Polymeric drug delivery of enzymatically degradable pendant agents: peptidyl-linked procainamide model system studies

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Abstract

Biodegradable polymeric drug delivery systems have become increasingly important for sustained release indications when a time-limited drug implant is required. A new methacrylic copolymer consisting of enzymatically-cleavable oligopeptidyl procainamide as pendant side chains was synthesized in a series of three reactions. Using high performance liquid chromatography (HPLC) analysis, procainamide release was monitored while incubating polymer specimens in the presence of a model enzyme, α-chymotrypsin, in a physiologic buffer at 37°C. It was found that the new polymeric drug conjugate was insoluble in aqueous solutions and it was relatively stable when not in the presence of the enzyme, releasing not more than 5 mg of drug/g polymer after 30 days incubation under physiologic conditions. However, in the presence of α-chymotrypsin, the procainamide side chains were gradually enzymatically cleaved over 20 days, and the rate of hydrolysis could be controlled by varying the enzymatic incubation conditions. The enzymatic rate dependency of each formulation was dependent upon the comonomer ratio and the degree of crosslinking. These two factors influenced the accessibility of the water-insoluble polymers to enzyme. It is concluded that procainamide release from an enzymatically degradable pendant-peptide link can be achieved in an enzymatically controllable manner. © 1997 Elsevier Science B.V. All rights reserved

Keywords: Biodegradable drug delivery systems; Cardiac arrhythmia; Controlled drug delivery; Methacrylic polymers; Polymeric prodrugs; Polymer synthesis; Procainamide

1. Introduction

Drug targeting to a specific tissue or organ has been the subject of creative and innovative re-

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search in medicinal and pharmaceutical chemistry since the beginning of the twentieth century. In many diseases (e.g. cancer, AIDS, rheumatoid arthritis, etc.) a considerable therapeutic advantage could be gained if drugs were delivered more selectively and in a controlled manner to their target sites. More particularly, it is conventionally accepted that efficient, compliant and reliable therapy requires that the drug resides as long as its therapeutic action is needed at a specific site, where it acts (by systemic absorption, binding, inhibition, etc.) as intact molecules. This concept has led to a development of a variety of physically-based controlled release dosage forms such as drug-dispersible matrices, coated tablets or particles, microcapsules, Aza's Oros® osmotic pump. Our present research focused on a the chemical approach of drug targeting, involving a specific degradable linkage of a drug to a carrier molecule, which may be a polymer. This chemical approach can provide a wide variety of possibilities to achieve drug targeting or site-specific delivery. Previous studies have already pointed out that peptidic side chains of N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers can be degraded by α-chymotrypsin (Drobnik et al., 1976; Kopecek and Rejmanova, 1979; Kopecek et al., 1981a), and lysosomal enzymes (Duncan and Lloyd, 1980; Duncan et al., 1982, 1983). These soluble HPMA copolymer conjugates are undergoing clinical evaluation as injectable agents for cancer chemotherapy (Duncan, 1992). Oligopeptidic side chains in synthetic matrix systems have been shown to degrade in-vivo when investigated with intravenous studies in rats (Kopecek et al., 1981b). Although all these studies were performed without drug, the results demonstrated the potential of the polymeric side-chains as drug carriers. Other reports have shown that chemically based systems may be produced by covalent coupling of the desired drug with an antibody carrier moiety and, if necessary, with a protective moiety (Rowland et al., 1975; Barton et al., 1991).

In this study, we synthesized and characterized a new water-insoluble methacrylic copolymer with a pendant pharmaceutical. The

synthesis can be quantitatively performed with a variety of factors that may control the drug release. The new polymeric pendant derivative was hydrolysed by proteolytic enzymes in vitro, followed by drug release. The release of the cleaved drug could allow not only the site-enhanced-specific delivery, but also as desired, it can result in sustained release of pharmacologically active concentrations at the active sites.

The present experiments have focused on procainamide as a model compound in a drug-polymer adduct system for potentially targeting the myocardium. In a previous publication, procainamide was coupled to the polysaccharides, dextran and inulin, after partial periodate oxidation (Schacht et al., 1984) and the soluble conjugates were shown to be captured by the rat visceral yolk sac by a mechanism of pinocytosis (Remon et al., 1984). Procainamide is generally administered orally or intravenously for the treatment of cardiac arrhythmias after acute myocardial infarction. As a drug-polymer adduct, procainamide could be delivered into either the endocardium by coronary artery injection during catheterization or open heart surgery for suppression of post-operative arrhythmias. The rationale for the choice of procainamide in a drug delivery system targeted to the myocardium is based on the following considerations. Due to the rapid metabolism of procainamide and the short duration of its action, blood levels must be maintained by high oral dosages. Furthermore, the high incidence of systemic adverse effects limits its usefulness for long-term systemic therapy. In addition, its chemical structure allows a reaction process that forms a covalent bonding with oligopeptide side-chains of macromolecules.

Thus, the goals of our study were as follows: (1) to formulate and synthesize procainamide-peptide-hydro gel linkage that would be enzymatically hydrolysable, (2) to assess the stability of this linkage under simulated physiologic condition in the absence of proteases, and (3) to investigate a model enzymatic drug delivery system involving procainamide-peptide-hydrogel linkages, which would be hydrolysed, following incubations with chymotrypsin.

2. Materials and methods

2.1. Materials

Procainamide (free base) and procaine were obtained from Sigma (MO). Methacryloyl chloride, methacrylic acid, and tetraethyleneglycol dimethacrylic acid were purchased from Polysciences, (PA) and were distilled before use. Dicyclohexylcarbodiimide (DCC) was obtained from Sigma and azo-*bis*-isobutyronitrile (AIBN) from Polysciences. Glycylglycine was purchased from Aldrich (WI). α-Chymotrypsin was obtained from Sigma. Potassium phosphate, ammonium acetate and sodium hydroxide pellets were obtained from J.T. Baker (NJ). Dimethylformamide (J.T. Baker) was distilled over calcium oxide and stirred with potassium hydroxide before use in reactions. Ethyl alcohol, acetone and acetic acid (J.T. Baker) were analytically pure. Acetonitrile (J.T. Baker) was high performance liquid chromatography (HPLC) grade.

2.2. Apparatus

Mass spectral analysis was performed with a Finnigan 4021 VG Analytical 70-250S (UK) using a fast atom bombardment (FAB) technique. Dithiothreitol (also denoted by us as 'magic bullet') was used to prepare the matrix sample.

HPLC analysis of procainamide was performed on a Waters Model 501 (MA), which was equipped with C_{18} column (Waters μBondapak™ C_{18}, 15 cm × 3.9 mm i.d., 10 μm). Procainamide was detected with a Waters 481 spectrophotometric detector at 274 nm. Data was recorded on a Waters 740 Data Module single-channel integrator.

2.3. Synthesis of methacryloylglycylglycine (MGG)

Glycylglycine (26.4 g, 0.2 mol) was dissolved with 8 g sodium hydroxide in 40 ml of double-distilled water at 0°C. A second solution containing 8 g NaOH in 40 ml water was prepared and cooled to 0°C. Methacryloyl chloride (19.5 ml = 20.9 g, 0.2 mol) was placed in an ice bath at 0°C.

Then, the two sodium hydroxide solutions were added dropwise and simultaneously into the methacryloyl chloride vessel. The mixture was left while stirring for 12 h at room temperature, then 1 N HCl solution was added until pH was brought to 2. The precipitated product was collected by filtration, redissolved in 800 ml of 50% ethyl alcohol and crystallized at 4°C. A recrystallization of the product yielded: 19.0 g (47.5%), with a mass spectrum of 200 (molecular ion), 126, 199, 69 (see Section 3, below).

2.4. Synthesis of methacryloylglycyglyl (diethylaminoethyl-p-amidobenzamide) ('MGGPA' monomer)

MGG (5.48 g, 27.4 mmol) and procainamide (6.45 g, 27.4 mmol) were dissolved in 60 ml of dimethylformamide. Approximately 6.22 g (30.14 mmol) of DCC were added and the mixture was stirred overnight at room temperature. The mixture was then filtered to remove the dicyclohexylurea precipitate. The solution was left for one more night at 4°C and then refiltered. Small amount of acetic acid was added for precipitation of unreacted procainamide and MGG, then the solution was filtered and evaporated to dryness under reduced pressure. The yield was 9.13 g (79.9%), and the mass spectrum of the product was 572 (M^+ + dithiothreitol), 418 (M^+), 236 (procainamide) (see Fig. 2 and Section 3, below).

2.5. Copolymerization

Copolymerization of MGGPA monomer and methacrylic acid was performed at four different comonomer (molar) ratios, 90:10, 80:20, 70:30, and 50:50, respectively. A stock solution (0.1 g/ml) of methacrylic acid was prepared in dimethylformamide. The following aliquots from this solution were taken with 0.5 g (1.2 mmol) MGGPA in a 5 ml teflon vial:

(1) to prepare a 90:10 monomer ratio, 0.115 ml (0.133 mmol);

(2) to prepare a 80:20 monomer ratio, 0.258 ml (0.30 mmol);

(3) to prepare a 70:30 monomer ratio, 0.440 ml (0.51 mmol);

(4) to prepare a 50:50 monomer ratio, 1.033 ml (1.20 mmol).

The volume was made up to 1.5 ml, followed by an addition of 45 mg of AIBN, the free-radical initiator. The obtained solution was then incubated for 12 h at 55°C under nitrogen atmosphere. After polymerization took place, the solvent was evaporated from the obtained slab. The slab was rinsed with acetone, flushed with an excess of double-distilled water, then freeze-dried for 24 h.

2.6. Crosslinking

The crosslinking was carried out during polymerization as above. Into the mixture solutions of MGGPA monomer and methacrylic acid, appropriate amounts of tetraethyleneglycol dimethacrylic acid were added from a 0.1 g/ml solution to obtain 0.5 or 1% (molar weight) cross-linkages.

2.7. In vitro degradation of procainamide-polymer conjugate

Two in vitro testing protocols were performed with the various copolymers obtained. In the first, the stability of the peptidyl linkage between procainamide and the macromolecule was tested in 0.05 M phosphate buffer (pH 7.4) at 37°C for 6 months. In the second series of experiments, the cleavage of the drug from the macromolecule was assessed by using α-chymotrypsin. Accurately weighed polymer slabs (0.2 g) were placed in 3 ml of phosphate buffer (0.05 M, pH = 7.8) at 37°C, then 0.2 ml of α-chymotrypsin aqueous solution (10 mg/ml) was added and mixed. In predetermined time intervals, the slabs were transferred to fresh enzyme/buffer solutions, and the previous incubation solutions were frozen until analyzed. All in vitro experiments were performed in duplicate.

2.8. Procainamide analysis

Procainamide containing samples were analyzed using a HPLC system with an isocratic mobile phase. The mobile phase consisted of 0.075 M ammonium acetate buffer (pH = 4.3) and acetonitrile at a 80:20 ratio. The flow rate was 1.3 ml/min. Specimens (0.3 ml) were taken from each sample (volume, 3.2 ml). Seventy microliters of procaine solution (internal standard, 1 mg/ml) were added to the specimens, which were vortexed; 20 μl samples were then injected into the HPLC system.
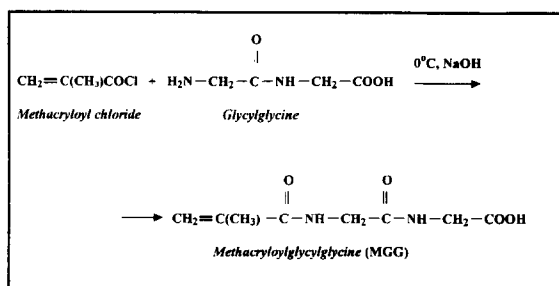
3. Results and discussion

3.1. Polymer preparation

Fig. 1 shows the schematic process in which the macromolecular drug conjugate was synthesized. In the first step (Fig. 1(a)) a Schotten-Baumann reaction between methacryloyl chloride and glycylglycine was performed in an ice bath with a dropwise addition of a sodium hydroxide solution. The recrystallized product (yield = 47%) was identified by mass spectral analysis as methacryloylglycylglycine (MGG). In the second step (Fig. 1(b)), the MGG was coupled with procainamide. The product was filtered and crystallized (yield = 79.9%), and was identified as the methacryloylglycylglycyl derivative of procainamide (MGGPA, see Fig. 2 for mass spectrum). Radical polymerization took place as the last step (Fig. 1(c)), in which the MGGPA monomer was mixed together with methacrylic acid monomer in dimethylformamide at the desired ratios. The reaction occurred at 55°C in the presence of azo-bis-isobutyronitrile as the initiator. The resultant polymer was thoroughly washed in acetone, and then rinsed with an excess of water. The dry weight was measured after the washed polymer had been freeze-dried.
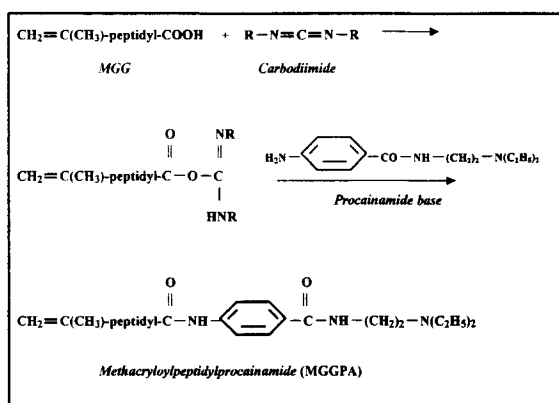
3.2. In vitro evaluation of drug cleavage

Fig. 3 demonstrates the stability of the procainamide-polymer adducts during a 6 month period in phosphate buffer (0.05 M, pH 7.4, 37°C). It was first observed that all conjugates including the uncrosslinked polymers were insoluble in water, keeping their physical structure for the whole incubation period. It should be noted that similar

Step I: Schotten-Baumann Reaction

$$CH_2=C(CH_3)COCl \ + \ H_2N-CH_2-\overset{O}{\overset{|}{C}}-NH-CH_2-COOH \ \xrightarrow{0^\circ C, \ NaOH}$$

Methacryloyl chloride   *Glycylglycine*

$$\longrightarrow \ CH_2=C(CH_3) \ -\overset{O}{\overset{\|}{C}}-NH-CH_2-\overset{O}{\overset{\|}{C}}-NH-CH_2-COOH$$

Methacryloylglycylglycine (MGG)

Step II: Monomer Synthesis

$$CH_2=C(CH_3)\text{-peptidyl-COOH} \ + \ R-N=C=N-R \ \longrightarrow$$

MGG   *Carbodiimide*

$$CH_2=C(CH_3)\text{-peptidyl-}\overset{O}{\overset{\|}{C}}-O-\overset{\overset{NR}{\overset{\|}{C}}}{\underset{HNR}{|}} \quad H_2N-\langle\ \rangle-CO-NH-(CH_2)_2-N(C_2H_5)_2$$

Procainamide base

$$CH_2=C(CH_3)\text{-peptidyl-}\overset{O}{\overset{\|}{C}}-NH-\langle\ \rangle-\overset{O}{\overset{\|}{C}}-NH-(CH_2)_2-N(C_2H_5)_2$$

Methacryloylpeptidylprocainamide (MGGPA)

Step III: Copolymerization

$$CH_2=C(CH_3)\text{-peptidyl-}\overset{O}{\overset{\|}{C}}-NH \longrightarrow \xrightarrow{+MA, \ initiator, \ 55^\circ C} ----[-CH_2-C(CH_3)-]_x---[-CH_2-C(CH_3)-]_y-$$

MGGPA

C=O
NH
(CH₂)₂
N
CH₂ CH₂
CH₃ CH₃

C=O
Peptidyl
C=O
NH
C=O
NH
(CH₂)₂
N
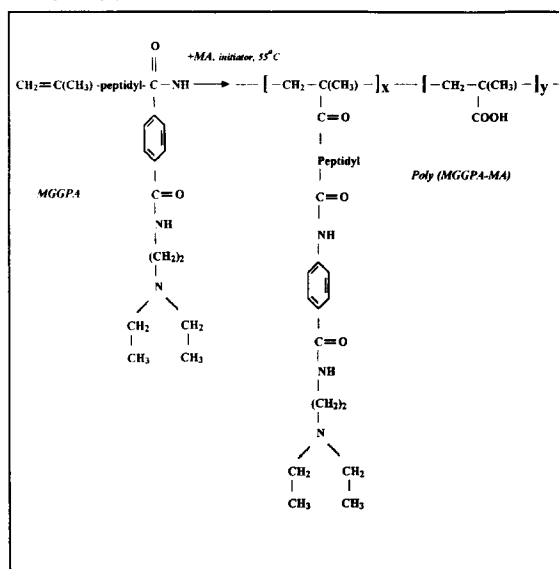CH₂ CH₂
CH₃ CH₃

COOH

Poly (MGGPA-MA)

Fig. 1. The synthetic methodology for producing a methacrylic copolymer bearing glycylglycyl(diethylaminoethyl-*p*-amidobenzamide) side groups. R is cyclohexyl, 'peptidyl' refers to glycyl-glycine and MA to methacrylic acid.
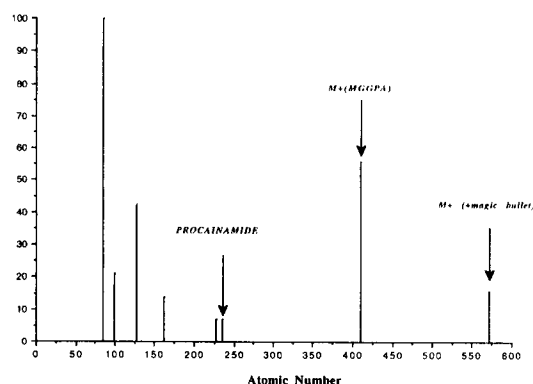

Fig. 2. Mass spectrum analysis of the monomer, methacryloylglycylglycyl derivative of procainamide.

phenomenon has also been reported by Sintov et al. (1993) while synthesizing uncrosslinked, water-insoluble methacrylic polymers containing pendant disaccharide groups. It was also observed that hydrolysis of procainamide took place in the course of this period, and that the drug release was dependent on the polymer crosslinking (0.5% molar weight) and the comonomer ratio. Fig. 4(a,b) describes the profiles of procainamide cleavage from the polymer side-chains. The oligopeptide 'spacer group' underwent chymotrypsin-catalyzed hydrolysis in a physiologic buffer. The extent of the enzyme hydrolysis was significantly greater than the water hydrolysis exhibited in Fig. 3.

The overall experimental results of the present studies demonstrated that: (1) Both the degree of cross-linking and the content of the drug-containing monomers in the copolymer were essential factors for the stability and the enzymatic degradation of the drug moiety along the polymeric backbone, (2) A hydrolytic process took place in a physiologic buffer (as shown in Fig. 3), resulting in a drug loss from the polymeric backbone. Nevertheless, the rate and extent of the cleavage were very low (maximum, 0.15 mg cleaved drug/polymer/day), and only a negligible content of the drug (max. 3 mg free drug/g polymer) was cleaved during the first 20 days of the testing. (3) In vitro degradation of the
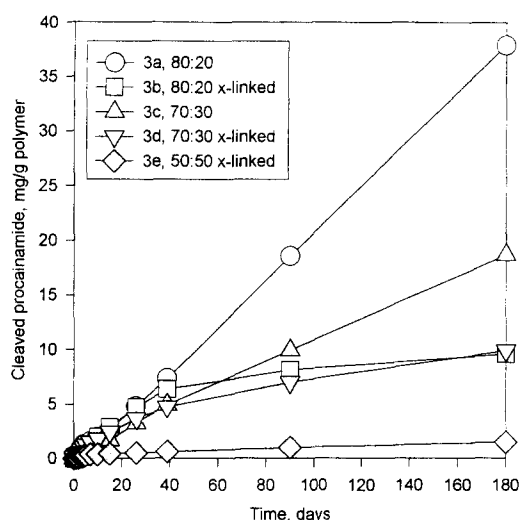
Fig. 3. Chemical stability of the polymeric drug conjugate in a physiologic solution is shown comparing free drug released from polymers of 80:20 MGGPA/MA comonomer ratio, free drug released from polymers of 70:30 MGGPA/MA comonomer ratio, and the comparisons between the cleavage/release profiles of three crosslinked polymers with different monomer ratios.

polymeric substrate with a model enzyme, α-chymotrypsin, was achieved. An interesting phenomenon was observed when polymers containing higher ratios of the monomeric drug hydrolysed faster in the stability tests and slower in the experiments which involved enzymes (Fig. 3(a,c) vs. Fig. 4(b)). This phenomenon indicates a possible formation of different sizes of amorphic regions in the polymeric network, which depends on the monomer ratio. These regions are the sites where the enzymes can be accessed and function. The hydrolysis rate in this enzymatic system can be defined as:

$$- \, \mathrm{d}C/\mathrm{d}t = KC \qquad (1)$$

where, K is the hydrolysis rate constant and C is the drug concentration per unit weight of the polymer at time t.

After integrating Eq. (1):

$$C = C_0 \mathrm{e}^{-Kt} \qquad (2)$$

where, C_0 is the initial concentration of the pendant drug.

At the end of the effective drug release period (t_e), which depends upon the accessible regions:
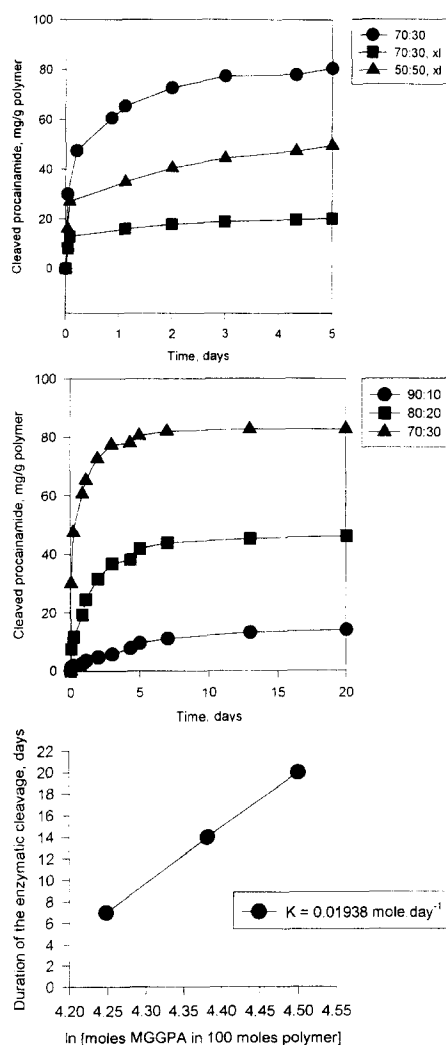

Fig. 4. Enzymatic cleavage of pendant procainamide in various drug-linked polymers. (a) Shown are comparisons between an uncrosslinked polymer with 70:30 MGGPA/MA ratio and the corresponding crosslinked polymer (1% molar weight) and a crosslinked polymer (1% molar weight) with lower MGGPA fraction (50:50 MGGPA/MA comonomer ratio), (b) In addition three uncrosslinked polymers manufactured with various MGGPA/MA comonomer ratios, (c) The relationship between the starting MGGPA/MA ratios in the copolymer as ln (moles) of MGGPA in 100 moles of polymer and the duration time of drug release from the cleaved side chains is also shown.

$$R_{\mathrm{e}} = KC_0 \mathrm{e}^{-Kt_{\mathrm{e}}} \qquad (3)$$

where, R_{e} is the rate of hydrolysis at t_{e}.

Since t_{e} is actually the duration of maximum availability of the released moiety (T) hence,

$$T = (1/K) \ln C_0 - (1/K) \ln(R_{\mathrm{e}}/K) \quad \text{or}$$

$$T = N \ln C_0 - M \qquad (4)$$

where, M and N are constants.

When plotting T versus C_0, a straight line is obtained (Fig. 4(c)). The enzymatic hydrolysis rate constant (K) of a series of such polymers with various drug content can be easily calculated from the slope of this linear plot.

Furthermore, the enzymatically hydrolysable drug linkage reported here suggests a number of ideal usages of this drug delivery approach. Sustained drug administration could be achieved by co-administration of relevant proteases, or by matrix degradation due to bioavailable enzymes as well. Specific applications might include optimizing regional gastrointestinal drug absorption. In addition, co-implants of enzyme incorporated in a controlled release polymer could also be carried out in order to achieve specific regional drug delivery rates. Furthermore, a variable rate drug infusion pump could be positioned next to an enzymatically hydrolysable polymer so as to vary regional drug delivery based on appropriate closed-loop feedback.

Regeneration of the pendant drug supply could be achieved with an appropriate prodrug consisting of an agent such as procainamide covalently linked to a reactive group such as an aldehyde, which could thereby bond to pendant peptide amino groups present after hydrolysis. Regeneration would also offer the possibility of varying the agent, by achieving regeneration with various other pharmaceuticals. In addition, should continuing drug activity become undesirable, such as in proarrhythmia, pendant drug linkages could also be prevented from undergoing hydrolysis by various capping reactions achieved through regional pertusion with appropriate reagents.

4. Conclusion

Polymeric carriers containing oligopeptide-linked drugs as side-chains could be ideal as they are relatively stable in the biological environment, while the amino acid sequences are susceptible to cleavage by specific proteases. The cleavage rate and extent may be easily optimized for specific proteolytic enzymes in the target tissue or organ by the following factors: (a) types of the comonomer (methacrylate, acrylate, hydroxyethyl methacrylate, or hydroxypropyl methacrylate), (b) degrees of the comonomer incorporation which also influence the degrees of swelling at the physiologic pH, (c) the length of the oligopeptide side-chain, (d) amino acid sequences in the oligopeptidic spacer, and (e) the degree of crosslinkage.

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