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Research Paper

PEGylation of Octreotide: II. Effect of N-terminal Mono-PEGylation on Biological Activity and Pharmacokinetics

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Purpose. To determine the optimal polyethylene glycol (PEG)-conjugate of octreotide by evaluating the effects of PEGylation chemistry on the biological activity and pharmacokinetic properties.
Methods. Octreotide was chemically modified by reaction with succinimidyl propionate monomethoxy PEG (SPA-mPEG, molecular weight 2000) or succinimidyl butyraldehyde-mPEG (ALD-mPEG, molecular weight 2000 and 5000). The structural conformation of PEG-octreotides was evaluated by circular dichroism (CD), the biological activity was assessed by measuring the decrease of serum insulin-like growth factor-I levels in rats, and a pharmacokinetic study was performed after subcutaneous administration in rats. The stability against acylation was investigated with poly(D,L-lactide-*co*-glycolide) (PLGA).
Results. ALD-mPEG was site-specific in PEGylating octreotide at the N-terminus. The mono-PEG-octreotides prepared with ALD-mPEG (mono-ALDPEG-octreotide), which alkyl bond preserves the amine's positive charge, showed complete preservation of biological activity, whereas the PEG-octreotides prepared with SPA-mPEG showed lower activity. In the CD analysis, the spectra of the mono-ALDPEG-octreotides were nearly superimposable with that of native octreotide. The mono-ALDPEG-5K-octreotide showed significantly improved pharmacokinetic properties compared with mono-ALDPEG-2K-octreotide as well as native octreotide. Both mono-ALDPEG-2K- and mono-ALDPEG-5K-octreotides were stable against acylation by degrading PLGA.
Conclusions. The mono-PEGylation of octreotide at N-terminus with ALD-mPEG produced a conjugate that is biologically and structurally active and stable against acylation by PLGA, and therefore it may serve as a candidate for somatostatin microsphere formulations.

KEY WORDS: biological activity; octreotide; site-specific PEGylation; pharmacokinetics; stability.

INTRODUCTION

Octreotide, a synthetic octapeptide analogue of somatostatin, is clinically used for the treatment of acromegaly and certain endocrine tumors (1,2). It has been commercially formulated in poly(D,L-lactide-*co*-glycolide) (PLGA) microspheres (Sandostatin LAR depot, Novartis Pharma, Basel, Switzerland) for intramuscular administration on a monthly basis. In addition to the convenience of a monthly injection, the depot preparations showed the same or even increased effectiveness compared with three-daily subcutaneous injections in lowering growth hormone and insulin-like growth factor (IGF)-I (3).

Although biodegradable poly(D,L-lactide) (PLA) and PLGA have been widely used for the long-term controlled release of peptides and proteins, the acidic microenvironment inside the matrix due to degradation of PLA and PLGA has been reported to cause instability (4–6). Recently, it was shown that peptide impurities formed within the degrading PLA or PLGA matrix by acylation with lactic and glycolic acid units (7–9) and that lactoyl and glycoyl adducts of octreotide formed after *in vitro* incubation of microspheres in phosphate buffer saline (10).

The covalent attachment of polyethylene glycol (PEG) to the peptide, PEGylation, appears to be a promising approach for stabilizing octreotide because it has been widely used to improve the chemical and biological stability of salmon calcitonin, a 32-amino-acid peptide (11,12). PEGylated octreotide (PEG-octreotide) showed much better stability than native octreotide against acylation in lactic acid solutions (13). In addition, an interaction study with hydrophilic and hydrophobic PLGA showed that N-terminal PEGylation could effectively prevent acylation of octreotide by degrading PLGA (14). The stability of N-terminally PEGylated octreotide may be attributed to the lowered nucleophilic reactivity of the Lys residue in octreotide and the steric hindrance of the PEG strand.

The objective of this study was to assess the best PEG-octreotide that is biologically active and stable against acylation with PLGA for microsphere delivery. Besides the advantageous effects of PEGylation such as stabilization and longer circulation half-life, the problem of preserving the biological activity of biomolecules after PEGylation is particularly im-

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portant with a small peptide. A number of factors, for example, PEGylation site, PEG size, and linkage between peptide and PEG, can affect the biological activity (15). Morpurgo *et al.* reported the effects of the type and location of PEGylation on the structural and biological activity of different mono-PEGylated somatostatin analogue RC160 (16). In the current study, PEG-octreotides with different PEGylation sites and linkages were prepared with two PEG reagents having different functional groups. PEGylation with the *N*-hydroxysuccinimidyl ester-activated PEG nonspecifically resulted in two mono- and one di-PEG-octreotide, whereas that with aldehyde-activated PEG produced N-terminally mono-PEGylated octreotide in a site-specific manner (17). Their structural conformations were evaluated by circular dichroism, and biological activity was assessed by measuring the decrease of serum IGF-I levels in rats. The pharmacokinetic parameters were assessed after subcutaneous administration to rats, and the stability against acylation by PLGA was evaluated.

MATERIALS AND METHODS

Materials

Octreotide acetate (MW 1019.26) was obtained from Bachem (Torrence, CA, USA). Succinimidyl propionate-monomethoxy PEG (SPA-mPEG, MW 2000) and butyraldehyde-mPEG (ALD-mPEG, MW 2000 and 5000) were purchased from Nektar Therapeutics (Huntsville, AL, USA). Hydrophilic 50:50 PLGA polymers (Resomer RG502H) was supplied by Boehringer Ingelheim (Ingelheim, Germany). Alpha-cyano-4-hydroxycinnamic acid (α-CHCA), Endoproteinase Lys-C (from Lysobacter enzymogenes, sequencing grade), and dithiothreitol (DTT) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade) was supplied from Fisher Scientific (Fair Lawn, NJ, USA). Trifluoroacetic acid (TFA) was obtained from Pierce (Rockford, IL, USA). All other chemicals were of analytical grade and used as obtained commercially.

PEGylation of Octreotide

Site-specific PEGylation of N-terminus in octreotide with ALD-mPEG was carried out in the presence of 20 mM sodium cyanoborohydride ($NaCNBH_3$) in 0.1 M acetate buffer at pH 5. The reaction was performed with different molar ratios of 1:1, 1:2, and 1:3 (octreotide:ALD-mPEG) at 4°C overnight. PEGylation with SPA-mPEG at a molar ratio of 1:1, 1:2, and 1:3 (octreotide:SPA-mPEG) was performed in 0.1 M sodium phosphate buffer (pH 6) for 1 h at room temperature. Each reaction was repeated in triplicate. The PEGylation reaction mixtures were loaded onto reversed-phase high-performance liquid chromatography (RP-HPLC) and the PEG-octreotides were isolated. The purified PEG-octreotides were freeze-dried after evaporation of organic solvent with a Speed-Vac (Eppendorf, Hamburg, Germany). The molecular weights of the PEG-octreotides were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Identification of PEGylation Sites

PEGylation sites were identified as described previously (18,19). PEG-octreotides were treated with DTT at a final concentration of 5 mM for 4 h to reduce the disulfide bond. Thereafter, enzymatic digestion with endoproteinase Lys-C was performed in 0.1 M Tris-HCl buffer (pH 8.0) at 37°C with an enzyme to substrate ratio of 1:100 (w/w) for 4 h. The concentrations of PEG-octreotides were 200 μg/ml. The Lys-C digests were directly analyzed by MALDI-TOF MS. The PEGylation sites were determined by measuring molecular weights of PEG-octreotide fragments digested with Lys-C.

RP-HPLC

The PEGylation reaction mixtures were loaded onto a HPLC system (2 LC-6A pumps, SIL-6B autoinjector, SPD-6AV detector and SCL-6B system controller from Simadzu Scientific Instruments, Inc., Columbia, MD, USA). A Prosphere C-18 column (4.6 × 250 mm, Alltech, Deerfield, IL, USA) with guard column (4.6 × 7.5 mm) was used with a mobile phase A (0.1% [v/v] TFA in water) and B (0.1% [v/v] TFA in acetonitrile). The gradient elution conditions for octreotides modified with SPA-mPEG and ALD-mPEG were 65:35 to 50:50 and 70:30 to 40:60 (A:B), respectively, for 20 min at a flow rate of 1.0 ml/min, and the chromatograms were recorded by UV detection at 215 nm.

MALDI-TOF MS

The molecular weights of the PEG-octreotides were obtained on a Kratos Kompact SEQ time-of-flight mass spectrometer (Manchester, UK) (20). The α-CHCA in 50% acetonitrile in water with 0.1% TFA was used as matrix. Data for 2-ns pulses of the 337-nm nitrogen laser were averaged for each spectrum in a linear mode, and a positive ion TOF detection was performed using an accelerating voltage of 20 kV.

Circular Dichroism

Circular dichroism (CD) spectra were recorded in the range of 190–250 nm with a Jasco J-710 spectropolarimeter (Easton, MD, USA) using a CD cell of 0.1-cm pathlength and a bandwidth of 1 nm. A scan speed of 20 nm/min was used with an average of five scans per sample. The spectra were expressed as the mean residue molar ellipticity in deg·cm^2/dmol. Peptide concentrations were precisely determined by RP-HPLC and set to 200 μg/ml in 0.1 M phosphate buffer (pH 7.4).

Biological Activity

Male Sprague-Dawley rats (n = 6 per sample), weighing 220–250 g, were used to evaluate biological activity of octreotide and PEG-octreotides. The animals were housed in groups of two in a well-ventilated environment under controlled temperature (22 ± 1°C) and humidity (60 ± 5°C), with food and water available *ad libitum*. The octreotide and PEG-octreotides dissolved in water for injection (WFI) were injected subcutaneously at the back of the neck at a dose of 200 μg peptide/kg. The injections were repeated at 2, 24, and 26 h. A control group received WFI. Blood samples were removed from the tail vein at 0, 2, 4, 24, 26, 28, and 30 h, centrifuged in Microtainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA), and the collected serum was frozen and stored at –20°C until analysis. The IGF-I levels were determined by radioimmunoassay (RIA) with a Rat IGF-I RIA kit having a sensitivity of 21 ng/ml (Diagnostic Systems Laboratories,

Webster, TX, USA). Serum IGF-I levels of the animal prior to administration of octreotide were set to 100% (initial value), and the averaged IGF-I levels after administration (at 26, 28, and 30 h) were expressed as percent of initial value.

Pharmacokinetic Study

Octreotide and PEG-octreotides in WFI (100 μg/ml) were subcutaneously injected into male Sprague-Dawley rats weighing 220–250 g (n = 6 per sample) at a dose of 100 μg/kg octreotide. Samples were taken at 0, 0.5, 1, 2, 3, 4, and 6 h, and the serum octreotide levels were determined by RIA (Peninsula Laboratories Inc., San Carlos, CA, USA). A standard calibration curve was constructed using native or mono-ALDPEG-octreotides. Pharmacokinetic parameters were determined using a noncompartmental pharmacokinetic model using WinNonLin 4.1 software (Pharsight, Mountain View, CA, USA).

Interaction with Polymers

One hundred milligrams of RG502H was added to 10 ml of octreotide or mono-ALDPEG-octreotide (peptide concentration of 200 μg/ml) in 0.1 M phosphate buffer (pH 7.4) at 37°C. Samples were collected at 1, 3, 7, 14, 21, 28, 35, 42, and 49 days, centrifuged, and the supernatant analyzed by RP-HPLC. The gradient elution conditions for octreotide and mono-ALDPEG-octreotide were 80:20 to 65:35 and 70:30 to 40:60 (A:B), respectively, for 20 min at a flow rate of 1.0 ml/min, and the chromatograms were recorded by UV detection at 215 nm.

Statistical Analysis

The biological activity and pharmacokinetic parameters were subjected to an unpaired Student's *t* test using Microsoft Excel Software. A p value <0.05 was considered as significant.

RESULTS AND DISCUSSION

Preparation and Characterization of PEG-Octreotides

General amine PEGylation using SPA-mPEG with *N*-hydroxysuccinimide (NHS) ester structure will modify the N-terminus and Lys residue of octreotide, whereas that with ALD-mPEG, having an aldehyde group, can be site-specific for the N-terminus in the presence of sodium cyanoborohydride at acidic pH (Fig. 1). Specific PEGylation at the

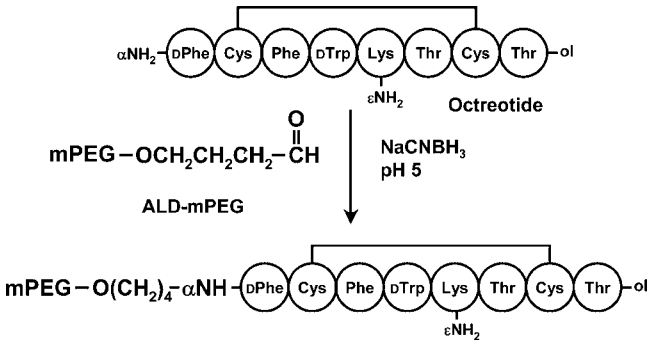

Fig. 1. Schematic diagram for the specific N-terminal PEGylation of octreotide.

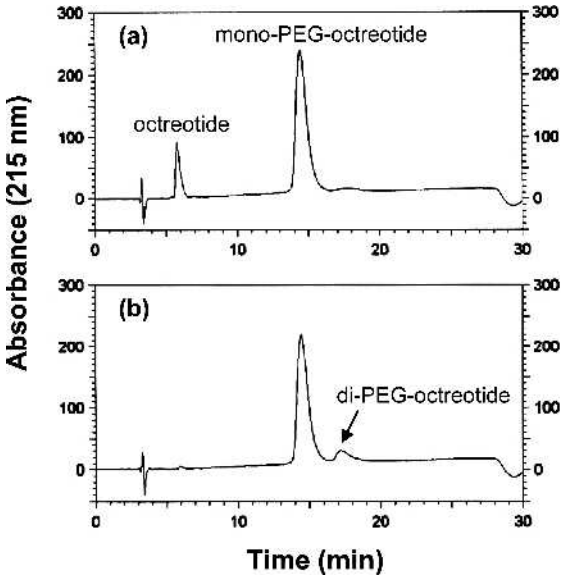

Fig. 2. RP-HPLC chromatograms of octreotides reacted with ALD-mPEG-2K. The reaction molar ratios (octreotide:ALD-mPEG-2K) were 1:2 (a) and 1:3 (b).

N-terminus is based on the difference in reactivity of the α-amino group in the N-terminus (pK_a = 7.8) and ε-amino group in the Lys residue (pK_a = 10.1) at acidic pH (21). The N-terminally site-specific PEGylation with ALD-mPEG forms a secondary amine linkage and thus preserves the positive charge on the N-terminal amino group, whereas the PEGylation with SPA-mPEG leads to an amide bond, which removes the charge on the amino groups of the N-terminus or Lys residue.

Figure 2 shows the RP-HPLC chromatograms of octreotide and ALD-mPEG-2K mixtures at different molar ratios. The mono-ALDPEG-octreotide was preferentially produced at a molar ratio of 1:3 (octreotide:ALD-mPEG-2K). Table I shows the characterization of the PEG-octreotides by MALDI-TOF MS after isolation by RP-HPLC and Lys C digestion. Three PEG-octreotides were produced with SPA-mPEG, that is, mono-SPAPEG-Phe^1-, mono-SPAPEG-Lys^5-, and di-SPAPEG-octreotide, while a single mono ALDPEG-Phe^1-octreotide was produced with ALD-mPEG-2K and -5K reagents. The PEGylation sites were determined by measurement of the mass change based on the PEG resistance to the proteolytic cleavage during Lys C digestion (18,19). As the octreotide has only one lysine residue, PEGylation at this site would show no mass change when treated with Lys-C after

Table I. Characterization of PEG-Octreotides by MALDI-TOF MS

Sample	Mass[a] of insect molecule	Mass after Lys C digestion
Mono-ALDPEG-2K-Phe^1-octreotide	3287	2934
Mono-ALDPEG-5K-Phe^1-octreotide	6505	6149
Mono-SPAPEG-2K-Phe^1-octreotide	3243	2930
Mono-SPAPEG-2K-Lys^5-octreotide	3242	3242
Di-SPAPEG-2K-octreotide	5534	5534

The molecular mass of octreotide is 1019 Da.
[a] The number is centroided mass value of the polydisperse peaks.

Table II. PEGylation of Octreotides at Various Molar Ratios

PEG	Molar ratio (octreotide:PEG)	% Composition in reaction mixture[a]			
		Octreotide	Mono-PEG-Phe[1]-octreotide	Mono-PEG-Lys[5]-octreotide	Di-PEG-octreotide
ALD-PEG-2K	1:1	52 ± 3.9	48 ± 3.9	—	—
	1:2	13 ± 2.2	83 ± 3.5	—	4 ± 1.0
	1:3	1 ± 0.2	89 ± 2.9	—	10 ± 1.2
ALD-PEG-5K	1:1	27 ± 4.1	73 ± 4.1	—	—
	1:2	8 ± 1.5	90 ± 3.0	—	2 ± 0.8
	1:3	—	76 ± 2.8	—	24 ± 2.8
SPA-PEG-2K	1:1	58 ± 2.6	28 ± 3.1	10 ± 2.2	4 ± 1.8
	1:2	23 ± 2.3	44 ± 3.8	13 ± 2.9	20 ± 1.4
	1:3	10 ± 1.1	44 ± 3.2	11 ± 1.9	35 ± 2.6

[a] Mean value ± SD, n = 3.

reduction of the disulfide bond with DTT. However, such treatment of the mono-PEG-octreotide modified at the N-terminus would result in the reduced mass fragment. The mass changes of approximately 350 Da in the mono-ALDPEG forms demonstrate site-specific PEGylation at the N-terminus. The identities of mono- and di-SPAPEG-2K-octreotides were also confirmed by measuring their intact and Lys-C treated masses.

To optimize site-specific PEGylation condition and investigate the site-specificity, the PEGylation reactions of octreotide with ALD-mPEG-2K, -5K, and SPA-mPEG-2K were monitored at different molar ratios (Table II). The PEGylation with ALD-mPEG-2K and -5K resulted in production of each mono-ALDPEG-octreotide with a yield of approximately 90%. When the reaction was performed at the molar ratio of 1:3 (octreotide:ALD-mPEG), the area percents of di-ALDPEG-2K- and di-ALDPEG-5K-octreotides in reaction mixtures were 10% and 24%, respectively. As the presence of di-ALDPEG-octreotide causes difficulty in separating the mono-ALDPEG-octreotide, the molar ratio of 1:2 was selected for isolating each mono-ALDPEG-octreotide. The PEGylation using SPA-mPEG-2K was performed at pH 6 because the α-amino group of the N-terminus is more susceptible to the PEGylation reaction at low pH (12). However, the yield of mono-SPAPEG-Phe[1]-octreotide did not exceed 44%.

Structural Study by Circular Dichroism

Figure 3 shows the CD spectra of octreotide and the mono-PEG-octreotides prepared with ALD-mPEG-2K, -5K, and SPA-mPEG-2K. The spectra of intact octreotide and mono-PEG-octreotides prepared with ALD-mPEG-2K and -5K were nearly superimposable in the range of 190 to 250 nm, suggesting that PEGylation using ALD-mPEG had no significant effect on the secondary structure of the octreotide. However, mono-PEG-octreotides prepared with SPA-mPEG-2K showed spectral changes in the range of 190 to 210 nm, which might indicate some conformational modification. This result strongly supports the importance of the positive charge for preserving the structural conformation after PEGylation, as previously reported by Morpurgo *et al.* with PEGylated RC160 (16). Concerning the possibility of the involvement of thiol group from the reduction of disulfide bond with N-terminal PEGylation using ALD-mPEG and $NaCNBH_3$, no spectral change of mono-ALDPEG-octreotides suggests that the disulfide bond is stable and not involved in the PEGylation process because the reduction of disulfide bond substantially changes the spectra (22).

Biological Activity

Octreotide substantially reduces growth hormone and IGF-I levels in patients with acromegaly (2). It binds with a high affinity to somatostatin receptor subtype 2 and 5 and Phe[3]-Trp[4]-Lys[5]-Thr[6] in the structure is known to be essential for biological activity (23). Serum IGF-I is an important

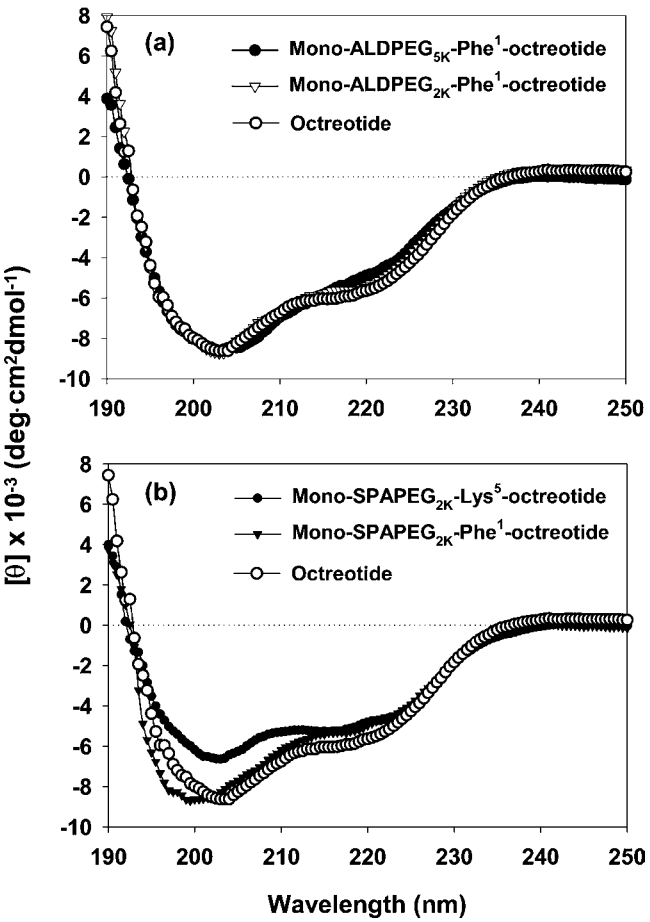

Fig. 3. Circular dichroism of octreotides modified with ALD-mPEGs (a) and SPA-mPEGs (b). The concentrations of PEG-octreotides were set equivalent to 200 μg/ml of octreotide.

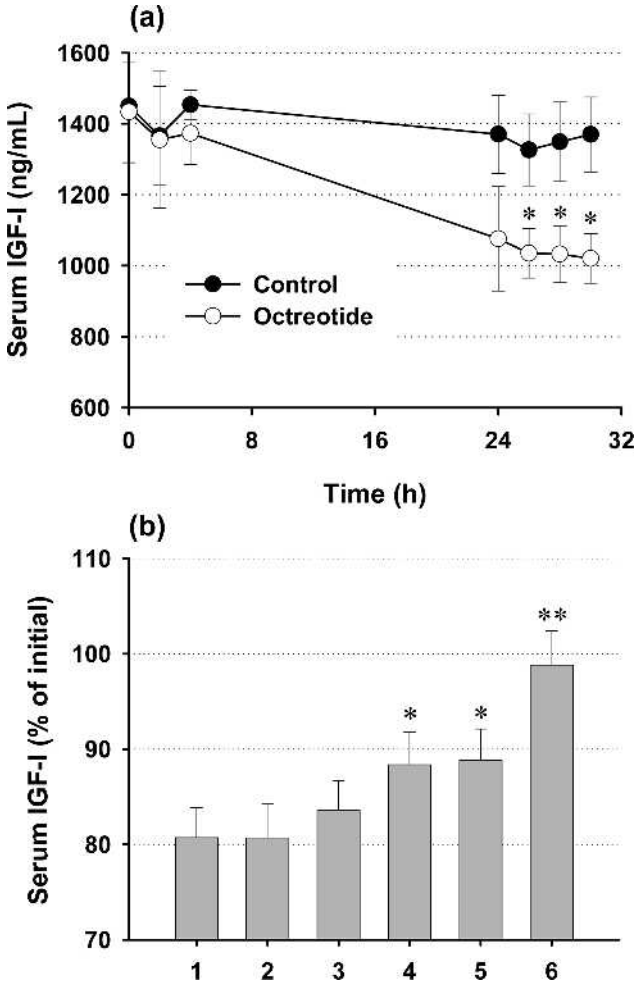

Fig. 4. Biological activities of octreotide and PEG-octreotides. (a) Effect of octreotide on rat IGF-I serum levels after subcutaneous administration at 0, 2, 24, and 26 h. *Significantly different from control. (b) The changes of rat IGF-I serum levels after 24 h after subcutaneous administration of octreotide and PEG-octreotides (1, octreotide; 2, mono-ALDPEG-2K-octreotide; 3, mono-ALDPEG-5K-octreotide; 4, mono-SPAPEG-2K-Lys^5-octreotide; 5, mono-SPAPEG-2K-Phe^1-octreotide; 6, di-SPAPEG-2K-octreotide). Significant differences from native octreotide: *$p < 0.05$; **$p < 0.01$. Mean ± SD, n = 6.

marker for diagnosis of acromegaly and monitoring the efficacy of treatment (24). The biological activity of PEG-octreotides was evaluated by measuring the capability to decrease serum IGF-I levels in rats. As the IGF-I levels drop slowly in response to somatostatin analogues, multiple samples were administered (25). Figure 4a shows the effect of octreotide serum IGF-I levels after subcutaneous administration to rats. After 24 h, native octreotide showed a reduction of approximately 20% in serum IGF-I levels compared with the initial level. Figure 4b represents the changes of IGF-I levels after 24 h in rats subcutaneously administered with native octreotide and PEG-octreotides. The mono-ALDPEG-octreotides prepared with ALD-mPEG-2K and -5K showed higher reductions of IGF-I levels than the PEG-octreotides prepared with SPA-mPEG. The reduction with mono-ALDPEG-2K-octreotide was comparable to native octreotide. Compared to mono-ALDPEG-2K-octreotide, the lower bioactivity of mono-ALDPEG-5K-octreotide may be attributed to the effect of steric hindrance of PEG on receptor binding. The biological activities of the two mono-SPAPEG-2K-octreotides with different PEGylation sites were not statistically different suggesting the PEGylation site might not have an effect on the biological activity. Di-SPAPEG-2K-octreotide appeared to be inactive. These results further support the importance of the positive charge for preserving the biological activity. The biological activity of somatostatin analogues depends on both their affinity for the cellular receptor and *in vivo* stability. The presence of the positive charge in the primary amino groups of octreotide may be related to the binding affinity to the receptor.

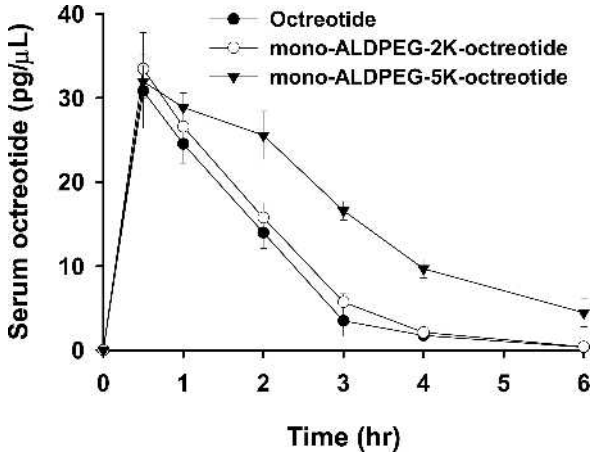

Fig. 5. Average serum concentration-time curves after subcutaneous administration of octreotide, mono-ALDPEG-2K-, and mono-ALDPEG-5K-octreotide. Mean ± SD, n = 6.

Pharmacokinetic Study

PEGylation has been successful in improving the pharmacokinetic properties of peptides and proteins by virtue of increasing the molecular mass and providing protection against proteolytic enzymes (26). Figure 5 shows the average serum concentration-time profiles of octreotide, mono-ALDPEG-2K-, and mono-ALDPEG-5K-octreotide after subcutaneous administration. Although the profile of mono-

Table III. Pharmacokinetic Parameters in Rats After Subcutaneous Administration of Octreotide and PEG-Octreotides (n = 6 per Sample)

	Octreotide	mono-ALDPEG-2K-octreotide	mono-ALDPEG-5K-octreotide
C_{max} (ng/ml)	30.8 ± 2.2	33.4 ± 3.2	31.9 ± 2.9
$t_{1/2}$ (h)	0.87 ± 0.3	0.83 ± 0.3	1.61 ± 0.6[a]
$AUC_{0\text{-last}}$ (ng · h/ml)	54.2 ± 4.9	62.7 ± 4.6	98.0 ± 6.7[a]
$AUC_{0\text{-}\infty}$ (ng · h/ml)	54.6 ± 4.8	63.2 ± 4.3	108.3 ± 6.5[a]
Cl (ml/h)	18.3 ± 1.9	15.8 ± 1.2	9.2 ± 1.4[a]
V_{ss} (ml)	22.9 ± 2.4	18.3 ± 1.4	21.5 ± 2.4
MRT (h)	1.46 ± 1.1	1.52 ± 0.8	2.78 ± 1.5[a]

Mean value ± standard deviation. C_{max}, maximum concentration; $t_{1/2}$, biological half-life; AUC, area under the curve; Cl, systemic clearance; V_{ss}, volume of distribution at steady state; MRT, mean residence time.

[a] Significantly different from native octreotide ($p < 0.05$).

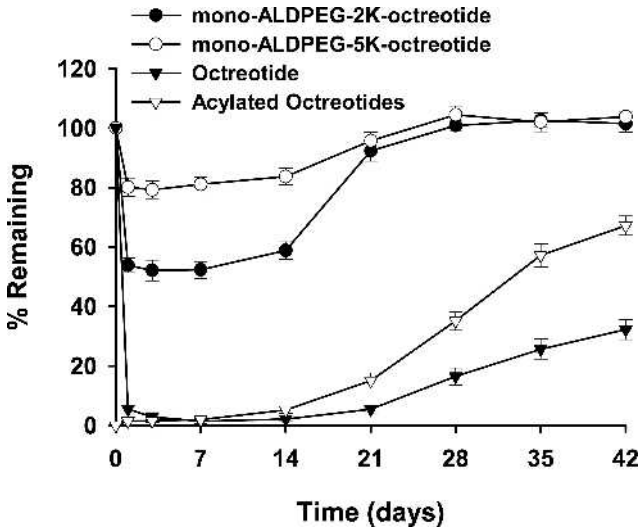

Fig. 6. Interaction of octreotide and mono-ALDPEG-octreotides with PLGA in 0.1 M phosphate buffer (pH 7.4) at 37°C. Mean ± SD, n = 3.

ALDPEG-2K-octreotide was not significantly different from that of native octreotide, mono-ALDPEG-5K-octreotide showed higher serum levels after 1 h compared with octreotide and mono-ALDPEG-2K-octreotide. The pharmacokinetic parameters are shown in Table III. The mono-ALDPEG-5K-octreotide had a longer circulation half-life ($t_{1/2}$: 1.61 h) than native octreotide ($t_{1/2}$: 0.87 h) and mono-ALDPEG-2K-octreotide ($t_{1/2}$: 0.83 h). The AUC was significantly higher for mono-ALDPEG-5K-octreotide than for native octreotide (108.3 *vs.* 54.6 ng·h/ml). The systemic clearance was significantly lower for mono-ALDPEG-5K-octreotide than for native octreotide (9.2 vs. 18.3 ml/h). The molecular size of PEG plays a crucial role in increasing the circulation time by decreasing the glomerular filtration rate and increasing resistance to proteolytic digestion (26). Knauf *et al.* examined the effects of PEG size on pharmacokinetic behavior using interleukin-2 modified with PEGs of different molecular mass ranging from 0.35 to 20 kDa (27). The circulation half-life remained unaltered with a molecular mass increase of 4 kDa, but increased at a molecular mass >8 kDa. In this study, the attachment of PEG-5K significantly increased the half-life and AUC of octreotide, whereas PEG-2K did not significantly alter the pharmacokinetic behavior.

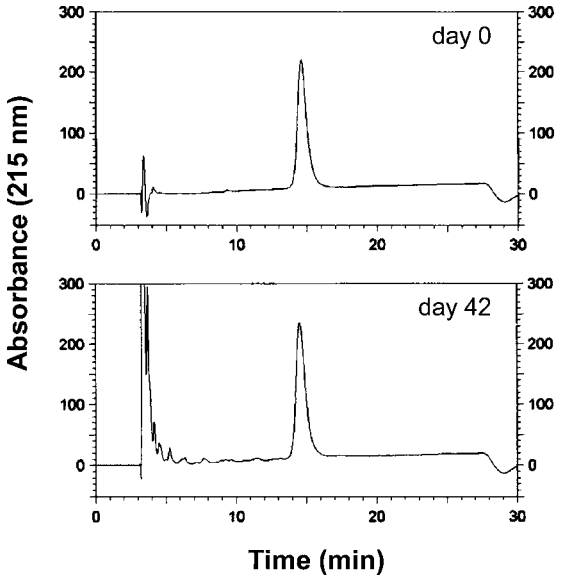

Fig. 7. RP-HPLC chromatograms of mono-ALDPEG-2K-octreotides incubated in 0.1 M sodium phosphate buffer (pH 7.4) at 37°C for 42 days.

Stability Against Acylation by PLGA

Figure 6 shows the interaction of mono-ALDPEG-octreotides with hydrophilic PLGA (RG502H) in 0.1 M phosphate buffer (pH 7.4) at 37°C. PEGylation of octreotide decreased the initial adsorption to PLGA. Although approximately 95% of the native octreotide was adsorbed within 1 day, the initial adsorption of mono-ALDPEG-2K and mono-ALDPEG-5K-octreotide was 46.2% and 19.9%, respectively. The increase of PEG size is shown to inhibit the interaction with PLGA due to the steric hindrance. As the adsorption is related to the ionic interaction between the carboxylic end groups of the polymer and the primary amino groups of peptide (14), PEG strands might interfere with attraction of the Lys residue to the polymer. At day 28, both mono-ALDPEG-2K- and mono-ALDPEG-5K-octreotides were entirely recovered in an intact form unlike native octreotide with 67% of acylated impurities at day 42. Figure 7 shows RP-HPLC chromatograms of mono-ALDPEG-2K-octreotides before and after incubation with RG502H at pH 7.4 for 42 days. Besides the peaks from the degraded PLGA before 10 min of retention time, only the intact mono-ALDPEG-2K-octreotide was observed with the same peak shape.

CONCLUSIONS

N-terminally PEGylated octreotides prepared with the alkyl bond in a site-specific manner showed better structural and biological activity than nonspecifically PEGylated octreotide with amide bond. Both mono-PEG-octreotides prepared with ALDPEG-2K and -5K were also stable against acylation by degrading PLGA. The attachment of ALDPEG-5K to octreotide significantly improved the pharmacokinetic properties compared with that of ALDPEG-2K.

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