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Uptake and transport of poly(*N*-vinylpyrrolidone-co-maleic acid) by the adult rat small intestine cultured in vitro: effect of chemical structure

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Abstract

To study the structure-activity relationships of polymer uptake and transfer across the gastrointestinal mucosa, poly(*N*-vinylpyrrolidone-co-maleic acid) (NVP MA) polymers of standardised molecular mass (20000 Da) were synthesised to contain side-chains of different charge or hydrophobicity. All polymers additionally contained tyrosinamide residues to permit radioiodination. Using an improved everted gut sac prepared from adult rat small intestine, the tissue accumulation and serosal transport of the polymers was measured in vitro over a 2 h incubation period. All polymers were captured by the tissue linearly with time (endocytic indices between 1.6 and 16 μl/mg protein per h), and then transferred slowly to the serosal fluid (endocytic indices between 0.18 and 2 μl/mg protein per h). The neutral NVP MA polymer showed the lowest rate of tissue association, but this was increased 5-fold by the presence of either negatively or positively charged groups. The maximum rate of transport across the mucosa was seen for the most negatively charged polymer derivative, this being equivalent to approx. 26% its rate of tissue accumulation. Increasing hydrophobicity of the polymer derivatives had a more pronounced effect on the rate of tissue capture, increasing it by up to 10-fold for the most hydrophobic derivative. However, in this case, the serosal transfer was only 10–15% the rate of tissue uptake. The data presented indicate that NVP MA polymers can be tailor-made for use in oral delivery systems. Substituent groups can be incorporated to promote tissue uptake or translocation across the gastrointestinal mucosa, or a combination of the two.

Key words: Polymeric drug carrier; Poly(*N*-vinylpyrrolidone-co-maleic acid); Everted gut sac; Intestinal uptake; Oral drug delivery

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1. Introduction

Synthetic polymers have been used for many years as excipients in oral dosage forms. More recently, however, sophisticated attempts have been made to optimise polymer characteristics specifically to allow control of gastrointestinal (GI) transit (reviewed by Gupta et al., 1990), mediate site-specific drug delivery within the GI tract (Saffran, 1992), to identify opportunities for controlled release by biological means (Kopečková et al., 1991), and to protect and promote uptake of peptide antigens (Morgan et al., 1991). The intestinal transit time for a formulation is 3–4 h from the pylorus to the ileo-caecal junction (Davis et al., 1986, 1987), and this interval is often too short to allow complete absorption of drugs. Park and Robinson (1984, 1985) studied systematically the bioadhesive properties of a number of polymers and concluded that polyanions with a high density of carboxyl groups had greatest potential as bioadhesives. However, this has proved an oversimplification and the ability of polyanions to act as oral bioadhesives in vivo seems to be dose-dependent (Harris et al., 1990). We have shown that cationic derivatives of the synthetic *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers bind strongly to rat small intestinal tissue in vitro (Bridges et al., 1988). HPMA copolymers containing pendant carbohydrate moieties such as galactosamine, mannosamine, glucosamine and fucosylamine show enhanced interaction with the small intestine in vitro and in vivo (Cartlidge et al., 1987; Bridges et al., 1988), and recently it has been suggested that HPMA copolymers containing fucose residues show region-specific bioadhesion towards guinea pig colon in vitro and in vivo (Ríhová et al., 1992; Kopečková et al., 1993).

In addition to their inherent bioadhesive properties, soluble polymers also have the potential to deliver covalently bound drugs, either intracellularly via the cell uptake process of endocytosis (Duncan, 1987), or alternatively, across cellular barriers by the mechanism of transcytosis. Although the latter is a relatively poorly efficient transport process in the gastrointestinal (GI) tract, we have shown, using an in vitro rat everted gut sac model, that GI mucosal cells endocytose macromolecules such as polyvinylpyrrolidone (PVP) (Bridges et al., 1978; Rowland and Woodley, 1981a), *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers (Cartlidge et al., 1986), proteins (Rowland and Woodley, 1981b; Naisbett and Woodley, 1989), and liposomes (Rowland and Woodley, 1981a–c). In each case a small proportion (< 1–5%) of substrate available in the culture medium is passed across the tissue from the mucosal to the serosal side. Before further development of synthetic polymers for use as bioadhesives, or platforms for delivery in oral pharmaceutical formulations, there is still a need to define clearly the structure-activity relationships of polymers that govern their interaction with the small intestinal epithelium and their transport across the mucosal barrier.

In this study we have used a series of modified (*N*-vinylpyrrolidone-co-maleic acid) (NVP MA) polymers to study the relationship between chemical structure of a polymer and its uptake by, and translocation across, the rat intestinal mucosa in vitro. NVP MA is an anionic, non-degradable, water-soluble synthetic polymer with a regular alternating structure previously developed as a polymeric drug-carrier (Azori, 1987). The parent polymer molecule has a narrow molecular mass distribution, and a number of functional groups suitable for chemical modification to give either model compounds or provide drug attachment sites (Csákvári et al., 1981). Drugs have been attached to NVP MA copolymers directly (Pató et al., 1982) or via oligopeptide spacers (Pató et al., 1984) from which they can be released by enzymes of the gastrointestinal tract (Móra and Pató, 1992; Woodley, 1992). Amidation of the polyanhydride with alkylamines yields a series of polymer derivatives with various hydrophobicity and ionic charge which are suitable to investigate the correlation between chemical structure and the biological-biochemical properties of the polymer (Pató and Tüdös, 1989). Here in this study, NVP MA polymers (of standardised molecular mass (20000 Da) and containing a small amount of tyrosinamide to facilitate radioiodination) were synthesised to contain different degrees of hydrophobicity, negative or positive charge (Fig. 1

and Table 1). Their rate of uptake by adult rat intestine, and transport across the tissue was measured in vitro using an everted gut sac method that we have previously described (Bridges, 1980).

2. Materials and methods

2.1. Synthesis of NVP MA copolymers

The parent NVP MA copolymer was synthesised by radical copolymerisation of *N*-vinylpyrrolidone with maleic anhydride in benzene at 60°C using azo-bis-isobutyronitrile as an initiator. Precipitated copolymer was then purified by chloroform extraction, and the chemical composition determined by IR, NMR and elemental analysis (Fehérvári et al., 1987). The molecular mass was determined by viscometry as previously described (Csákvári et al., 1981). Synthesis of the copolymer derivatives used in this study is summarised in Table 1. First the parent NVP MA copolymer was dissolved in dry dimethylformamide (DMF) and 2 molar% (calculated on the basis of anhydride groups) of tyrosinamide was coupled to the backbone, to permit radiolabelling of the molecule with [^{125}I]iodide. The resultant product was precipitated with diethyl ether and its tyrosinamide content was determined by UV spectroscopy as 1.5 molar%. The structure is shown in Fig. 1. This material was divided into several fractions for preparation of each derivative (100%

Table 1
Summary of the synthesis of pendant side chains on the parent NVP MA backbone

Reactant	Product Side-chains: R_1 R_2		Polymer no.
H_2O	→	-COO^-	
		-COO^-	1
NH_3	→	-$CONH_2$	
		-COO^-	2
$HO(CH_2)_2NH_2$	→	-$CONH(CH_2)_2OH$	
		-COO^-	
$+HO(CH_2)_2NH_2$ + CME-CDI	→	-$CONH(CH_2)_2OH$	
		-$CONH(CH_2)_2OH$	3
Z-$NH(CH_2)_5NH_2$	→	-$CONH(CH_2)_5$NH-Z	
		-COO^-	
$+$Z-$NH(CH_2)_5NH_2$ + CME-CDI	→	-$CONH(CH_2)_5$NH-Z	
		-$CONH(CH_2)_5$NH-Z	
$+H_2/Pd$	→	-$CONH(CH_2)_5NH_3^+$	
		-$CONH(CH_2)_5NH_3^+$	4
$C_4H_9NH_2$	→	-$CONHC_4H_9$	
		-COO^-	5
$C_8H_{17}NH_2$	→	-$CONHC_8H_{17}$	
		-COO^-	6

Full details are given in section 2. CME-CDI, (*N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate); Z, carbobenzyloxy-.

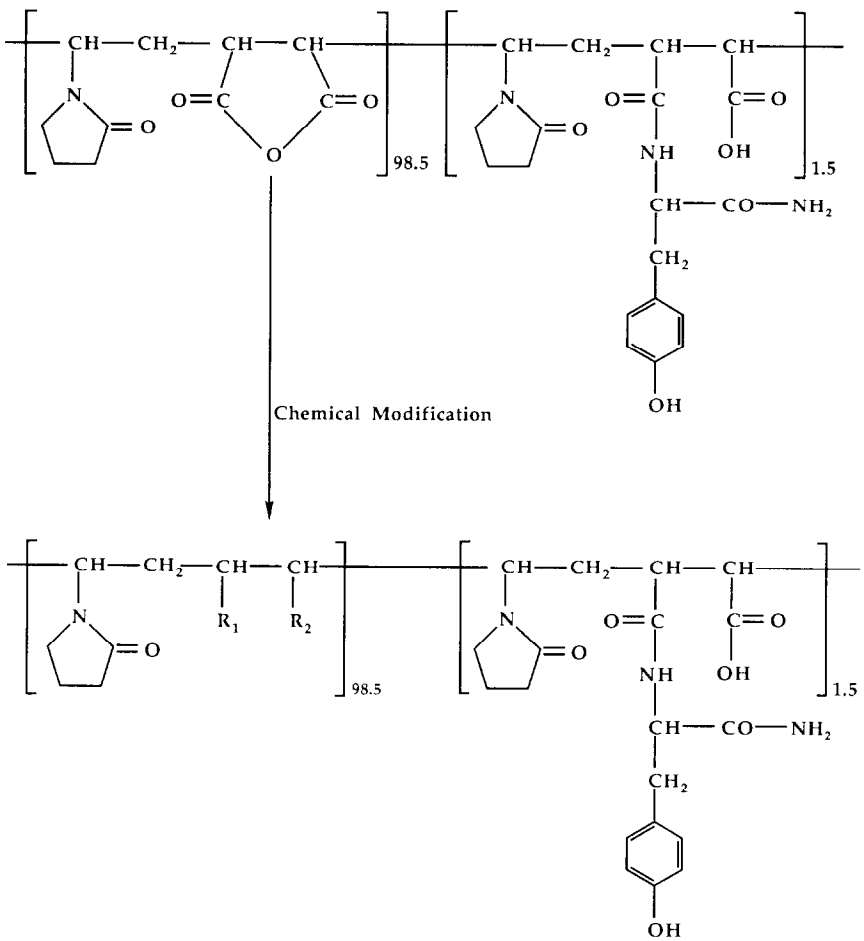

Fig. 1. Structure of parent poly(*N*-vinylpyrrolidone-co-maleic acid) (NVP MA) with the addition of tyrosinamide and modification with pendant side-chains.

COOH content determined by titrimetric analysis).

The copolymer derivatives were prepared as follows (shown in summary in Table 1): One fraction of the parent tyrosinamide polyanhydride was hydrolysed by 2% (w/v) sodium bicarbonate solution, with pH adjustment to pH 7.2, to produce copolymer 1. The unsubstituted semi-amide derivative (copolymer 2) was prepared by dissolving the polyanhydride in liquid ammonia. To four of the fractions, dissolved in dimethylformamide, the following were added at 2 molar excess: 2-aminoethanol, 1-carbobenzyloxyamino-5-aminopentane, *n*-butylamine and *n*-octylamine. The hydroxyethylamide separated from the reaction mixture while the others were precipitated by diethyl ether. All of these products were dissolved in distilled water and ultrafiltered after adjusting the pH to 7.2. Ultrafiltration was performed using Amicon UM2 membranes until no free amine could be detected by ninhydrin in the low molecular weight fraction. The purified solutions were freeze-dried and the products (copolymers 5 and 6) characterised by their N content, determined by elemental analysis. Copolymers 3 and 4 (diamide derivatives) were prepared from the monohydroxyethylamide and 1-carboxbenzyloxyamino-pentylamide derivatives as follows: the semi-amides were dissolved in water and excess of the appropriate amino components and

water-soluble [*N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CME-CDI)] were added. The carbobenzyloxy diamide precipitated, while the hydroxyethyl derivative was ultrafiltered directly. The carbobenzyloxy derivative was deblocked in ethanolic solution by catalytic hydrogenation (Pd/charcoal), the catalyst filtered off, and the solution evaporated. The residue was dissolved in water, the pH adjusted to 7.2 with 0.1 N HCl, and the product ultrafiltered. The high molecular mass fractions were freeze-dried to yield coploymer products 3 and 4. Carboxyl and amino contents were verified by titration with 0.1 N NaOH or 0.1 N HCl respectively. All the derivatives were characterised by elemental analysis and IR spectroscopy.

2.2. Radioiodination

Each NVP MA (5–10 mg) derivative was radiolabelled with $Na^{125}I$ using a modified chloramine T method (Duncan et al., 1984), and following the reaction, free [^{125}I]iodide was removed by extensive dialysis against 1% (w/v) sodium chloride. The amount of [^{125}I]iodide in the reaction mixture and resultant preparations was estimated using paper electrophoresis (Whatman No. 1 filter paper, run in sodium barbitone buffer (0.05 M, pH 8.6) for 25 min at 400 mV, 10–15 mA) The specific activities of the labelled polymers were approx. 20 μCi/mg.

2.3. Uptake by everted intestinal sacs

^{125}I-labelled NVP MA copolymer derivatives were incubated with everted intestinal sacs using the improved gut sac culture method originally described by Bridges (1980). This technique uses tissue culture medium (TC199) to ensure tissue viability for an incubation period up to 2 h. Adult male Wistar rats (250–300 g) were humanely killed, and the entire small intestine quickly excised and flushed through with 0.15 M saline. The intestine was everted over a notched glass rod and placed in oxygenated TC199 medium at 37°C. The whole length of the intestine was filled with fresh, oxygenated medium and this was then divided into sacs of approx. 1.5 cm in length (their contained tissue culture medium is henceforth designated serosal fluid). Each sac was placed in an Erlenmeyer flask containing 10 ml of TC199 medium and subsequently incubated with radiolabelled NVP MA copolymer (2 μg/ml) for periods up to 120 min. At the appropriate times, the sacs were removed and the serosal fluid collected. The sacs were then washed 4 times in ice-cold 0.85% (w/v) saline and dissolved in 1.0 M NaOH (25 ml). Samples of the medium, serosal fluid, and the tissue digest were assayed for radioactivity. The protein content of the digest was determined using the Lowry method as modified by Peterson (1983). In each experiment, duplicate sacs were taken at each time point, and the data shown represent between four and 10 separate experiments performed with each copolymer. The uptake and transport of the radiolabelled NVP MA copolymers was expressed as a clearance rate, or endocytic index, as first defined by Williams et al. (1975). The endocytic index expresses uptake (or serosal transport) as an equivalent μl of the culture medium whose contained substrate is captured (or transported)/mg tissue protein per h. Use of this clearance unit permits comparison of the rate of uptake, or transport, for a variety of different substrates.

2.4. Intestinal sac viability

When using tissue culture systems to monitor physiological processes it is important to confirm tissue viability for the duration of the experiment. This is particularly true when potentially toxic substrates are added to the culture medium (such as synthetic polymers of different charge and hydrophobicity). Here the ability of the intestinal sacs to maintain a glucose concentration [inside] > [outside] was taken as evidence of integrity and tissue viability. The tissue culture medium contains 5.6 mM glucose, and at 10, 30 and 100 min of incubation the glucose concentration in the medium and in the serosal fluid was measured using a glucose oxidase-based test kit (Boehringer Mannheim).

Table 2
Ability of everted adult rat intestinal sacs to concentrate glucose in the presence of NVP MA copolymers

Polymer code no. (charge)	Ratio of glucose concentration (medium : serosal) [a]		
	(Incubation time, min)		
	10	30	100
1 (2−)	1:1.09	1:1.28	1:1.67
2 (1−)	1:1.13	1:1.22	1:1.47
4 (2+)	1:1.05	1:1.21	1:1.34

[a] Intestinal sacs were incubated with NVP MA copolymers 1, 2 and 4 and the glucose concentration in the incubation medium and the serosal fluid determined at each time point as described in the Methods. Each ratio represents the mean of three values.

3. Results

3.1. Sac viability

Table 2 shows that at all time points there was a higher concentration of glucose in the serosal fluid than found in the external culture medium, and that the concentration ratio [internal]:[external] increased progressively with time. This indicates that the sacs were intact and biologically viable throughout the time course of experiments in which the tissue was exposed to either cationic or anionic NVP MA copolymers.

3.2. Tissue accumulation of NVP MA copolymers

Tissue uptake of all the NVP MA copolymers was linear with time, thus enabling endocytic indices to be calculated by regression analysis. Fig. 2 shows examples of the tissue uptake seen. The mean endocytic indices observed for tissue uptake and serosal transfer of all the copolymers tested are shown in Fig. 3. The uncharged derivative (copolymer 3) showed the slowest rate of uptake, with an endocytic index of 1.6 ± 0.345 μl/mg protein per h. The anionic derivatives, copolymer 2 (1 −) and copolymer 1 (2 −) showed a greater rate of uptake than the uncharged derivative, with endocytic indices of 3.90 ± 0.37 and 7.82 ± 1.04 μl/mg protein per h, respectively. However, a higher rate of tissue uptake, an endocytic index of 9.61 ± 1.62 μl/mg protein per h, was seen with the polycationic derivative, being 6-fold higher than that observed for the uncharged copolymer. It should be noted, however, that increasing the hydrophobicity of the NVP MA derivatives by addition of pendant side chains with increasing numbers of C atoms led to rates of tissue uptake greater than that of the charged coploymers, and the cationic copolymer (no. 4)

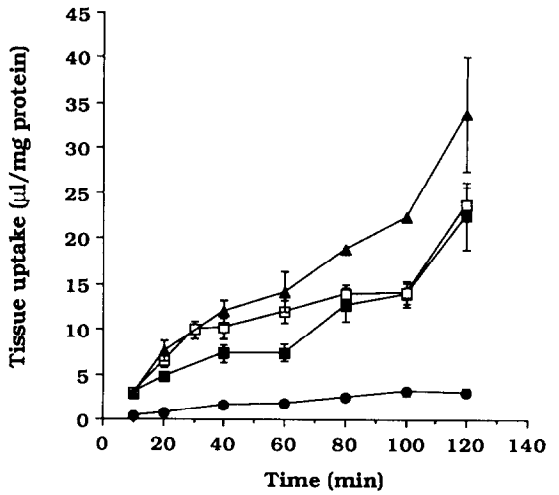

Fig. 2. Tissue accumulation of NVP MA copolymers by rat everted gut sacs. The uptake of copolymer 1 (2−) (■), copolymer 3 (neutral) (●), copolymer 4 (2+) (□), and copolymer 6 (C8 side-chain) (▲) is shown. Each point is mean ± S.E. of 3–9 replicates.

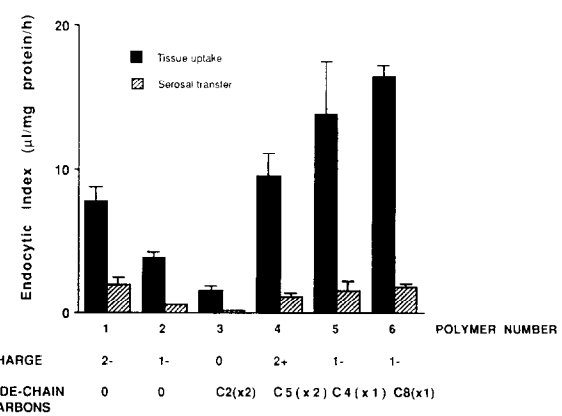

Fig. 3. Structure activity relationships of NVP MA copolymer uptake and transfer across the GI for all copoymers in the series. Values are means ± S.E. of 3–9 replicates. Copolymer numbers refer to Table 1.

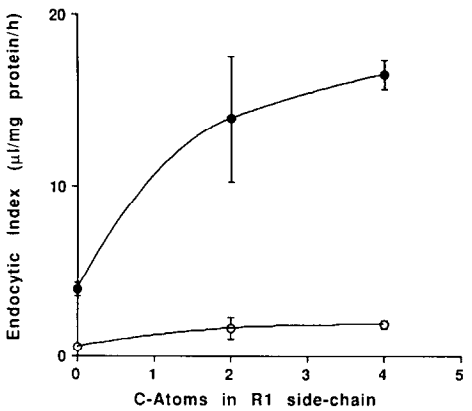

Fig. 4. The relationship between tissue uptake and transfer to serosal side and the hydrophobicity of derivatives with a single aliphatic pendant side-chain and one negative charge per copolymer repeat unit (copolymers 2, 5 and 6). Tissue uptake (●); serosal transfer (○). Values are the means ± S.E. of 4–6 replicates.

contained five side C atoms in position R_1 and R_2. Copolymers 5 and 6, containing pendant hydrophobic side-chains (four and eight side-chain C atoms), showed 3.6- and 4.2-fold greater endocytic indices (13.9 ± 3.7 and 16.5 ± 0.84 μl/mg protein per h), respectively, than copolymer 2 which might be viewed as the nearest control in this series (they all contain one negative charge per unit). The relationship between the C-chain length and endocytic index for tissue and serosal uptake can be seen in Fig. 4.

3.3. Transfer of NVPMA copolymers to the serosal side of everted sacs

All the copolymers were transported across the tissue into the serosal fluid, and the radioactivity recovered there increased linearly with time. In general the rate of transport across the tissue showed a direct correlation with the rate of tissue uptake, and usually was equivalent to 10–15% of the rate of uptake in to the tissue. However, the parent polyanion (copolymer 1) was the exception. This polymer displayed a rate of transfer across the tissue (2.01 ± 0.48 μl/mg protein per h) that was 11.2-fold greater than that shown by the uncharged copolymer 3 (0.18 ± 0 μl/mg protein per h). The rate of serosal transfer of this polyanion was equivalent to 26% of the tissue uptake rate. The relationship between tissue uptake and serosal transfer for this polymer is shown in Fig. 5. With the polycationic derivative (copolymer 4), while the rate of uptake into the tissue was slightly higher than the polyanion, there was considerably less material transferred across the mucosal wall, with the rate of mucosal transfer being 12% of the tissue uptake rate. The hydrophobic copolymers, 5 and 6, were transported across the everted sacs at a rate which directly correlated with their endocytic index measured for tissue uptake, the rates of transfer being 1.63 ± 0.63 and 1.9 ± 0.23 μl/mg protein per h, respectively. The overall relationship between the rate of serosal transfer and NVP MA chemical composition is also summarised in Fig. 3, with the hydrophobicity data shown again in Fig. 4.

4. Discussion

Soluble synthetic polymers of weight average molecular weight 20000–40000 and bearing no net charge, such as PVP and HPMA copolymers, have been shown to be captured by adult rat intestine cultured in vitro with an endocytic index of 0.6–0.8 μl/mg protein per h, and they are

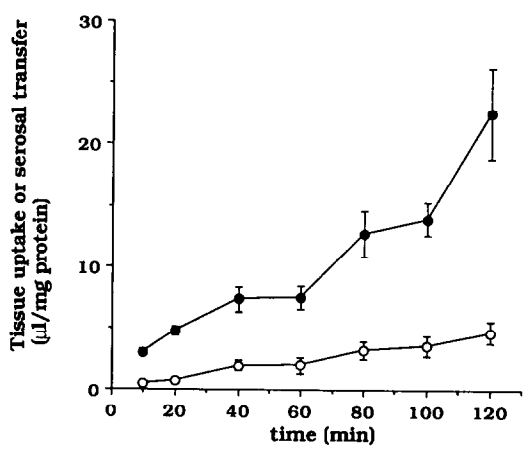

Fig. 5. Relationship between tissue uptake and serosal transfer shown for the polyanionic copolymer 1 (2−). Tissue uptake (●) and serosal transfer (○). All points are the mean ± S.E. of 6–9 replicates.

subsequently transported to the serosal side of the tissue at a rate of approx. 0.12–0.33 μl/mg protein per h (Bridges, 1980; Rowland and Woodley, 1981a; Cartlidge et al., 1986). Cartlidge et al. (1986) further demonstrated that uptake of HPMA copolymers by rat everted gut sacs in vitro was a molecular weight-dependent process, with the highest tissue accumulation and transport across observed with the highest molecular weight HPMA copolymer fraction (> 400 000). The endocytic indices for uptake and transport of this fraction were reported as 3.22 ± 0.36 and 0.49 ± 0.13 μl/mg protein per h, respectively. The family of NVP MA copolymers used in this current study have the advantage that their molecular weight could be standardised, therefore it was possible to investigate, independently of molecular weight effects, the relationship between the physico-chemical characteristics of these polymers and their rate of tissue association and transport across the GI mucosa. It can be seen that the neutral NVP MA copolymer 3 has an endocytic index for tissue uptake of 1.6 ± 0.345 μl/mg protein per h which is approximately twice that reported previously for PVP and HPMA copolymers in the same model system. However, the rate of transfer of this neutral NVP MA polymer to the serosal fluid was similar to that reported previously for HPMA copolymers and PVP.

Although each ^{125}I-labelled NVP MA polymer showed a progressive increase in tissue association with time, this could theoretically represent either their internalisation by epithelial cells via the endocytic pathway, or, alternatively, simply reflect adsorption of polymer to the epithelial cell surface which is not dislodged during the rigorous washing procedure. In most cases, there was good correlation between the rate of tissue uptake of NVP MA copolymer derivatives, and the rate of appearance of radioactivity in the serosal fluid (approx. 10–15% of the rate of uptake by the tissue), indicating that copolymer was indeed captured by the mechanism of endocytosis and subsequently a proportion of the material internalised was translocated to the serosal side within the limits of the 2 h culture period used. The fact that the tissue accumulation is continued over the incubation period, and only a proportion of the material taken up is transferred to the serosal fluid could suggest one or more of the following explanations; in part tissue accumulation of polymer represents progressive mucosal surface binding (this seems unlikely); that serosal transfer is the rate-limiting step which delays material intracellularly; or alternatively that there are two distinct pathways for transport, one leading to lysosomal deposition and the other acting via a transcytosis route.

The charged NVP MA copolymers (both cationic and anionic polymers) displayed a higher endocytic index for tissue association than the neutral counterpart (copolymer 3), and in the case of the polyanions, there was a clear relationship between greater polymer charge density and a higher endocytic index (Fig. 3). These observations are consistent with the reports in the literature suggesting that both polycations and polyanions have bioadhesive properties. However, it was interesting to note the anionic parent copolymer was transported across the tissue more rapidly than any of the other derivatives. In fact, as a proportion, the rate of transfer to serosal fluid was equivalent to more than 26% of the endocytic index measured for tissue uptake, which was higher than any of the other copolymers studied. It is known that polyanions can inhibit intracellular vesicle fusion events (D'Arcy Hart and Young, 1975) and it has been suggested that the anionic dextran sulphate may be orally active as a drug and hence traverse the GI mucosa (Abrams et al., 1989). These observations certainly suggest that anionic NVP MA copolymers offer greatest potential to promote mucosal to serosal transfer of covalently bound drugs.

Polycations in general are known to interact strongly with negatively charged cell membranes (Quinton and Philpott, 1973), and in the case of a cationic derivative of HPMA, the copolymer was found to bind strongly to the liver cell membranes after intravenous administration. Subcellular fractionation showed that the polymer was poorly endocytosed by liver cells in vivo remaining fixed at the cell surface (McCormick et al., 1986). Recently, polycationic derivatives of HPMA have been studied in the everted gut sac

system and the uptake into the tissue shown to be enhanced several fold compared with uncharged copolymer of similar molecular weight (Blundell, unpublished). However, the maximal tissue uptake achieved (endocytic index = 2.6 μl/mg protein per h) was lower than observed with NVP MA in the current study. As shown in Fig. 3, the cationic NVP MA copolymer had a rate of transfer to the serosal side that was similar to that seen for most of the other derivatives so it is impossible to speculate whether or not cationic polymers may be processed via a pathway distinctly different from that used by the anionic derivatives. Further studies involving subcellular fraction of the tissue after exposure to NVP MA polymers would clarify this point.

Introduction of hydrophobic side-chains into NVP MA copolymers, in combination with a single anionic side chain per unit (copolymers 5 and 6), produced the highest tissue endocytic indices (Figs. 3 and 4). However, although the rates of transfer across the tissue were higher than seen for the neutral polymer, they were lower than for the anionic polymer 1. These observations are in accord with previous studies that have demonstrated the ability of hydrophobic residues to enhance membrane adsorption of polymers, and thus promote non-specific adsorptive endocytosis. Using the rat visceral yolk sac as a model to study endocytosis in vitro, we have shown that HPMA copolymers bearing pendant tyrosinamide residues (Duncan et al., 1984) and poly(α,β-hydroxyethylaspartamide) containing tyramine residues (Duncan et al., 1982) displayed endocytic indices which were related to the number of tyrosinamide or tyramine containing side-chains present in the polymer. In particular, substitution of 10 mol% or more caused a marked increase in membrane association with a 10-fold increase in endocytic index. A similar observation has been seen using the everted sac gut sac system, though the increase in endocytic index was only 5-fold (Blundell et al., 1993). In the case of the NVP MA copolymers in the current study, hydrophobic and anionic side groups in combination produced the considerable enhancement of tissue uptake. Copolymer 3 contained two two-carbon side chains per polymer chain unit, but was uncharged and gave the lowest tissue uptake and transfer across the serosa.

NVP MA copolymers may provide an interesting drug delivery system. The whole body distribution, elimination kinetics and acute toxicity of the copolymer have been documented previously (Azori et al., 1986). However, this study serves to illustrate the importance of studying carefully the structure activity relationships of any candidate polymer prior to development for use in a specific oral delivery application. To facilitate transfer across the GI it would seem that the anionic parent NVP MA copolymer offers the best opportunity to promote transfer across the mucosal barrier. Cationic, anionic and hydrophobic derivatives all appear better bioadhesives than the neutral analogue. In addition, if such polymers are used for covalent attachment to drugs, it has been shown previously that the kinetics of the enzymatic release of a drug analogue linked to the NVP MA copolymers via a peptide linkage is modulated by the overall charge on the copolymer (Móra and Pató, 1992) and therefore this is another feature that must be borne in mind when ultimately considering the desired location for, and rate of, drug release.

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