.1 M mannose inhibits the binding only in a limited extent (Fig. 5). A different pattern was seen using isolated enterocytes (Fig. 8). Enterocytes, similarly to the mucus layer, also bind selectively fucosylamine-containing HPMA copolymers and this attachment is directly correlated with the amount of fucosylamine (Fig. 7). There were no binding differences between the enterocytes isolated from the small intestine and those isolated from the colon (Fig. 7). However, the binding sites on the colon and small intestine enterocytes seem to be of different types as the pattern of inhibition of binding by free sugars (0.1 M fucose, 0.1 M glucose, 0.1 M mannose) is different in the colon and small intestine enterocytes (Fig. 8).

Interaction of carbohydrate-containing HPMA copolymers with the mucosal surface of guinea pig colon (and small intestine) reflects the complexity of these surfaces. An integral part of the apical membrane of gut mucosal cells is the glycocalyx, which is a uniform layer of filamentous glycoprotein (Egberts et al., 1984). Superimposed on this surface coat is the mucus gel, composed of glycoproteins (mucins) with high molecular weight ($> 10^6$). The last layer is formed by an unstirred water layer which is discrete from the rest of gut contents (Thomson and Dietschy, 1984). The movement of molecules through this unstirred layer will involve simple diffusion and can be rate-limiting to the adsorption of hydrophobic molecules (Humphrey, 1986). Allen (1984) reported that mucus is impermeable for macromolecules of the size of pepsin, while others demonstrated the penetration and uptake of macromolecules by rat and human intestine in vivo (Kilpatrick et al., 1985). Gardner (1988) and Pusztai (1989) suggested that small quantities of intact proteins do cross the GI tract in animals and adult humans, and that this is a physiologically normal process. It is obvious that the mucus coat must be sufficiently permeable or porous to allow access of nutrients to the digestive enzymes and transport systems of the brush border surface in the intestine. It was shown that the ability of a drug to diffuse through a mucus gel could be directly correlated with its absorptive behavior within the GI tract (Nimmerfall and Rosenthaler, 1980). We have also observed that the binding is inversely proportional to the molecular weight of HPMA copolymers (Fig. 4). These results indicate that the diffusion of polymers through the mucus layer is equally important in binding of HPMA copolymers. Our finding that a considerable amount of radiolabeled HPMA associated with everted sacs was detached after treatment with EDTA and DTT (Fig. 6) suggests that a considerable part of fucosylamine containing HPMA copolymers bind to the mucus layer. However, some molecules could penetrate and reach epithelial cells as it was reported that unmodified ^{125}I-labeled HPMA copolymers are accumulated by rat jejunal sacs in vitro and that these polymers are even transported across the tissue to the serosal side (Bridges, et al., 1987, 1988; Cartlidge, et al., 1987). However, after ad-

ministration of irritants or ulcerogenic agents (Menguy and Desbaillets, 1967a, b; Kent and Allen, 1968; Johansson and Lindquist, 1971) or under some pathological conditions like ulcerative colitis, Crohn's disease, benign and malignant colonic polyps or colorectal cancer (Rhodes, 1989) the composition of oligosaccharide side chains of the mucins in the mucus layer is altered (Pfeiffer, 1981). Alteration in sialomucin content, in particular a diminished proportion of *O*-acylated sialic acid variants, occurs in both early (Greaves et al., 1984) and established colorectal adenocarcinoma (Culling et al., 1977) and ulcerative colitis (Reid et al., 1984). Changes in mucus structure or in mucus secretion may be responsible for the greater susceptibility of mucus to enzymatic degradation. Consequently, mucus thickness is reduced (McCormick et al., 1990), its permeability to high molecular weight molecules increased and the binding to the underlying epithelial cells (enterocytes) becomes more important.

The sugar binding structures in mucus could be either of intrinsic or extrinsic origin. Endogenously secreted proteins or glycoproteins could behave as lectins (adhesins), or the binding could be (partially) due to lectins from food temporarily incorporated in the mucus layer or finally, the sugar containing HPMA copolymers could attach to the colonic enteric bacteria, which are trapped within the mucus gel. Experiments are in progress to answer these questions by isolating the lectin-like molecules and by using germ free animals.

From the results obtained so far it appears that fucose binding molecules are present on the surface membrane of enterocytes isolated from small intestine and from the colon. Molecules with similar, but not quite identical specificities are also present in the mucus layer of the colon. Both types of molecules have an affinity for fucosylamine containing HPMA copolymers. The binding increases with time, and is dependent on the sugar content and molecular weight of the copolymer molecule and can be inhibited by fucose and glucose.

In summary, by the synthesis of tailor-made HPMA copolymers we were able to mimick a bioadhesive process occurring in the GI tract of guinea pigs.

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