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An Injectable and *In Situ*-Gelling Biopolymer for Sustained Drug Release Following Perineural Administration

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Abstract

Study Design—This study evaluated whether the aggregation behavior of a thermally responsive elastin-like polypeptide (ELP) prolongs protein residence time at the dorsal root ganglion (DRG). This work involves development of a sustained-release drug delivery vehicle to provide high and sustained levels of biologic therapeutics to the dorsal root ganglion while minimizing systemic exposure.

Objective—To study the potential of the ELP biopolymer to sustain release and lower systemic exposure of bioactive peptides following perineural administration.

Summary of Background Data—Anticytokine treatment for lumbar radiculopathy may offer clinical improvement, but exposes patients to systemic toxicities of immunosuppression. ELPs are environmentally responsive polypeptides that undergo a phase transition on heating to form an insoluble aggregate. Drug conjugates with ELP exhibit both temperature-sensitivity and *in vitro* bioactivity. Monomer resolubilization yields solution-phase molecules, and this reversible aggregation behavior may create a perineural drug depot to sustain drug delivery to an inflamed nerve.

Methods—This experiment involved 48 rats in which radiolabeled ELPs (aggregating or soluble) were injected overlying the L5 dorsal root ganglion. Animals were killed at 6 different time points, and radioactivity associated with the injected segment, serum, and other tissues was evaluated.

Results—The aggregating ELP demonstrated a 7-fold longer perineural half-life compared with the soluble ELP. This supports the hypothesis that the aggregating ELP forms a depot from which slow resolubilization and clearance provides sustained, local protein release. Furthermore, serum radioactivity reached a lower peak for the aggregating group, demonstrating slower absorption of the aggregating protein into the systemic circulation.

Conclusion—These results suggest that ELP aggregation confer the benefit of perineural compartment longevity for bioactive therapeutics delivered fused with this carrier. This may sustain release of potent immunomodulator therapeutics to treat local neuroinflammation. Desirable features include delivery of high local doses and protection against systemic exposure and associated toxicity.

Keywords

perineural; radiculopathy; controlled release; drug delivery; elastin-like polypeptide; biodistribution; dorsal root ganglion

The pathophysiology of radiculopathy associated with nucleus pulposus (NP) herniation from the intervertebral disc may involve both mechanical deformation and biochemical irritation.¹ Whereas mechanical compression of the nerve root causes histopathological changes and pain behavior in animal models,^{2,3} the trigger and sequence of biochemical events accompanying noncompressive disc-herniation remain unclear.¹ Contact of nucleus pulposus tissue with the dorsal root ganglion (DRG) elicits inflammatory histopathology, slowed nerve conduction velocities, and pain behavior^{4–6} that may be mediated by tumor necrosis factor alpha (TNF α).⁷ Higher biomarker expression of astrocyte and microglia activation³ and histopathological evidence of macrophage infiltration⁸ further implicate the nonspecific inflammatory basis of this disease. Systemic immunomodulatory therapeutics targeting TNF α attenuate these effects in animal models^{9–11} and may provide clinical relief of sciatica in humans.^{12–14} However, conventional systemic delivery of anti-TNF α medications expose patients to toxicity associated with relative immunosuppression.¹⁵ While systemic inflammatory diseases such as rheumatoid arthritis, Crohn disease, and psoriasis demand parenteral treatment, absent serum and cerebrospinal fluid biomarkers of inflammation suggest that disc-herniation radiculopathy is primarily a local inflammatory process.¹⁶ These considerations motivate development of perineural drug delivery vehicles that maximize dose and sustain release of therapeutic agents at the DRG.

Local drug depots can sustain drug release at a target site. Epidural liposomes containing alfentanil,¹⁷ morphine,^{18,19} and bupivacaine,^{20,21} prolong analgesic effects with few systemic toxicities. These systems are highly effective for entrapment of small molecules, sustaining release for several hours after epidural administration. Biodegradable copolymers of glycolic acid and lactones increase the duration of epidural lidocaine block from 61 to 170 minutes in rabbits while decreasing serum exposure.²² Difficulty arises when applying these systems to delivery of protein biomacromolecules because of the need for organic solvents and exposure to drying and shear stresses during the microencapsulation process that make protein instability and maintenance of native structure significant concerns. Furthermore, these polymers suffer from low drug-to-carrier ratio, and the entrapped protein may be simultaneously degraded during the release process. Drugs with low aqueous solubility will phase-separate and undergo spontaneous depot release, such as butamben suspensions that prolong antinociceptive effect in a rat model.²³ Thermally responsive polymers may be delivered in aqueous solution below physiologic temperature and spontaneously undergo a phase change on heating to form a gel-like depot. Poly-(*N*-isopropylacrylamide) and polyoxyethylene-polyoxypropylene block copolymers effectively entrap drugs through this mechanism and provide for sustained release.²⁴ The elastin-like polypeptides (ELPs) are thermally responsive polypentapeptide biopolymers of sequence Val-Pro-Gly-Xaa-Gly where the Xaa residue can be any amino acid other than proline.^{25,26} The phase transition temperature can be specifically genetically designed by modifying 2 orthogonal variables: Xaa amino acid residue identity, and ELP molecular weight.²⁷ As a recombinant biopolymer, ELPs have advantages of high-yield production in *Escherichia coli*,²⁸ demonstrated biocompatibility and nonimmunogenicity,²⁹ and facile genetic engineering to produce recombinant proteins with bioactive peptide fusion partners.^{30,31} This carries the potential advantage of simultaneous expression of the protein drug and polypeptide carrier to yield chimeric proteins with bidomain functionality. Of specific interest for this study, the ELP phase transition leads a soluble polypeptide below a characteristic transition temperature (T_i) to form insoluble, micron-sized, supramolecular

complexes on being raised above T_t .³² This aggregation behavior prolongs the half-life of ELPs after intra-articular administration.³³

The objective of this study is to evaluate biodistribution of a depot-forming ELP following perineural delivery in a rat model. Two ELPs of similar molecular weight were selected—one transitioning below physiologic temperature to ensure depot formation, and a second with a transition temperature above 75°C to guarantee solubility. Biodistribution of tritium-labeled ELPs was quantified over 2 weeks, with the specific interests of quantifying prolongation of protein residence at the injected segment, attenuation of serum exposure, and bioaccumulation in remote tissues.

Methods

Synthesis of ELPs

Two different ELPs were expressed in *E. coli* and purified using inverse transition cycling (ITC).³⁴ ELP-(V)₁₂₀ (MW = 49.9 kDa) exclusively contained Val as the guest residue and was selected because of a subphysiological T_t of 29°C. The soluble ELP-(VA₈G₇)₁₂₈ (MW = 49.0 kDa) contained Val, AL, and Gly as guest residues with a frequency of 1:8:7, and served as a control because of similar molecular weight but a T_t of 78°C ensuring *in vivo* solubility.

Radiolabeling of ELPs by Reductive Methylation

Purified ELPs were reconstituted in PBS at 36 µmol/L and reductively methylated using a method described by Jentoft and Dearborn³⁵ with ³[H]-sodium cyanoborohydride (American Radiochemical Corp., St. Louis, MO) as the radioactivity source. The reaction mixture was dialyzed 5 times against 3 L of deionized water for 8 hours each, concentrated using an Amicon Ultra-15 centrifugal filter of 5 kDa MWCO (Millipore, Billerica, MA), and then subjected to 2 further rounds of ITC. Proteins were sterile-filtered through a 0.22 µmol/L filter and tritium incorporation was evaluated using absorbance spectro-photometry to quantify ELP concentration and liquid scintillation counting to quantify associated radioactivity (5 mL of Bio-Safe II, RPI Corp., Mt. Prospect, IL) using a Packard Tricarb B1900-TR scintillation counter (Packard Instruments, CT). Analogous nonradioactive alkylation permitted evaluation of the transitioning behavior of the modified proteins

***In Vitro* Stability of ELPs**—Proteolytic degradation by active enzymes under physiologic or pathologic conditions could lead to loss of the tritium radiolabel from the ELP molecule. Consequently, an experiment was designed to evaluate the *in vitro* stability of both ³[H]-ELP-(V)₁₂₀ and ³[H]-ELP-(VA₈G₇)₁₂₈ by incubating quadruplicate replicates of ³[H]-ELP (4 nmol in 1 mL) at 37°C in rat serum (Invitrogen, Carlsbad, CA) or PBS. One hundred microliters aliquots were withdrawn at 1, 2, 4, 7, 10, and 14 days. A 10 kDa MWCO microcentrifugation filter (Millipore, Billerica, MA) was used to separate radioactivity associated with smaller degradation products from larger parent ELP molecules. Total radioactivity in both the filtrate and retentate was measured by liquid scintillation counting (LSC) in 5 mL of Bio-Safe II, and the fraction of radioactivity in the filtrate was computed. Two-factor analysis of variance (ANOVA) and *post hoc* Dunn's tests evaluated ELP sequence and incubation buffer dependency of filtrate fraction of radioactivity. An average degradation rate was computed for each ELP in serum using linear least-squares regression, and derived parameters were compared using a Student *t* test.

***In Vivo* Perineural Administration of Radiolabeled ELPs**—Forty-eight Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used for biodistribution studies of ³[H]-ELP following perineural administration. Rats in the experimental group received the aggregating ³[H]-ELP-(V)₁₂₀ and those in the control group received soluble ³[H]-

ELP-(VA₈G₇)₁₂₈. While drug administration to the disc-nerve interface is accomplished by transforaminal injection under fluoroscopic guidance,³⁶ the small size of these structures in the rodent model precluded our use of a guided injection procedure.³⁷ The DRG was exposed using an open procedure involving paraspinal muscle dissection followed by drilling a 1 mm hole in the transverse process using a Midas A3 drill bit.^{38–40} Each ELP sequence had 4 animals for each of 6 sacrifice time points. Animals were anesthetized with intraperitoneal pentobarbital (40 mg/kg), opened with a 15 mm midline dorsal lumbar incision, and a 10 mm paramedian incision through the dorsal lumbar fascia. Twenty-five microliters of 25 μ mol/L [³H]-ELP solution was injected through the defect after which the hole was sealed with bone wax. Dorsal lumbar fascia was closed with 3-0 silk sutures, and skin was closed with staples. Animals in the experimental group receiving [³H]-ELP-(V)₁₂₀ were killed at 0, 6 hours, and 1, 4, 7, and 14 days. Animals in the control group receiving [³H]-ELP-(VA₈G₇)₁₂₈ were killed at 0, 1, 4, 12, 24, and 48 hours. For sacrifice, animals were administered 60 mg/kg intraperitoneal pentobarbital after which a blood sample was obtained via retro-orbital bleed. After this, the following tissues were harvested in order: brain, C2 motion segment, left gastrocnemius muscle, liver, kidney, adjacent segment (L4), and injected segment (L5).

Tissue Solubilization and Liquid Scintillation Counting

The isolated tissues and serum samples were weighed and incubated in an alkaline tissue solubilizer (GE Healthcare, Piscataway, NJ) at 55°C until they were completely solubilized. The solutions were decolorized (3% hydrogen peroxide, 55°C for 1 hour) and the radioactivity was quantified by LSC. Radioactivity in the injected segment at the earliest time point was interpreted as an average injected dose (ID) for experimental and control groups. Each tissue or fluid sample was normalized by the corresponding injected dose. Radioactivity associated with the L5 (injected) and L4 (adjacent) regions were expressed as fractions of ID associated with the segment. Data for organs (brain, liver, and kidney) and the C2 motion segment were expressed as fraction of ID per gram of tissue. The fraction of injected [³H]-ELP in the serum compartment was estimated by scaling up the measured radioactivity in a serum sample to the total blood volume predicted by animal body mass.⁴¹

Biodistribution Analysis

The clearance half-life ($t_{1/2}$) of the protein depot was evaluated by fitting the time-dependent radioactivity at the injected segment to first-order kinetics by a monoexponential decay model using nonlinear least squares regression. The derived rate constant of absorption (k_a) is related to the half-life by the equality $t_{1/2} = \ln 2/k_a$. The $t_{1/2}$ values for the aggregating ELP-(V)₁₂₀ and the soluble ELP-(VA₈G₇)₁₂₈ were compared by a Student *t* test. One-factor ANOVA with *post hoc* Dunn's tests were used to detect an effect of time on the presence of [³H]-ELP in the noninjected tissues (muscle, liver, kidney, C2 segment, L4 segment) and serum. The maximum serum fraction observed for each ELP was also evaluated.

Results

Tritium-Radiolabeling of ELPs by Reductive Methylation

The specific radioactivity of the ELP-(V)₁₂₀ and ELP-(VA₈G₇)₁₂₈ proteins after reductive methylation were 2628 and 876 mCi/mmol, respectively. The inverse phase transition behavior of both ELPs was only slightly modified following nonradioactive methylation, with observe transitions occurring at 26°C for ELP-(V)₁₂₀ and at 82°C for ELP-(VA₈G₇)₁₂₈. This observation suggests that the important physical properties of ELPs were not modified following radiolabeling, thus permitting their use to contrast biodistribution of aggregating and soluble ELP formulations in this study.

***In Vitro* Stability of ELPs**

There was evidence of degradation of ^3H -ELP to fragments of molecular weight less than 10 kDa for both soluble and aggregating ELP formulations in rat serum (Figure 1, ANOVA, $P < 0.05$). There was no corresponding degradation effects noted for incubation of either ^3H -ELP in PBS. *Post hoc* analysis reveals that filtrate fraction of radioactivity rises above background levels for the soluble ELP from 24 hours onwards (Dunn's, $\alpha = 0.05$), whereas the aggregating ELP reaches such difference at 7 days and beyond (Dunn's, $\alpha = 0.05$). The soluble ELP was more extensively degraded than the aggregating ELP at every sampling time, likely because of greater accessibility to serum proteases than aggregating ELPs that physically separate from aqueous solution into an insoluble coacervate phase. The degradation rate for ELP-(VA₈G₇)₁₂₈ averaged $0.56\% \pm 0.05\%$ /day, significantly faster (Student *t* test, $P = 0.002$) than for than for ELP-(V)₁₂₀ averaging $0.33\% \pm 0.05\%$ /day.

***In Vivo* Biodistribution of Radiolabeled ELPs After Perineural Administration of Radiolabeled ELPs**

All 48 rats survived to the desired time point, with no deaths, infections, permanent neurologic deficits, or wound dehiscence. Drilling through the transverse process was consistently performed without gross damage to the underlying exiting nerve root.

Fractions of soluble and aggregating ^3H -ELP per ID at the L5 injected level decreased monotonically and was well described by first-order exponential kinetics (Figure 2). The computed half-life of release for the soluble ELP was 5.5 ± 0.5 hours whereas a significantly longer 39 ± 5 hours characterized the aggregating ELP as summarized in Table 1 (Student *t* test, $P < 0.01$).

The fractions of ^3H -ELP per ID in the serum compartment are shown in Figure 3, with both formulations peaking at 24 hours and steadily thereafter decaying to below detectable values. The maximum fraction of soluble ^3H -ELP-(VA₈G₇)₁₂₈ was $23 \pm 5\%$ ID, while that for the aggregating ^3H -ELP-(V)₁₂₀ was only $1.6 \pm 0.3\%$ ID.

Distribution of the ^3H -ELP to peripheral tissues are shown in Figure 4, and immediate postinjection analysis is summarized in Table 2. Time-dependent variation in radioactivity was observed in the kidney for both soluble (ANOVA, significant effect of time, $P < 0.01$) and aggregating (ANOVA, significant effect of time, $P < 0.05$) proteins. Maximum values for the soluble ELP were reached at 24 hours with a value of $1.2 \pm 0.4\%$ ID/g (or $1.0 \pm 0.1\%$ ID/kidney), whereas maximum values for the aggregating ELP were reached at 96 hours with a value of $1.8 \pm 0.5\%$ ID/g (or $1.9 \pm 0.8\%$ ID/kidney). All levels of ^3H -ELP dropped below 1% for the soluble ELP by 48 hours and for the aggregating ELP by 168 hours. Neither aggregating nor soluble ELPs accumulated in the brain, muscle, liver, and C2 motion segment above baseline detection; and none of these tissues demonstrated time-dependent radioactivity (ANOVA, no significant effects of time, $\alpha = 0.05$). Table 3 summarizes the residual tissue radioactivity after 14 days among the animals injected with aggregating ELP.

Discussion

Local irritation of the DRG can generate symptomatic changes of painful radiculopathy, detected as histologic changes in the DRG and dorsal horn,^{42,43} electrophysiological changes in nerve conduction,⁴⁴ and behavioral¹ changes in the affected subject. Animal models have demonstrated therapeutic effect on systemic pretreatment with anticytokine agents.^{9,45,46} Local therapeutic delivery to this region is attractive to treat the local pathology and minimize systemic toxicities. In this study, a thermally responsive polypeptide, ELP, was evaluated for its ability to prolong the residence time of a potential drug carrier delivered locally to the DRG.

ELP was hypothesized to increase polypeptide residence time by forming insoluble aggregates at temperatures above body temperature that reversibly separate from an aqueous phase, thus slowing their clearance. As a consequence, a significant 7-fold prolongation in perineural residence time was observed compared with an ELP that remains soluble at body temperature. Vascular and lymphatic absorption of ELP is expected to only clear solution-phase particles, which includes only a fraction of the aggregating ELP-(V)₁₂₀. This process of clearing the soluble fraction overlying an ELP aggregate thermodynamically drives disaggregation forming the basis of this drug delivery system. This contrasts with the soluble ELP-(V₅A₂G₃)₁₂₈ molecules that do not aggregate *in vivo* and are hence all accessible for clearance. The formation of multimeric particles following delivery to the perineural space will also increase the apparent molecular weight of ELP with concomitant slowing of diffusion, as assessed by dynamic light scattering.²⁴ Prolongation in protein residence time suggests that aggregation occurs in the perineural space and is responsible for delayed clearance from around the DRG.

This delayed absorption of the aggregating ELP also lowers serum fraction of radiolabel (maximum $1.6 \pm 0.3\%$ ID) compared with the soluble ELP (maximum $23 \pm 5\%$ ID). Reduction of serum drug concentration is important when for local drug depots to reduce systemic toxicity. This is particularly important when delivering bioactive peptides to the perineural space, such as anti-TNF agents or other immunomodulating proteins. These drugs elicit untoward immunosuppression on significant systemic exposure, and the ability of the aggregating ELP to reduce serum compartment peak exposure is an important feature of this delivery system. Liu *et al*⁴⁷ have shown intravascular ELP elimination half-life to be 8.37 hours. The clearance mechanism is unclear, but the rise and fall of kidney-associated radioactivity in synchrony with serum in our experiment suggests a glomerular filtration route. Although additional studies are necessary to definitively implicate renal or hepatic clearance, we have demonstrated feasibility of delivering high local drug concentrations while attenuating systemic exposure. We further observe the remote C2 level did not accumulate radiolabel above background for either ELP. This supports local peptide delivery because even high circulating protein doses very inefficiently reaches desired neural structures.

Drug clearance after perineural injection is predominantly by vascular and lymphatic absorption, with the latter more prominent for large soluble polypeptides. While previous work suggests ELP aggregation to prolong residence time after injection into the anatomically confined knee joint,³³ the perineural region is less anatomically defined and access is more challenging because of complex bony anatomy. The lumbar DRG lies distal to the confluence of dura and peripheral nerve sheath, medial to the transverse process in the rat^{37–39} and either foraminal or intraspinal in human radiographic⁴⁸ and cadaveric⁴⁹ studies. The foraminal zone, an osteofibrous canal, is bounded superiorly and inferiorly by pedicles of adjacent vertebrae. The intervertebral disc and posterior margins of adjacent vertebrae form the anterior boundary. The posterior limit comprises the pars interarticularis, the superior facet joint, and the ligamentum flavum.⁵⁰ Target neural structures are directly visualized by surgical procedures such as lumbar laminectomy, or less invasively accessed by fluoroscopy-guided injection specifically delivering therapeutics to the disc-nerve interface.

While herniated disc material triggers proinflammatory cascades and contributes to radiculopathy pain and disability,^{51–54} it is unclear whether addressing cytokine expression at the disc-nerve interface is sufficient or whether subepineurial effects have greater importance. Symptomatic resolution after disc resorption implicates the former,^{55–57} and local perineural therapeutics address the interaction between nucleus pulposus and the DRG epineurium. An ongoing clinical trial is evaluating epidural etanercept to treat painful radiculopathy. This biodistribution study shows that an ELP depot sustains release and attenuates extraspinal spread. Applications include gel phase entrapment of small molecules, conjugation of these molecules to ELP to further slow clearance, or expression of immunomodulator proteins fused

with ELP. This delivery system is limited by the fixed volume in which spinal agents must be delivered beyond which extraspinal spread occurs. Maximum doses are limited by drug solubility and the effects of high doses on surrounding tissue.³⁷ This biodistribution study is limited in its ability to address the animal's *in vivo* response to ELP exposure because of the small mass of radiolabeled peptides used and the short follow-up. An evaluation of nonspecific complement response and inflammatory cell activation will require both *in vitro* testing of ELP material safety and *in vivo* testing with exposure to higher doses. However, it is noteworthy that a prior study of recombinant ELP application as a cardiovascular device coating demonstrated reduced platelet microparticle release with fewer thrombotic emboli.⁵⁸ Furthermore, additional studies of host generation of antibodies against bioactive peptide fusions with ELP must be evaluated once a desired therapeutic and the anticipated doses are selected.

Covalent attachment of active drugs to ELP carriers is readily performed,^{30,59,60} and *in vitro* bioactivity of ELP-based therapeutics has been thoroughly reviewed.⁶¹ Chemical conjugation of ELP with doxorubicin yields a thermally sensitive compound that retains *in vitro* cytotoxicity against squamous cell carcinoma (FaDu) cells equivalent to free doxorubicin.⁵⁹ Fusion protein activity has been demonstrated for oligopeptide cell penetrating peptides and oncogene inhibitors recombinantly expressed with an ELP partner. Penetratin-ELP readily translocates across cell membranes, while ELP-H1 decreases *c-myc* transcriptional activation and inhibits *in vitro* proliferation of breast carcinoma (MCF-7) cells. Ternary fusion penetratin-ELP-H1 exhibits tridomain functionality.³⁰ Finally, ELP has been expressed fused with the anti-inflammatory protein, interleukin-1 receptor antagonist (IL1Ra), with effective antagonism of IL1 pro-inflammatory effects on thymocytes, lymphocytes, and fibrochondrocytes.⁶² The retention of ELP-IL1Ra bidomain functionality are especially relevant as they suggest the possibility of local delivery of complex biologic therapeutics for radiculopathy.

This study demonstrated feasibility of a locally administered drug delivery system based on thermally induced ELP phase separation. Perineural injection of an aqueous protein solution triggers formation of an insoluble aggregate from which individual molecules disaggregate over time. This behavior substantially increases ELP half-life in the perineural compartment, while also reducing ELP fraction in the serum compartment with little extraspinal redistribution. This biocompatible and nonimmunogenic^{29,63} biopolymer has the additional benefit of facile expression fused with bioactive polypeptides. While this work demonstrates ELP aggregation to sustain protein release with slowed elimination from the perineural compartment, future work will evaluate therapeutic efficacy of this drug delivery system in an animal model of lumbar radiculopathy secondary to disc herniation.

Key Points

- This study demonstrates the feasibility of sustaining delivery of peptide-based therapeutics to the dorsal root ganglion.
- This recombinant biopolymer is biocompatible and nonimmunogenic, and undergoes supramolecular aggregation to form an insoluble coacervate phase on reaching body temperature.
- We show that this aggregation behavior prolongs local residence time of the ELP after perineural delivery, and slows its absorption into systemic circulation as reflected in its lower peak serum concentration as compared with a soluble, control protein.

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Figure 1.

Incubation of both soluble and aggregating ELP sequences in PBS or rat serum at 37°C. Significant effects (ANOVA) were detected for both tested factors: ELP sequence ($P < 0.01$) and incubation conditions ($P < 0.01$). Serum degradation exceeded baseline from day 1 onwards for the soluble ELP (*, Dunn's tests, $\alpha = 0.05$) and from day 7 onwards (*, Dunn's tests, $\alpha = 0.05$). Greater fraction of degradation products were measured for the soluble ELP at nearly all time points (#, Dunn's tests, $\alpha = 0.05$).

Figure 2.

Clearance of ELP from the perineural delivery to the right L5 DRG is well described by a first-order exponential model for both soluble (upper, $r^2 = 0.97$) and aggregating (lower, $r^2 = 0.94$) sequences. Data are expressed as mean $\pm$ SD ($n = 4$) and presented with first-order exponential decay fits and 95% confidence intervals. Half-lives are 5.5 ± 0.5 hours for the soluble ELP and 39 ± 5 hours for the aggregating ELP (Student t test, $P < 0.01$).

Figure 3.

Serum fraction of soluble and aggregating ELP, normalized to the injected dose. Data are expressed as mean $\pm$ SD ($n = 4$) with the maximum serum fraction of both ELPs observed at 24 hours. Maximum magnitudes were $23 \pm 5\%$ ID for the soluble ELP and $1.6 \pm 0.3\%$ ID for the aggregating ELP.

Figure 4.

Profile of soluble (upper) and aggregating (lower) ELP distribution to peripheral tissues, normalized to the injected dose. Data are expressed as mean $\pm$ SD ($n = 4$). Accumulation of radioactivity was noted in the kidney for soluble ELP (significantly higher at 24 hours than all other times, Dunn's tests, $\alpha = 0.05$) and for aggregating ELP (significantly higher at 96 hours than 0, 6, and 336 hours, Dunn's tests, $\alpha = 0.05$). The soluble ELP did not accumulate in the brain, liver, or muscle (ANOVA, $P > 0.05$); but did peak at the C2 motion segment at 24 hours (significantly higher than at 0 and 48 hours, Dunn's test, $\alpha = 0.05$). The aggregating ELP did not accumulate in the brain, liver, C2 motion segment, or muscle (ANOVA, $P > 0.05$).

Table 1

Physical Properties of Aggregating and Soluble ELP and Their Pharmacokinetic Parameters After Perineural Injection

	ELP-(VA ₈ G ₇) ₁₂₈	ELP-(V) ₁₂₀	<i>P</i>
MW (Da)	48990	49867	
<i>T_t</i> (°C)	26	82	
<i>k_a</i> (h ⁻¹)	0.125 ± 0.012	0.017 ± 0.002	<0.01 [*]
<i>t</i> _{1/2} (h)	5.5 ± 0.5	39 ± 5	<0.01 [*]
<i>t</i> _{10%} (h)	18.3 ± 1.7	126 ± 14	<0.01 [*]
<i>C_{max,serum}</i> (%ID)	23 ± 5	1.6 ± 0.3	

* Significantly different by Student's *t* test at 0.05 level of significance.

MW indicates molecular weight; *T_t*, transition temperature; *k_a*, absorption rate constant; *t*_{1/2}, half-life; *t*_{10%}, time to 10% dose; *C_{max,serum}*, maximum observed serum fraction of radioactivity.

Table 2

Tissue Distribution of Soluble and Aggregating ELP Immediately After Injection

Tissue	Scale	ELP-(VA ₈ G ₇) ₁₂₈ (% ID)	ELP-(V) ₁₂₀ (% ID)
Injected segment	Per organ	100 ± 8	100 ± 16
Adjacent segment	Per organ	9 ± 4	8 ± 5
C2 segment	Per gram	0.16 ± 0.15	0.08 ± 0.04
Brain	Per gram	0.6 ± 1.0	0.20 ± 0.14
Kidney	Per gram	0.08 ± 0.04	0.14 ± 0.18
Liver	Per gram	0.10 ± 0.07	0.10 ± 0.15
Muscle	Per gram	0.05 ± 0.07	0.12 ± 0.07
Serum	Per organ	1.6 ± 1.1	0.5 ± 0.3

No significant differences between ELP distribution between soluble and aggregating ELPs immediately after injection as assessed by Student's *t* test at 0.05 level of significance.

Table 3

Tissue Distribution of Aggregating ELP at 2 Weeks

Tissue	Scale	ELP-(V) ₁₂₀ (% ID)
Injected segment	Per organ	0.8 ± 0.4
Adjacent segment	Per organ	0.10 ± 0.06
C2 segment	Per gram	0.11 ± 0.08
Brain	Per gram	0.049 ± 0.005
Kidney	Per gram	0.43 ± 0.09
Liver	Per gram	0.06 ± 0.04
Muscle	Per gram	0.08 ± 0.03
Serum	Per organ	0.44 ± 0.12