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Soluble *N*-(2-hydroxypropyl)methacrylamide copolymers as a potential oral, controlled-release, drug delivery system. I. Bioadhesion to the rat intestine in vitro

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Summary

The interaction of *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers containing pendent sugar residues or quaternary ammonium groups with adult rat intestine was examined in vitro. Copolymers containing sugar residues had a greater affinity for intestinal tissue than unmodified copolymer, but the cationic derivative displayed the greatest tissue association. The extent of tissue association was dependent upon the sugar residue present, and increased in the order fucose > mannose > galactose. Eversion of intestinal tissue caused an increase in the tissue association of copolymers containing galactose attached directly to the copolymer backbone, but had no effect if the sugar residue was attached via a glycylglycyl spacer. Measurement of HPMA–copolymer binding to specific regions of the intestine revealed that galactose-containing copolymers had slightly higher affinity for the duodenum/first-part jejunum, whereas fucose-containing copolymers adhered more to distal regions particularly the third-part jejunum. The cationic derivative bound strongly to all regions. The HPMA copolymers described here could be useful in oral delivery formulations, to slow gastrointestinal transit time of drug, and also facilitate binding to specific regions of the gastrointestinal tract, allowing site-specific drug delivery.

Introduction

N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymers have been developed as targetable drug-carriers (Kopeček, 1982, 1984; Kopeček and Duncan, 1987) particularly for use in cancer chemotherapy (Kopeček, 1986; Duncan et al., 1987). Factors which govern their body distribution following intravenous, subcutaneous and intraperitoneal administration have been described previously, including molecular weight (Cartlidge et al., 1987; Seymour et al., 1987a) incorporation of carbohydrate residues (Duncan et al., 1986; Seymour et al., 1987b) and incorporation of antibodies (Říhová et al., 1986, 1988). Recently, Cartlidge et al. (1987) have shown that incorporation of galactosamine residues into HPMA copolymers alters their progress along the gastrointestinal tract. Here we have studied the influence of various substituent groups in HPMA copolymers on their bioadhesion to rat small intestine in vitro.

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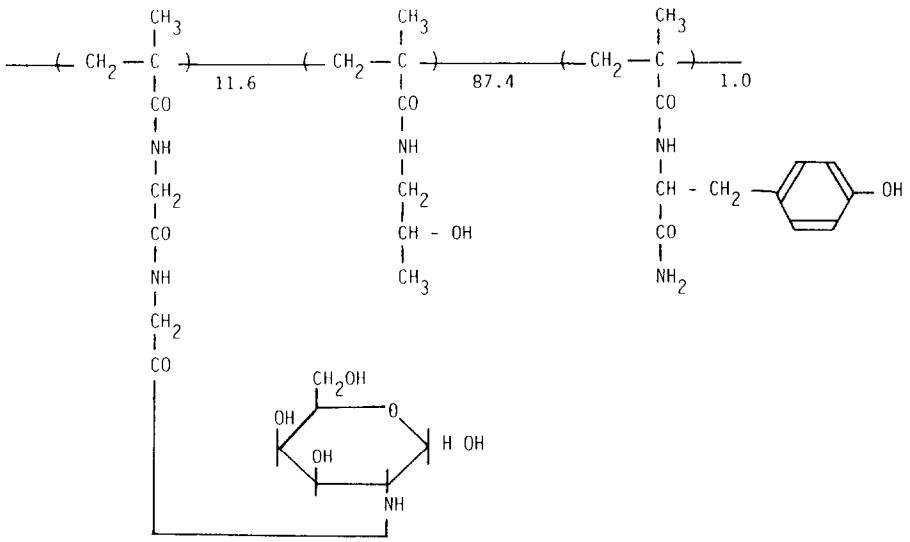
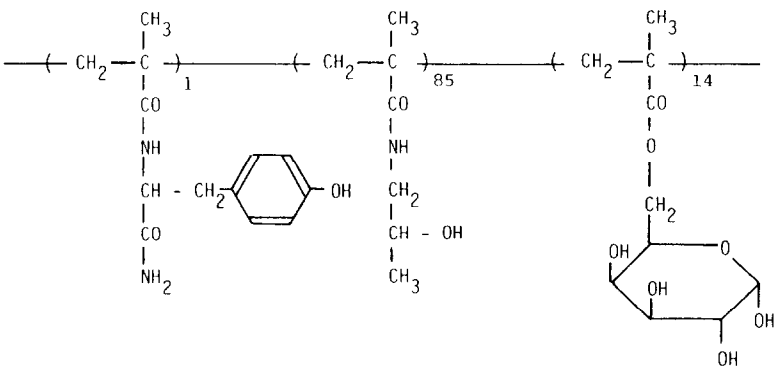
Polymers 1 - 6 e.g. Polymer 3



Polymers 7 and 8 e.g. Polymer 7



Polymer 9

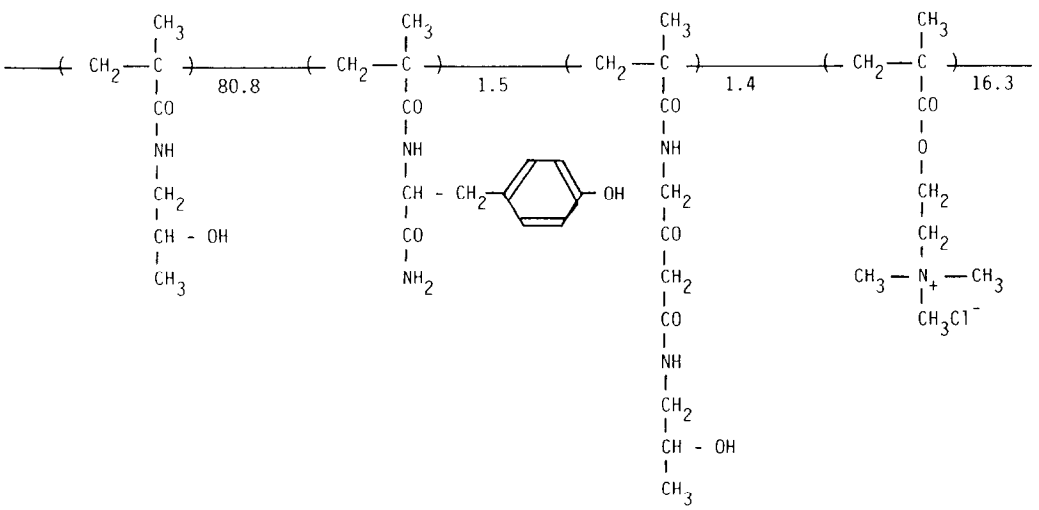


Fig. 1. Chemical structure of HPMA copolymers.

Oral administration is still the most convenient and commonly employed route for drug administration. However, use is often limited because the gastrointestinal transit time of many drugs is too rapid (approximately 3 h). In animals and man the duration of most currently available oral sustained-release formulations is approximately 8–12 h. To date it has not been possible to localize a drug formulation in a selected region of the gastrointestinal tract, e.g. colon, permitting localized drug delivery (Park et al., 1984; Park and Robinson, 1985). Several approaches have been proposed to slow transit of drugs in the gastrointestinal tract. Osmotically controlled dosage formulations (Oros) have been developed (John et al., 1985), but their rate of drug release is strongly influenced by the local ion concentration, a factor often subject to considerable variability in the intestine. Large particles (up to 5 mm in diameter) can delay stomach emptying, and thereby prolong transit time (Davis, 1985), but this phenomenon is relatively short-lived (maximum 12 h), particularly when the drug delivery system is administered (to dogs or humans) in the absence of food (Hinder and Kelly, 1977; Davis et al., 1986). Polymers that adhere specifically to mucin/epithelial surfaces have been proposed as potential bioadhesives for use in oral drug delivery formulations. Using human conjunctival epithelial cells as a model system, Park et al. (1984b) found that highly charged carboxylated polyanions had good potential as a bioadhesive for drug delivery.

We believe that polymers having the necessary physicochemical properties to promote mucin

TABLE 1

Chemical characteristics of HPMA copolymers

Code no.	Side-chain composition	Side-chain content [a]	M_w [b]	M_w/M_n
1	P< TyrNH$_2$ / Gly-Gly-aminopropanol	3.3 4.6	15 000	1.4
2	P< TyrNH$_2$ / Gly-Gly-galactosamine	1.1 7.5	33 000	1.2
3	P< TyrNH$_2$ / Gly-Gly-galactosamine	1.1 11.6	27 000	1.2
4	P< TyrNH$_2$ / Gly-Gly-mannosamine	1.0 11.1	28 000	1.3
5	P< TyrNH$_2$ / Gly-Gly-glucosamine	1.3 11.6	28 000	1.3
6	P< TyrNH$_2$ / Gly-Gly-fucosylamine	1.0 4.0	25 000	1.4
7	P< TyrNH$_2$ / galactose	1.0 14.0	131 000	n.d.
8	P< TyrNH$_2$ / galactose	1.0 99.0	402 000	n.d.
9	TyrNH$_2$ P<—Gly-Gly-aminopropanol O-CH$_2$-CH$_2$N$^+$(CH$_3$)$_3$Cl$^-$	1.0 1.4 16.3	62 000	2.3

P = HPMA polymer backbone.

n.d. = not determined.

[a] Content of Tyrosinamide was determined spectrophotometrically.

[b] The M_w and polydispersity was determined using Sepharose 4B/6B (1 : 1) chromatography, column calibrated using poly(HPMA) fractions.

and/or brush border interaction will ultimately prove very useful in oral drug delivery formulations, both as a method of controlling gastrointestinal transit time, and in the case of region-specific interaction, enabling region-specific drug delivery. In certain cases an elevation in the concentration of drug transported across the mucosa may be achieved if the drug can be delivered in the most appropriate region. However, there is a need to investigate fundamental structure–activity relationships governing polymer interactions with gastrointestinal tract.

In the current study HPMA copolymers were synthesized to contain carbohydrate residues (galactosamine, mannosamine, glucosamine, fucosylamine or galactose) or cationic groups, (quaternary ammonium groups), they also contained a small amount (approximately 1 mol%) of tyrosinamide to permit radioiodination for monitoring of polymer fate. Structure of the polymers used, and their compositions are shown in Fig. 1 and Table 1, respectively. To investigate their binding to adult rat intestine in vitro, polymers were incubated (periods up to 30 min) with intestinal rings (either everted or non-everted), taken from rat duodenum, jejunum or ileum. Incubations were also carried out in the presence and absence of competing calf serum proteins.

Materials and Methods

Chemicals

[^{125}I]Iodide (preparation IMS 30) was from Amersham International, U.K. Tissue culture medium 199 and foetal calf serum were from Gibco, Bio-Cult, Paisley, U.K. Calf serum was heat inactivated at 56 °C for 30 min prior to use.

Synthesis and characterization of HPMA copolymers

Synthesis of HPMA copolymers containing aminosugars (Duncan et al., 1986; Seymour et al., 1987b), trimethylammonium chloride groups (McCormick et al., 1986) and galactose bound directly to the polymer backbone (Chytrý et al., 1987) have been described previously.

In summary, polymers 1–6 (Table 1) were prepared by, firstly synthesizing a polymeric precursor by radical precipitation copolymerization of HPMA, methacryoyltyrosinamide and methacryloylglycylglycine *p*-nitrophenyl ester (Rejmanová et al., 1977). Polymeric precursor was then subject to aminolysis by reaction with aminopropanol (polymer 1), galactosamine (polymers 2 and 3) mannosamine (polymer 4), glucosamine (polymer 5) or fucosylamine (polymer 6). A synthetic procedure described by Chytrý et al. (1987) was used to increase the amount of galactose in the HPMA copolymer to 99 mol%. Polymers 7 and 8 were prepared by copolymerization of *N*-methacryloyltyrosinamide, 1.2 : 3.4-di-*O*-isopropylidene-6-methacryloyl-D-galactopyranose and HPMA, followed by deblocking of the protected galactose units with 80% HCOOH. Polymer 9 was prepared by copolymerization of HPMA, methacryloyloxyethyltrimethylammonium chloride, *N*-methacryloyltyrosinamide and *N*-methacryloylglycylglycine *p*-nitrophenyl ester in a mixture of acetone and dimethylsulfoxide (20 : 1) followed by aminolysis of the *p*-nitrophenyl ester groups with 1-aminopropan-2-ol (McCormick et al., 1986). The content of carbohydrate residues was estimated as previously described. Galactosamine, mannosamine and glucosamine content was measured according to the method of Plummer (1976) and Cheng and Boat (1978). Fucosylamine content was estimated from kinetic measurement of the reaction between polymeric precursor and fucosylamine. The amount of sugar bound was proportional to the decrease in absorbance of leaving *p*-nitrophenyl groups (assessed using UV spectroscopy). The content of galactose in copolymers 7 and 8 was determined by the phenol–sulfuric acid method (Dubois et al., 1956). The amount of methacryloyloxyethyltrimethylammonium chloride units in copolymer 9 was determined by elemental analysis (content of chlorine). Molecular weight was estimated by GPC on a Sepharose 6B/4B (1 : 1) column calibrated with fractions of poly HPMA.

All polymers were radiolabelled using the chloramine-T method (Duncan et al., 1981).

Incubation of rat intestinal rings with ^{125}I-labelled HPMA copolymers

Intestinal rings were incubated with ^{125}I-labelled copolymers after the method described by Bridges et al. (1978) for incubation of rat jejunal sacs. Male Wistar rats (300–350 g) were killed by cervical dislocation. The intestine, from the pyloric sphincter to the jejuno-ileal junction, was removed and placed immediately in medium 199 at 37 °C, and continuously gassed with 95% CO_2/5% CO_2. The intestine was washed with oxygenated gravity-fed medium 199, using gentle manipulation. In certain experiments the intestine was everted by eversion over a thin (2 mm diameter) glass rod.

In all cases the intestine was divided into 0.5 cm length rings using a sharp scalpel, excluding the areas of gut-associated lymphoid tissues (Peyers patches). Intestinal rings were placed in Erlenmeyer flasks (3 to a flask) containing 10 ml of oxygenated medium 199 at 37 °C. Where specified, calf serum was added at a concentration of 10% v/v. All incubations were carried out in a shaking water bath and after a preincubation period of 5 min, radiolabelled polymer was added (at 2 μg/ml or 10 μg/ml as indicated). Flasks were reoxygenated immediately after addition of the radiolabelled substrate. At various time intervals up to 30 min, rings were removed from a flask and washed 4 × 2 min (20 ml each wash) in ice-cold saline (0.85%).

The intestinal rings were then solubilized in 1 M NaOH (5 ml) for 2 h at 37 °C. Duplicate samples (1 ml) were taken from the resultant digest and assayed for radioactivity. Protein concentration was measured using the method described by Lowry et al. (1951). Accumulation of radiolabelled polymer by the tissue was expressed as the amount of polymer (ng) captured per mg tissue protein.

In the above experiments the intestinal rings were randomly taken from the gastrointestinal (GI) tract. However, to seek evidence for region-dependent interaction rings were taken selectively from 4 different regions. These were duodenum/first part jejunum (pyloric sphincter to ligament of Trietz), second part jejunum (proximal third of remaining small intestine), third part jejunum (middle third) and ileum (remaining third).

Results

Tissue association of ^{125}I-labelled HPMA copolymers over a 30 min time period by intestinal rings (taken from the length of the gastrointestinal tract and then randomized) is shown in Fig. 2. Polymer association with intestinal tissue generally increased with time over 30 min and polymers containing galactosamine (polymer 2), fucosylamine (polymer 6) and cationic residues (polymer 9) all associated more readily with the

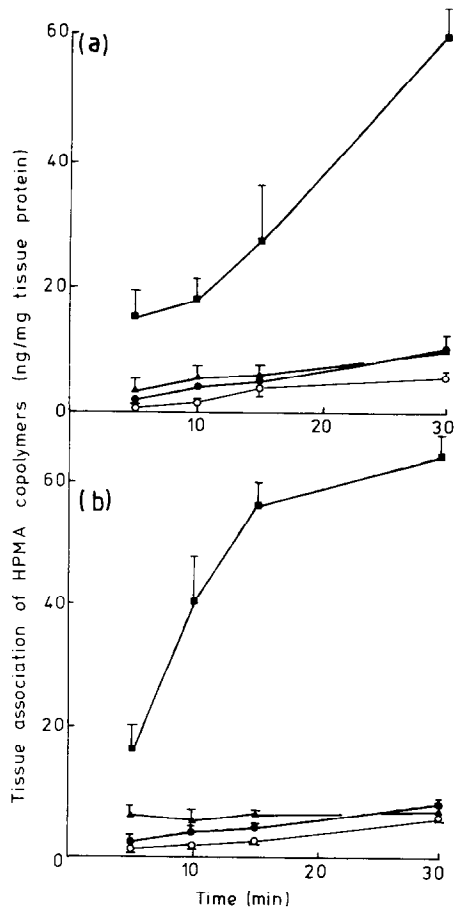


Fig. 2. Association of ^{125}I-labelled HPMA copolymers with non-everted rat intestinal rings (taken randomly), over a 30 min incubation period. Tissue association of copolymer 1 (○——○), copolymer 2 (●——●), copolymer 5 (▲——▲) and copolymer 9 (■——■) is shown. Experiments were carried out in the presence (panel a) or absence of foetal calf serum (panel b) and ^{125}I-labelled copolymers were added at a concentration of 10 μg/ml.

tissue than unmodified HPMA copolymer (polymer 1). However, the polycationic derivative showed by far the greatest affinity for the intestinal rings, both in the presence and absence of serum proteins (Fig. 2a and b). In the presence of calf-serum (Fig. 2a) tissue association of the cationic HPMA copolymer was much lower over the first 15 min compared with tissue association measured in the absence of serum. However, by 30 min this difference was no longer apparent.

A sample time of 15 min was chosen for subsequent experiments investigating tissue association of radiolabelled polymer to permit a comparative study involving a large number of different HPMA copolymers. Polymers were incubated with randomized intestine rings (as above) that were either, non-everted, or everted, and in both cases experiments were carried out in the presence and absence of calf serum (Fig. 3). Inclusion of serum had no measurable effect. HPMA copolymers containing sugar residues showed a higher tissue association than the unmodified HPMA copolymer (polymer 1) throughout, with the exception of the galactose-containing HPMA copolymers (polymers 7 and 8) which showed no greater tissue affinity than polymer 1 when incubated with non-everted tissue (Fig. 3a). Evertion of this tissue (Fig. 3b) seemed to increase tissue association of these polymers. Of the sugar-containing polymers, the fucosylamine-containing polymer (polymer 6) consistently showed the greatest tissue level, the measured association being approximately 2.4 × the value measured with polymer 1. Although tissue association of copolymers containing glycyl-glycylgalactosamine side-chains was twice the control value, the degree of sugar-substitution did not influence this interaction (see polymers 2 and 3). Once again the polycationic derivative, polymer 9, showed by far the greatest tissue association irrespective of the state of evertion of the tissue. Although the different polymers associate

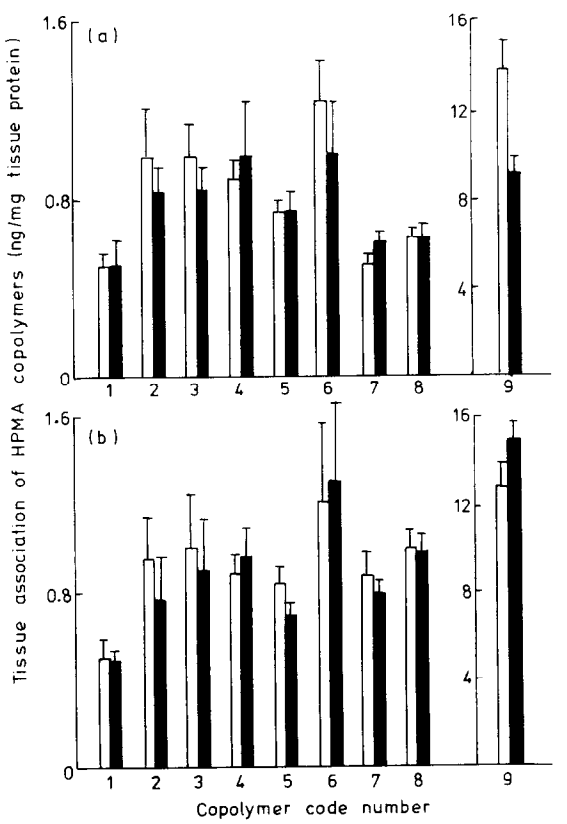


Fig. 3. Association of a number of ^{125}I-labelled HPMA copolymer derivatives (15 min) with rat intestinal rings taken at random. Copolymer (code numbers indicated, 2 μg/ml) were incubated with non-everted (panel a) or everted (panel b) intestinal rings, in the presence (■) or absence (□) of foetal calf serum.

TABLE 2

Association of ^{125}I-labelled HPMA copolymers with adult rat intestine expressed as a percentage of the substrate available [a]

Copolymer code no.	Everted intestine [b]		Non-everted intestine	
	Without calf serum	With 10% calf serum	Without calf serum	With 10% calf serum
1	4.1±0.4	4.1±0.4	4.4±0.5	4.7±1.0
2	6.8±1.7	6.6±1.7	8.7±1.9	7.2±0.9
3	7.7±1.8	7.7±0.2	8.9±1.3	7.5±0.8
4	8.3±1.1	8.3±1.1	7.8±0.7	8.6±0.2
5	5.9±0.5	5.9±0.5	6.5±0.5	6.4±0.7
6	11.2±3.1	11.3±3.1	10.7±1.6	8.9±0.2
7	6.8±0.5	6.8±0.5	4.7±0.4	5.1±0.7
8	8.3±0.7	8.3±0.7	5.4±0.4	5.2±0.7
9	55.3±4.9	64.2±4.8	58.9±5.2	39.2±3.8

[a] Rat intestinal rings (taken randomly) were incubated with ^{125}I-labelled HPMA copolymers under conditions shown.

[b] Data are expressed as the mean percentage (±S.E.) of available ($\times 10^2$) substrate removed from the incubation medium during the 15 min incubation period. Each flask contained approximately 2.5 mg of intestinal tissue (protein equivalent).

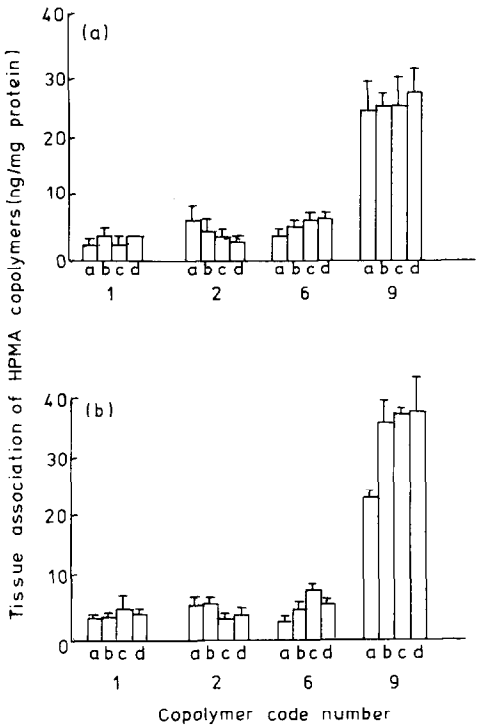


Fig. 4. Tissue association of ^{125}I-labelled HPMA copolymers after incubation with non-everted intestinal rings taken from defined regions of the small intestine. The code number shown indicates the copolymer studied and the intestinal regions are indicated as follows: (a) duodenum/first part jejunum, (b) second part jejunum, (c) third part jejunum, and (d) ileum. These regions are defined in the Methods section.

with an intestinal tissue to differing extents, in no case was more than 1% of the available substrate removed from the culture medium during the experiment (Table 2).

Association of polymers with non-everted intestinal rings taken from specific regions of the intestinal tract (as defined in the methods section) is shown in Fig. 4. When incubated in the presence of calf serum (Fig. 4a) it can be seen that the two sugar-containing polymers appear to adhere preferentially to particular regions of the gastrointestinal tract, depending on the particular sugar residue they contain. For example, the galactose-containing polymer 2 bound twice as well to region (a) (duodenum/first part jejunum) compared to region (d). In contrast the fucosylamine-containing polymer showed greatest affinity for more distant regions (c and d) (Fig. 4a). For polymer 6 these phenomena were not influenced by the presence of calf serum proteins. However, interaction of polymer 2 and the cationic derivative (polymer 9) was different in the absence of serum (Fig. 4b). Polymer 9 bound to all the intestinal regions to a much greater extent than any other polymer.

Discussion

Incorporation of cationic groups, or carbohydrate residues, into HPMA copolymers was shown to promote their interaction with the small intestine in vitro. The short duration of the reported experiments, 15 min or up to 30 min, might suggest that the observed accumulation of radioactivity by the intestinal rings could be due to simple adsorption but the nature of HPMA copolymer interaction with intestinal tissue is not clear from data reported here. It is known that macromolecules, including ^{125}I-labelled polyvinylpyrrolidone (PVP) and unmodified ^{125}I-labelled HPMA copolymers are accumulated by rat jejunal sacs in vitro by the mechanism of fluid-phase pinocytosis and, what is more, these polymers are transported across the tissue to the serosal side (Bridges and Woodley, 1987; Cartlidge et al., 1987). It was shown that the amounts of polymer pinocytosed were relatively small, approximately 0.05% of the substrate available per hour. In this study the measured rate (over 30 min) of uptake of polymer 1 (Fig. 2) was equivalent to 0.96 μl/mg protein/h, a value consistent with that reported previously for pinocytic uptake of unmodified HPMA copolymers (0.86 μl/mg protein/h) and PVP (0.76 ± 0.07 μl/mg protein/h) (Bridges and Woodley, 1987; Cartlidge et al., 1987). It is clear that the experimental conditions used maintain tissue viability for the duration of the experiments (Bridges, 1980) but further experiments are necessary to define clearly whether the association of polymers with rat intestinal rings is by a mechanism of simple adsorption, pinocytic uptake or a combination of both.

Interaction of soluble polymers with the intestine is very complex. Polymers may bind (or become entangled within) the glycoproteins that

constitute mucin, they can interact with the many components that are trapped within the mucin matrix, or alternatively, they may bind to the exposed brush border membrane on the mucosal surface. Previously, it has been demonstrated that synthetic polyanions interact strongly with mucin, e.g. polycarboxylic acids (Park and Robinson, 1984a). This is perhaps surprising as most mucopolysaccharides are negatively charged (Allen and Leonard, 1985) and therefore should not attract polyanions electrostatically. In complete contrast we find that the cationic HPMA copolymer (copolymer 9) has by far the greatest affinity for intestinal tissue, association being consistently 5–10 times greater than that observed for any other HPMA copolymers (Fig. 3). Extent of accumulation of this polycation was largely independent of both state of the tissue (i.e. everted or not), and presence of serum proteins. The latter suggest that serum proteins cannot compete with polymer for mucin-related or cell-surface binding sites and the former suggests that interaction is not limited by any mucin depletion which visibly occurs during the evertion process.

The polycationic HPMA copolymer shows high affinity for all parts of the small intestine with little regional specificity (Fig. 4) although presence of calf serum in the incubation medium does slightly alter quantitatively polymer affinity for all regions. More recently (Bridges et al., 1987) have shown that the same cationic HPMA copolymer binds to stomach and small intestine in vivo following administration into the stomach of rats by intubation and this interaction markedly delays transit during the first 5 h after administration. The observed interaction is not surprising as it has been known for many years that low molecular weight quaternary ammonium compounds adhere to intestinal mucus and this affects their absorption in vivo (Levine and Pelikan, 1961).

In the past, polycations have usually proved unsuitable for biomedical use due to their inherent toxicity. Synthetic polyamino acids such as poly-L-lysine and poly-L-ornithine are extremely toxic to cells in μg quantities and the mechanism of their toxicity has been discussed at length (e.g. Quinton and Philpott, 1973). In contrast HPMA copolymers containing quaternary ammonium groups (16.3 mol%), similar to those used in this study do not display the same toxicity problems when incubated with P388D_1 cells in vitro (McCormick et al., 1987). McCormick et al. (1987) showed poly-L-lysine to be highly toxic at a concentration 10 μg/ml, whereas the cationic HPMA copolymer did not display toxicity at concentrations less than 500 μg/ml. Histological examination of adult rat small intestine after administration in vivo of copolymer 9 (total dose 100 μg) has shown no loss of cellular integrity although after 5 h there was some evidence of increased goblet cell discharge (Bridges et al., 1987). Dose-dependency of these effects are now being investigated in more detail.

The association of carbohydrate-containing HPMA-copolymers, although less than seen for the cationic derivative, was significantly greater than measured for unmodified copolymer. Interestingly, tissue eversion caused a 30% increase in the association of polymers 7 and 8, presumably attributable to exposure of specific binding sites not previously available (serum proteins do not compete for this interaction), but polymers containing galactosamine attached to polymer via glycylglycyl spacer (polymer 2), bound equally well to everted and non-everted tissue. These observations may suggest that there is an intestine-related binding site which can recognize and bind galactose. When the sugar is bound directly to the polymer backbone it appears that steric hindrance prevents binding to non-everted tissue, eversion either reveals receptors not otherwise expressed or removes mucus allowing closer access of polymer to existing binding sites. The fact that polymers containing pendent galactosamine bound via a diglycyl spacer associate equally well with everted and non-everted tissue suggest the latter to be true. Hepatocytes contain a specific membrane receptor that recognizes terminal galactose residues on glycoproteins (Bezouška et al., 1985) and we have shown previously that HPMA copolymers containing galactose (cf. polymers 7 and 8) bind with lesser affinity than those containing diglycylgalactosamine (Duncan et al., 1986; Chytrý et al., 1987). Of course this particular phenomenon will be important in the design of polymers for use in vivo in respect of their penetration into

the mucus layer, and/or in relation to barrier effects of the unstirred layer in the intestine (Dietschy and Westergaard, 1977). It is also important that the diglycyl aminosugar side-chain has been shown to be non-degradable following exposure to a wide variety of enzymes (Duncan et al., 1984) and is, therefore, unlikely to liberate aminosugar prematurely during passage along the gastrointestinal tract.

The particular sugar-residue incorporated into the polymer appears to be important in determining the extent of polymer bound (fucosylamine > mannosamine > galactosamine), and also the region of the intestine to which the polymer will most effectively bind (Fig. 4). Although the regional differences reported seem relatively insignificant if judged by their magnitude, their importance would be amplified in context of the total GI tract surface area available in vivo. Delivery of drug to specific regions may be able to promote absorption of particular drugs, e.g. some water-soluble vitamins (ascorbic acid and vitamin B_{12}) are largely absorbed in the distal region of the small intestine (Rose, 1985), and also allow local treatment of GI tract-related disorders.

It is not surprising that synthetic polymers containing carbohydrates should interact with intestinal tissue (or the mucus matrix) in a carbohydrate-dependent manner. Pathogenic bacteria bind to mucosal lectins. *Shigella flexneri* adhere to guinea pig mucosal cells via a fucose- and glucose-specific interaction (Izhar et al., 1982), and the lectin responsible for *E. coli* interaction with colonic mucosa has been partially purified (Ashkenazi 1986). Although the data reported here provide evidence that interaction of HPMA copolymers with the gastrointestinal tract can be manipulated by modifying the polymers chemically, the mechanism of polymer–tissue interaction remains unclear. Light microscopy using colloidal gold–polymer conjugates has indicated that polymers are associated with the intestinal mucus, particularly the acid mucopolysaccharide component. Electron microscopy has shown that these polymer–gold conjugates are not internalized by the enterocyte within the 15 min incubation period, but remain attached to the extracellular mucus and cell debris (results not shown). There is much evidence now that intestinal cells themselves produce lectin-like mucosal adhesins (Izhar et al., 1982; Ashkenazi, 1986) which are soluble, and can be washed away from the enterocyte surface using saline solution. These mucosal adhesins have been shown, in the presence of calcium, to cause a fucose- or mannose-sensitive agglutination of certain invasive enteropathogens (Ashkenazi, 1986).

HPMA copolymers described here could be useful for modifying GI transit time, and perhaps in certain cases, allow a drug formulation to bind with increased affinity to specific regions of the GI tract. Using this system is would be possible to design a drug delivery system that would facilitate both targeting and sustained-release. To enable controlled drug release, peptidyl side-chains have been incorporated into HPMA copolymers tailormade for hydrolysis by chymotrypsin (Kopeček et al., 1981) mixture of lysosomal enzymes (Duncan et al., 1984), cathepsin B (Rejmanová et al., 1983), and specific brush border enzymes (Kopeček et al., 1987a). For use in oral formulations these HPMA copolymers can be prepared as soluble, single-chain polymers (as described here), polymers crosslinked below the gel point (Rejmanová et al., 1981) or as biodegradable hydrophilic gels (Ulbrich et al., 1982).

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References

Allen, A. and Leonard, A., Mucus structure. *Gastroenterol. Clin. Biol.*, 9 (12) bis Suppl. (1985) 9–12.

Ashkenazi, S., Adherence of non-fimbriate entero-evasive *coli* 0124 to guinea pig intestinal tract in vitro and in vivo. *J. Med. Microbiol.*, 21 (1986) 177–123.

Bezouška, K., Táborský, O., Kubrycht, J., Pospíšil, M. and Kocourek, J., Carbohydrate structure dependent recogni-

tion of desialylated serum glycoproteins in the liver and leucocytes. Two complementary systems. *Biochem. J.*, 227 (1985) 345–354.

Bridges, J.F., Millard, P.C. and Woodley, J.F. The uptake of liposome entrapped ^{125}I-labelled poly(vinylpyrrolidone) by rat jejunum in vitro. *Biochim. Biophys. Acta*, 544 (1978) 448–451.

Bridges, J.F., *A. Study of Macromolecule Absorption in vitro by the Small Intestine of Adult Rats*, Ph.D. Thesis, University of Keele, Staffordshire, U.K., 1980.

Bridges, J.F. and Woodley, J.F., A'study of macromolecule absorption in vitro by the small intestine of adult rats, In preparation.

Bridges, J.F., Woodley, J.F., Duncan, R., Kopečková, P. and Kopeček, J., In vitro and in vivo evaluation of *N*-(2-hydroxypropyl)methacrylamide copolymers as potential oral drug delivery system. *Proc. Int. Symp. Controlled Release Mater.*, 14 (1987) 14–15.

Cartlidge, S.A., Duncan, R., Lloyd, J.B., Kopečková-Rejmanová, P. and Kopeček, J., Soluble, crosslinked *N*-(2-hydroxypropyl)methacrylamide copolymers as potential drug carriers 3. Targeting by incorporation of galactosamine residues. Effect of route of administration. *J. Controlled Release,* 4 (1987) 265–278.

Cheng, P.W. and Boat, T.F., An improved method for the determination of galactosaminitol, glucosaminitol, glucosamine and galactosamine on an amino acid analyzer. *Anal. Biochem.*, 85 (1978) 276–779.

Chytrý, V., Kopeček, J., Leibnitz, E., O'Hare, K., Scarlett, L. and Duncan, R., Copolymers of 6-*O*-methylacryloyl-D-galactose and *N*-(2-hydroxypropyl)methacrylamide: targeting to liver after intravenous administration to rats. *New Polymeric Materials*, 1 (1987) 21–28.

Davis, S.S., The design and evaluation of controlled release systems for the gastrointestinal tract. *J. Controlled Release*, 2 (1985) 27–38.

Davis, S.S., Hardy, J.G. and Flara, J.W., Transit of pharmaceutical dosage forms through the small intestine. *Gut*, 27 (1986) 886–892.

Dietschy, J.M. and Westergaard, H., The effect of unstirred water layers on various transport processes in the intestine. In Czaky, T.Z. (Ed.), *Intestinal Absorption and Malabsorption*, Raven, New York, 1975, pp. 197–209.

Dubois, M., Gilels, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F., Calorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28 (1956) 350–356.

Duncan, R., Rejmanová, P., Kopeček, J., and Lloyd, J.B., Pinocytic uptake and intracellular degradation of *N*-(2-hydroxypropyl)methacrylamide copolymers. A potential drug delivery system. *Biochim. Biophys. Acta*, 678 (1981) 143–150.

Duncan, R., Cable, H.C., Lloyd, J.B., Rejmanová, P. and Kopeček, J., Polymers containing enzymatically degradable bonds, 7. Design of oligopeptide side-chains in poly *N*-(2-hydroxypropyl)methacrylamide copolymers to promote efficient degradation by lysosomal enzymes. *Macromol. Chem.*, 184 (1984) 1997–2003.

Duncan, R., Seymour, L.C.W., Scarlett, L., Lloyd, J.B., Rejmanová, P. and Kopeček, J., Fate of *N*-(2-hydroxypropyl) methacrylamide copolymers with pendent galactosamine residues after i.v. administration to rats. *Biochim. Biophys. Acta*, 880 (1986) 62–71.

Duncan, R., Kopečková, P., Strohalm, J., Hulme, I., Cable, H.C., Pohl, J., Lloyd, J.B. and Kopeček, J., Anticancer agents coupled to *N*-(2-hydroxypropyl)methacrylamide copolymers. 1. Evaluation of daunomycin and puromycin conjugates in vitro. *Br. J. Cancer*, 55 (1987) 165–174.

Hinder, R.A. and Kelly, K.A., Canine gastric emptying of solids and liquids. *Am. J. Physiol.*, 233 (1977) E335–E340.

Izhar, M., Nuchamowitz, Y. and Merelman, D., Adherence of *Shigella flexneri* to guinea pig intestinal cells is mediated by mucosal adhesin. *Infection and Immunity*, 35 (1982) 1110–1118.

John, V.A., Shotton, P.A., Moppert, J. and Theobold, W., Gastrointestinal transit of oral drug delivery systems in healthy volunteers. A short report. *Br. J. Clin. Pharmacol.*, 19 (1985) 2035–2065.

Kopeček, J., Rejmanová, P. and Chytrý, V., Polymers containing enzymatically degradable bonds, 1. Chymotrypsin catalyzed hydrolysis of *p*-nitroanilides of phenylalanine and tyrosine attached to side-chains of copolymers of *N*-(2-hydroxypropyl)methacrylamide. *Makromol. Chem.*, 182 (1981) 799–809.

Kopeček, J., Targeting of anticancer drugs by water soluble carriers. *Polymer Preprints*, 27 (1986) 11–12.

Kopeček, J., Biodegradation of polymers for biomedical use. In Benoit, H. and Rempp, P. (Eds.), *IUPAC Macromolecules*, Pergamon, Oxford, 1982, pp. 305–320.

Kopeček, J., Controlled biodegradability of polymers – a key to drug delivery systems. *Biomaterials*, 5 (1984) 19–25.

Kopeček, J. and Duncan, R., Targetable polymeric prodrugs. *J. Controlled Release*, in press (1987).

Kopeček, J., Kopečkova, P., Woodley, J.F. and Duncan, R., (1987a) unpublished data.

Levine, R.R. and Pelikan, E.W., The influence of experimental procedures and dose on the intestinal absorption of onium compound, benzomethamine. *J. Pharmacol Exp. Ther.* 131 (1961) 319–327.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193 (1951) 265–267.

McCormick, L.A., Seymour, L.C.W., Duncan, R. and Kopeček, J., Interaction of a cationic *N*-(2-hydroxypropyl)methacrylamide copolymer with rat visceral yolk sacs cultured in vitro and rat liver in vivo, *J. Bioactive and Compatible Polymers*, 1 (1986) 1–19.

McCormick, L.A., Duncan, R. and Kopeček, J., Biocompatibility of soluble polymers developed as drug-carriers, *Abstracts from Proc. Biointeractions '87*, Butterworth Scientific, London, 1987, p. 31.

Park, K. and Robinson, J.R., Bioadhesive polymers as platforms for oral-controlled drug delivery: a method to study bioadhesion, *Int. J. Pharm.*, 19 (1984a) 107–127.

Park, K., Wood, R.W. and Robinson, J.R., Oral controlled

release systems. In Langer R.S. and Wise, D.L. (Eds.), *Medical Applications of Controlled Release, Vol. I*, CRC Press, Boca Raton, FL, 1984b, pp. 160–201.

Park, K. and Robinson, J.R., Physico-chemical properties of water insoluble polymers important to mucin/epithelial adhesion. *J. Controlled Release*, 2 (1985) 47–57.

Plummer, T.H., A simplified method for determination of amino sugars in glycoproteins. *Anal. Biochem.*, 73 (1976) 532–536.

Quinton, P.M. and Philpott, C.W., A role for anionic sites in epithelial architecture. Effects of cationic polymers on cell membrane structure. *J. Cell Biol.*, 56 (1973) 787–796.

Rejmanová, P., Labský, J. and Kopeček, J., Aminolyses of monomeric and polymeric p-nitrophenyl esters of methacryloylated amino acids. *Makromol. Chem.*, 178 (1977) 2159–2163.

Rejmanová, P., Obereigner, B. and Kopeček, J., Polymers containing enzymatically degradable bonds, 2. Poly[*N*-(2-hydroxypropyl)methacrylamide] chains connected by oligopeptide sequences cleavable by chymotrypsin. *Makromol. Chem.*, 182 (1981) 1899–1915.

Rejmanová, P., Pohl, J., Baudyš, M., Kostka, V. and Kopeček,J., Polymers containing enzymatically degradable bonds. 8. Degradation of oligopeptide sequences in *N*-(2-hydroxypropyl)methacrylamide copolymers by bovine spleen cathepsin B. *Makromol. Chem.*, 184 (1983) 2009–2020.

Říhová, B., Kopeček, J., Kopečková-Rejmanová, P., Strohalm, J., Plocová, D. and Semorádová, H., Bioaffinity therapy with antibodies and drugs bound to soluble synthetic polymers. *J. Chromatogr.*, 376 (1986) 221–233.

Říhová, B., Kopečková, P., Strohalm, J., Rossmann, P., Větvička, V. and Kopeček, J., Antibody directed affinity therapy applied to the immune system. In vivo effectiveness and limited toxicity of daunomycin conjugates to HPMA copolymers and targeting antibody. *Clin. Immunol. Immunopathol.* (1988) in press.

Rose, R.C., Intestinal absorption of vitamins. *J. Inherited Metabolic Disease*, 8 (1985) 13–17.

Seymour, L.C.W., Duncan, R., Strohalm, J. and Kopeček, J., Effect of molecular weight (M_w) of *N*-(2-hydroxypropyl) methacrylamide copolymers on body distribution and rate of excretion after subcutaneous, intraperitoneal and intravenous administration to rats. *J. Biomed. Mater. Res.*, (1987a) in press.

Seymour, L.C.W., Duncan, R., Kopečková-Rejmanová, P. and Kopeček, J., Potential of sugar residues attached to *N*-(2-hydroxypropyl)methacrylamide copolymers as targeting groups for the selective delivery of drugs. *J. Bioactive and Compatible Polymers*, (1987b) in press.

Ulbrich, K., Strohalm, J. and Kopeček, J., Polymers containing enzymatically degradable bonds 6. Hydrophilic gels cleavable by chymotrypsin. *Biomaterials*, 3 (1982) 150–154.