Characterization and *In Vivo* Study of Sustained-Release Formulation of Human Growth Hormone Using Sodium Hyaluronate

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Purpose. Aiming at once-a-week injection, a novel sustained release formulation of recombinant human growth hormone (SR-hGH) using sodium hyaluronate was developed for the treatment of children who have growth failure due to the lack of adequate secretion of endogenous growth hormone.

Methods. SR-hGH was produced in the form of solid microparticle using a Niro spray dryer and characterized by Malvern particle size analysis, scanning electron microscopy (SEM), size exclusion chromatography (SEC), reverse phase–high-performance chromatography (RP-HPLC), and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). After *in vitro* release test, pharmacokinetic and pharmacodynamic studies were carried out in beagle dogs. SR-hGH was dispersed in medium-chain triglyceride (MCT) and administered at a dose of 1.0 mg hGH/kg subcutaneously.

Results. SR-hGH microparticles were successfully produced with a mean particle size of 5.6 ± 1.0 μm. Physicochemical analysis with SEC, RP-HPLC, and SDS-PAGE showed that hGH extracted from SR-hGH was intact and comparable to that of hGH bulk standard indicating no structural change in hGH during the formulation processes. Monomeric content of hGH recovered from SR-hGH was 97.4% by SEC analysis, and its purity was 96% by RP-HPLC analysis. *In vitro* release test showed the sustained-release characteristics of SR-hGH up to 48 h with the complete release of hGH loaded. The continuous and monotonous release profile observed in *in vitro* release test was supported by pharmacokinetic study in beagle dogs. Delayed absorption of hGH was observed with C_{max} of 69.5 ± 8.0 ng/ml and T_{max} between 10 and 12 h. The administration of SR-hGH induced elevation of serum insulin-like growth factor-I (IGF-I) level for 6 days with a maximum value higher than the predose level by ca. 350 ng/ml. After 6 days, IGF-I level returned to the initial baseline level.

Conclusions. Sustained-release formulation of hGH for once-a-week injection was successfully developed using high-molecular-weight sodium hyaluronate. No adverse effect was observed during and after the *in vivo* test using beagle dogs.

KEY WORDS: formulation; human growth hormone; lecithin; sodium hyaluronate; spray drying; sustained release.

INTRODUCTION

Human growth hormone (hGH) is an endocrine hormone produced and stored in the anterior pituitary gland. hGH has been used for the treatment of children with short stature caused by growth hormone deficiency in a way of hormone replacement therapy (1). Since the launch of recombinant products of hGH in the market in 1986, drug availability has greatly increased and the inherent risk of infection by using pituitary-derived hGH products has drastically decreased (2–4). Although hGH is secreted into the circulation in a pulsatile pattern, recent clinical studies have shown that continuous infusion of hGH via a pump resulted in elevated insulin-like growth factor-I (IGF-I) levels comparable to those of daily injections (5,6). This indicates that pulsatile hGH release may not be required for clinical efficacy. With improved patient compliance, the sustained-release formulation may increase efficacy with suitable pharmacokinetic release profile.

The controlled delivery of protein drugs has been of substantial therapeutic interest with the rapidly increasing protein drug market. There have been a lot of research efforts to develop a sustained-release formulation of protein drugs (7–12). For the first time, Genentech and Alkermes developed Nutropin Depot as a sustained-release formulation of hGH using poly(lactic glycolic acid) [PLGA] microparticles (13–16). PLGA has been used for sustained-release formulation of steroids (17) and small-molecular-weight peptides (18,19), and the performance of these formulations was proven to be successful. However, formulation of a macromolecule, such as hGH, using PLGA may be much more difficult than that of small-molecular-weight peptide due to the hydrophobicity of PLGA. Recently, these delivery systems have been reported to have complexities such as protein denaturation by hydrophobic interaction and inflammation associated with the degradation of PLGA (12).

Hyaluronic acid (HA) is a natural linear polysaccharide composed of alternating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine with β(1→4) interglycosidic linkages (20). The sodium salt of hyaluronic acid, sodium hyaluronate, is biodegradable, biocompatible, and viscoelastic with a wide molecular weight range from 1,000 to 10,000,000 Da. HA is the only nonsulfated glycosaminoglycan (GAG) that is abundant in synovial fluid and extracellular matrix (ECM) (20). HA acts to control tissue hydration and is present in hydrated networks with collagen fibers in the ECM (21). It also constitutes the backbone of cartilage proteoglycan (22). HA plays pivotal roles in wound healing and in promoting cell motility and differentiation during development (23). HA has unique viscoelastic properties, which make it important for the lubrication function of the synovial joint fluid and vitreous humor in the eye (24,25). Because of its various functions and physicochemical properties, HA and modified HA have been extensively investigated and used for arthritis treatment (24), ophthalmic surgery (25), drug delivery (26,27), and tissue engineering (28). A number of strategies for the modification of HA through carboxyl and hydroxyl groups have been developed including esterification of HA (27), chemical modification of HA with amine compounds after derivatization with carbodiimide (29), and crosslinking of HA using divinyl sulfone (30), glycidyl ether (31), or dialdehyde after modification of HA with adipic dihydrazide (32). However, chemical modification of HA has generally been carried out in highly alkaline or acidic solutions and

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at elevated temperatures. These reaction conditions cannot be applied for the inclusion of sensitive molecules such as protein drugs or living cells during the preparation of polymer network hydrogels (32).

In this work, a novel sustained-release formulation of hGH using sodium hyaluronate has been developed aiming at once-a-week injection. Without any chemical modification, spray-dried HA solid microparticle with a high molecular weight worked as an excellent protein drug reservoir. Morphological analysis was carried out with a Malvern particle size analyzer and scanning electron microscopy (SEM). Physicochemical analysis of hGH extracted from SR-hGH was carried out by size exclusion chromatography (SEC), reversed phase–high-performance liquid chromatography (RP-HPLC), and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). After *in vitro* release test, pharmacokinetic and pharmacodynamic studies of SR-hGH were carried out in beagle dogs. This paper describes the stability of hGH in SR-hGH during and after the formulation using a spray dryer and its sustained-release characteristics as demonstrated in *in vitro* and *in vivo* tests.

MATERIALS AND METHODS

Materials

The following raw materials were used in this study: human growth hormone (Eutropin, lot no. UTB5008, LG Life Sciences, Taejon, Korea), Eutropin in-house standard (lot no. UTS8001, LG Life Sciences, Taejon, Korea), sodium hyaluronate (Hyal 2000, LG Life Sciences), lecithin (Lipoid E80, Lipoid GmbH, Ludwigshafen, Germany), sodium phosphate monobasic (reagent grade, Junsei Chemicals, Tokyo, Japan), sodium phosphate dibasic (reagent grade, Junsei Chemicals), medium-chain triglyceride (MCT, Lipoid GmbH), and water for injection (WFI, LG Life Sciences).

The following equipments were used in this study: stirred cell (Amicon, Beverly, MA, USA), tip sonicator (Vibra cell, Sonics and Materials, Newtown, CT, USA), spray dryer (Mobile minor 2000, Niro, Denmark), vacuum drying oven (Heraeus, GmbH, Hanau, Germany), particle size analyzer (Master sizer MS20, Malvern, Worcestershire, England), spectrophotometer (Varian, Palo Alto, CA, USA), scanning electron micrograph (JSM 6340F, JEOL, Peabody, MA, USA), Karl Fischer Titrator (Mettler-Toledo, Columbus, OH, USA), HPLC (Waters 2690 Separations Module, Waters 996 PDA detector, Waters Dual λ Absorbance Detector, Waters, Milford, MA, USA), and SDS-PAGE (power supply: PS2500, Hoeffer, San Francisco, CA, USA; and scanner: GT9600, Epson, Long Beach, CA, USA).

Preparation of SR-hGH

SR-hGH was produced under aseptic conditions. Bulk solution of hGH was phosphate-buffer (pH = 7.4, 10 mM) changed using a stirred cell and then filtered with 0.2-μm filter for sterilization. Sodium hyaluronate supplied aseptically was dissolved in WFI using a mechanical stirrer under aseptic conditions. Lecithin was predispersed in WFI using a magnetic stirrer and then sonicated with a tip sonicator. The dispersion was filtered with 0.2-μm filter for sterilization. Sodium hyaluronate solution and lecithin dispersion were mixed with a mechanical stirrer for 1 h, and then hGH solution was added to the mixed solution. The ratio of hGH:HA:lecithin was optimized to be 1:3:0.5 for sustained release of hGH. The final mixed solution was spray-dried aseptically using a Niro spray dryer, which was operated under the following conditions: inlet temperature of 100°C, outlet temperature of 50°C, and feeding rate of 1 L/h. After spray drying, the recovered powder was further vacuum dried for 2 days. Three batches of SR-hGH were produced at the same conditions and tested for reproducibility in this study.

Characterization of SR-hGH

Particle Morphology

Particle size analysis of SR-hGH was carried out using Malvern particle size analyzer. SR-hGH (10 mg) was dispersed in MCT (10 ml) with a vortex mixer and then analyzed. The mean particle size was determined as a volume median diameter at 50% point of entire volume distribution. After vacuum drying for 2 days, water content of SR-hGH was measured with a Karl Fischer Titrator in order to define an accurate composition of SR-hGH. Morphological analysis of SR-hGH was carried out by scanning electron microscopy (SEM). Powder samples were coated with gold-platinum under a high vacuum. SEM photographs were taken at a magnification of 1,000.

hGH Extraction from SR-hGH

After reconstitution of SR-hGH with phosphate buffer, 0.5 ml of the solution was mixed with equal volume of ethanol for HA precipitation. After centrifugation, supernatant was taken into a new microtube. Recovery of hGH in extraction steps was more than 95%, determined by comparison between the measured amount by BCA protein assay (33) and theoretically loaded amount based on the composition ratio of SR-hGH. From the recovery data, the amount of hGH in SR-hGH was estimated and used for the determination of dose for *in vivo* test. This supernatant solution of hGH was used for SEC-HPLC, RP-HPLC, and SDS-PAGE according to European Pharmacopoeia supplement 2000.

SEC-HPLC Analysis

SEC-HPLC was used in order to identify hGH and determine the monomeric hGH content in SR-hGH. SEC-HPLC provides information on the size of the protein and the presence of aggregates. The extracted hGH samples from SR-hGH were analyzed using TSK G2000SW$_{XL}$ column (5 μm, 7.8 × 300 mm, TOSO Co., Tokyo, Japan). As a mobile phase, a buffer of 0.063 M sodium phosphate (pH 7.0)/*n*-propanol (97/3) was used at a feeding rate of 0.6 ml/min. The concentration of extracted hGH sample was approximately 1 mg/ml and injection volume was 20 μl. The detection was carried out by UV monitoring at 214 nm. Impurity related to dimer or higher molecular weight multimers could be determined using this method.

RP-HPLC Analysis

RP-HPLC could identify and quantify native form of hGH in SR-hGH. Contents of impurities, such as desamido and oxidized forms of hGH, could be determined by RP-HPLC. The extracted hGH samples from SR-hGH were analyzed using Vydac214ATP54 (5 μm, C_4, 4.6 × 250 mm, Grace

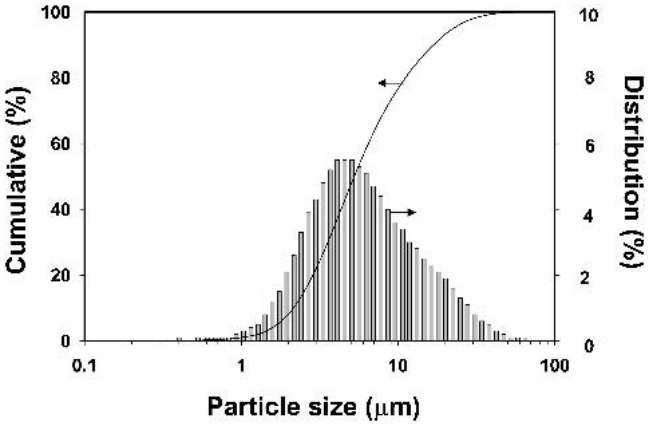

Fig. 1. Size distribution of SR-hGH microparticles.

Vydac, Hesperia, CA, USA) column that was maintained at 45°C. As a mobile phase, 0.05 M Tris (pH 7.5)/*n*-propanol (71/29) was used at a feeding rate of 0.5 ml/min. The concentration of extracted hGH sample was approximately 1 mg/ml and injection volume was 20 μl. The detection was implemented by UV monitoring at 220 nm (15).

SDS-PAGE Analysis

SDS-PAGE was performed to identify hGH and determine the fragments or covalent dimers of hGH in SR-hGH. The extracted hGH samples from SR-hGH were mixed with SDS reducing sample buffers containing dithiothreitol and boiled at 90°C for 2 min. The samples of 10 μg were loaded onto 15% SDS-PAGE gel (16 cm × 16 cm with 0.75-mm thickness). After electrophoresis, the gels were stained with Coomassie blue.

In Vitro Release Test

A new *in vitro* release test method was designed for water-soluble SR-hGH. SR-hGH powder (50 mg) in a vial was dispersed with MCT (1 ml) using a vortex mixer. Aliquot of this oil suspension (0.1 ml) was added respectively to the top of phosphate buffer (5 ml, pH = 7.4, 10 mM) aqueous solution in a test tube that was prepared as many as sampling numbers (7 in this study). The final two-phase solution was kept at 37°C. After incubation for predetermined sampling time period, the phosphate buffer solution (4 ml) was carefully taken from aqueous phase in the lower part of the test tube. The quantification of hGH released from SR-hGH was carried out using Lowry protein method (34).

In Vivo Test in Beagle Dogs

Four groups of 6 beagle dogs at the age of 5~6 months were tested in this study. The weights of the animals were in the range of 7.8~9.9 kg. Three different batches of SR-hGH were dispersed in MCT by gentle inversion and injected for groups 1 to 3 subcutaneously at a dose of 1.0 mg/kg. MCT satisfying the European Pharmacopoeia for injection was filtered through 0.22-μm filter unit and used as an injection vehicle. MCT is also in US generally regarded as safe (GRAS) list and regarded as nontoxic and a nonirritant. Preliminary studies showed that MCT does not cause any influence for hGH pharmacokinetics. For group 4, hGH of daily injection formulation was administered subcutaneously at a dose of 0.15 mg/kg for 7 days. The total amount of hGH delivered for a week was same in four groups, respectively. The plasma hGH and IGF-I concentrations were measured with ELISA kits (Diagnostic Systems Laboratory, Webster, TX, USA).

RESULTS AND DISCUSSION

Characterization of SR-hGH

SR-hGH microparticle was successfully produced by spray-drying method. The advantage of spray drying is that both mass and energy are transferred in a very short time keeping the product temperature well below 50°C in our operating conditions. Though the water remains on the surface of sprayed droplet, its transfer is immediate. All the energy is used for evaporation and the droplet remains at a constant temperature. According to the Malvern particle size analysis, the particle sizes for three batches of SR-hGH were 6.8 μm, 4.9 μm, and 5.3 μm, respectively, with a mean value of 5.6 ± 1.0 μm. As shown in Fig. 1, the particle size distribution was in the range 1~50 μm, and 95% of the population was less than 20 μm. With small particle size, SR-hGH could be easily injected through a 26-gauge needle, which may significantly contribute to the patient compliance.

Figure 2 shows nonaggregated and uniform microparticles of SR-hGH with a mean particle size of ca. 5 μm. The result was consistent with that of Malvern particle size analysis. The surface of sodium hyaluronate microparticles containing hGH was coated with lecithin to provide microparticle surfaces with affinity to oil phase. The resulting SR-hGH microparticle was well dispersed in MCT.

The recovery of hGH extracted from SR-hGH was 96.7 ± 3.5% based on the amount of hGH added to a feeding solution in the optimal ratio of hGH:HA:lecithin = 1: 3:0.5. When the ratio of hGH to HA decreased from 1:1 to 1:3 in the preliminary study, hGH released more slowly from SR-hGH with a smaller initial burst. The hGH content in SR-hGH microparticle was 21.5 ± 0.8% (w/w). Figure 3 shows SEC chromatogram of hGH extracted from SR-hGH, along with those of bulk hGH solution used for SR-hGH and Eutropin

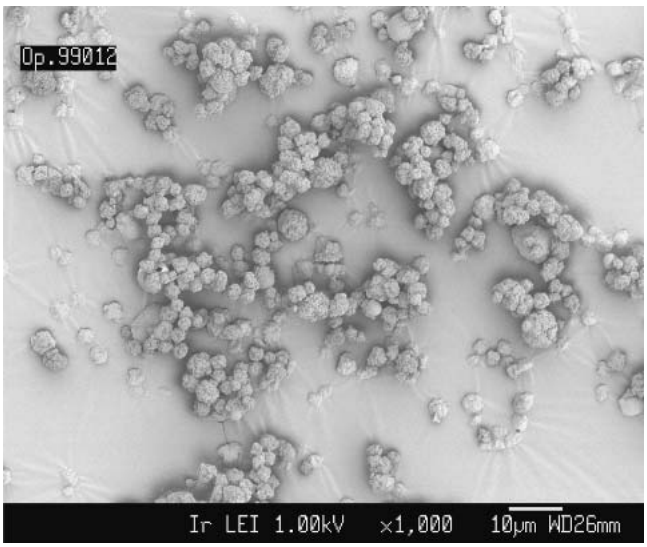

Fig. 2. Scanning electron microscopy of SR-hGH microparticles.

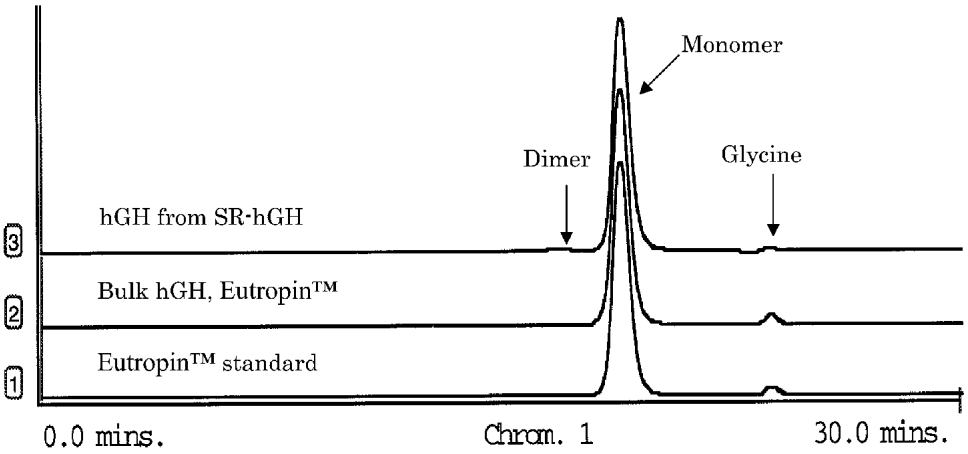

Fig. 3. Size exclusion chromatography of ① hGH (Eutropin) in-house standard, ② bulk hGH solution used for SR-hGH, and ③ hGH extracted from SR-hGH.

in-house standard. Main peak at a retention time around 19 min matches well to that of monomer in standard hGH. Peak around 17 min corresponds to the dimer and peak around 23 min to the glycine. There existed a small peak corresponding to dimer in SR-hGH chromatogram. The monomeric hGH contents for three batches of SR-hGH are listed along with that for Eutropin in-house standard in Table I. The monomeric hGH content of SR-hGH higher than 97% was comparable to that of Eutropin in-house standard and met the specification for impurity related to dimer or higher molecular weight mass not to be more than 6.0% for somatropin for injection (15).

Impurities related to hGH, such as oxidized and desamido forms of hGH, were quantified by RP-HPLC. Figure 4 shows RP-HPLC chromatograms of Eutropin in-house standard, bulk hGH solution used for SR-hGH, and hGH extracted from SR-hGH. There was no significant difference among the chromatograms. RP-HPLC for three batches of SR-hGH resulted in purities higher than 96%, which were consistent and comparable to that of Eutropin in-house standard (Table I). Content of impurities related to hGH less than 5% in SR-hGH satisfied the specification not to be more than 13% for somatropin for injection (15).

The fragments or covalent dimers of hGH in SR-hGH were analyzed by SDS-PAGE. Figure 5 shows the analysis of hGH by SDS-PAGE under reducing conditions. The major band for SR-hGH located in the same position at ca. 22 kDa with that for Eutropin in-house standard. On the contrary, band for a small amount of dimer in SR-hGH was detected at ca. 44 kDa. The amount of dimer in SR-hGH was estimated to be less than 1% based on the density.

All these results from SEC-HPLC, RP-HPLC, and SDS-PAGE confirmed that hGH remained intact during and after the formulation processes of SR-hGH. According to the recent reports, protein drugs are susceptible not only to thermal degradation but also to other types of denaturation (35), which can be overcome by adequate formulation using divalent metal ion and/or surfactant such as polysorbate-20 (36). In this work, lecithin and sodium hyaluronate was thought to contribute for the protection of hGH from denaturation.

In Vitro Release Test

The hGH release from SR-hGH was presented as a percentage (%) of cumulative amount released to the expected total amount based on the compositions of solid substances used for the preparation of SR-hGH. The release profile was consistent for three batches, as shown in Fig. 6. For all three batches, more than 80% of total loaded hGH was released within 24 h, and more than 90% of total loaded hGH was released in 48 h. According to the analysis with a Karl Fischer, the average water content of SR-hGH was 9.7% due to the

Table I. The Structural Intactness of hGH Extracted from SR-hGH

			Purity (%)	
		Recovery of hGH (%)	Content of monmeric hGH in SR-hGH	Content of native hGH in SR-hGH
	Methods	BCA assay	SEC	RP-HPLC
Standard hGH	UTS8001	N/A	100	97.0
	SR-hGH (1)	100	98.0	95.2
SR-HGH	SR-hGH (2)	93	98.0	96.2
	SR-hGH (3)	97	98.3	97.1

SR-hGH, sustained-release human growth hormone; SEC, size exclusion chromatography; RP-HPLC, reverse phase–high-performance liquid chromatography; BCA, bicinchoninic acid.
The amount of protein was determined by each of the indicated analysis methods. The data represent mean values of triplicate experiments with standard deviations less than 1%.

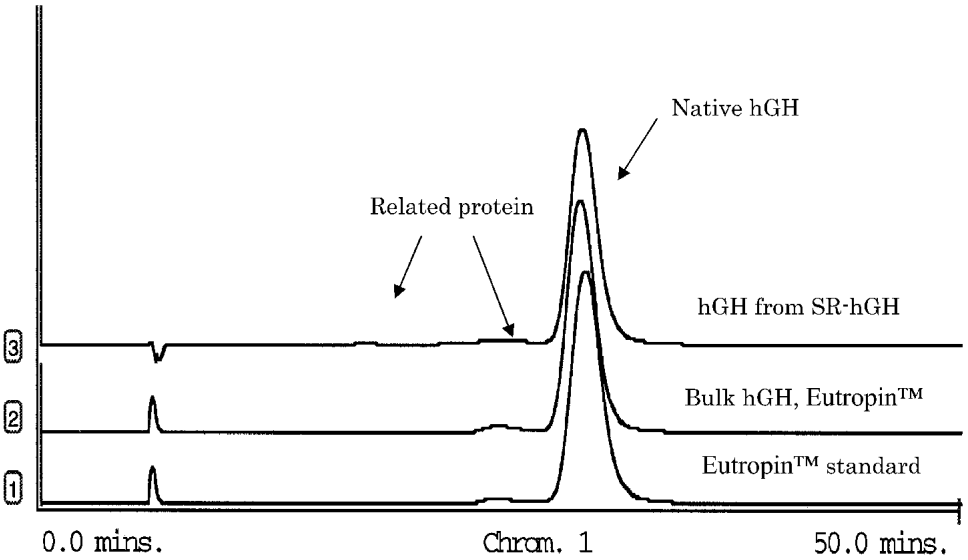

Fig. 4. Reverse phase–high perfomance liquid chromatoscopy of ① hGH (Eutropin) in-house standard, ② bulk hGH solution used for SR-hGH, and ③ hGH extracted from SR-hGH.

hygroscopic nature of sodium hyaluronate. Considering its water content, almost all the hGH was thought to be released in 48 h. The continuous and monotonous release profiles follow first-order release kinetics, which can be expressed in the following equation: hGH released (%) = 100 × [1 – exp(–0.091 t)]. In a word, *in vitro* release test showed the sustained-release characteristics of SR-hGH up to 48 h with complete release of hGH loaded.

In the case of PLGA formulation, many proteins encapsulated are often released in multiphasic manner. Considerable amount of loaded protein drug is released in a very early time, which is called as an "initial burst." After the "initial burst," release rate is drastically reduced, and finally no protein comes out even though considerable amount of protein remains in the particles. According to the *in vitro* release test of PLGA formulation with hGH (14), unreleased hGH in PLGA formulation was almost 30% after 28 days. A large amount of unreleased hGH might be ascribed to the strong hydrophobic interaction between hGH and PLGA.

In Vivo Test Using Beagle Dogs

Three groups of 6 male beagle dogs received single subcutaneous injections of SR-hGH at a dose of 1.0 mg hGH/Kg, respectively. In a comparative study, Eutropin bulk at a dose of 0.15 mg/kg was administered daily for a week subcutaneously. By a single administration, the release of hGH from SR-hGH continued for 72 h or longer with T_{max} between 10 and 12 h, whereas serum hGH concentration by a daily injection of conventional aqueous formulation dropped to baseline level within 12 h (Fig. 7). The C_{max} of serum hGH concentration resulted from SR-hGH was 69.5 ± 8.0 ng/ml, which was lower than that by the conventional aqueous formulation

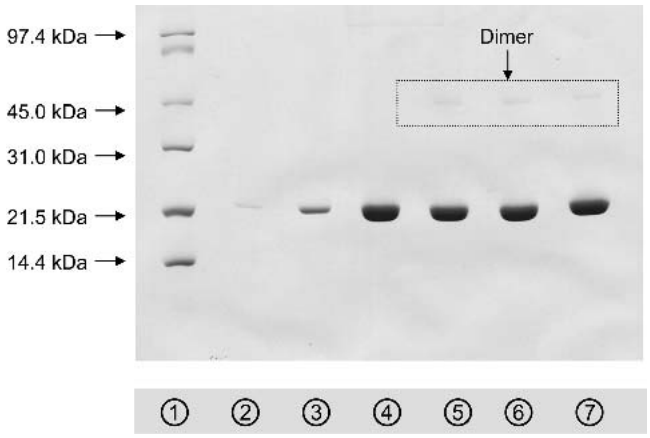

Fig. 5. SDS-PAGE for ① low-range MW marker, ② hGH (Eutropin) in-house standard (0.1 μg, lot no. UTS8001), ③ hGH (Eutropin) in-house standard (1.0 μg), ④ hGH (Eutropin) in-house standard (10 μg), and ⑤, ⑥, ⑦ hGH extracted from three different batches of SR-hGH (10 μg).

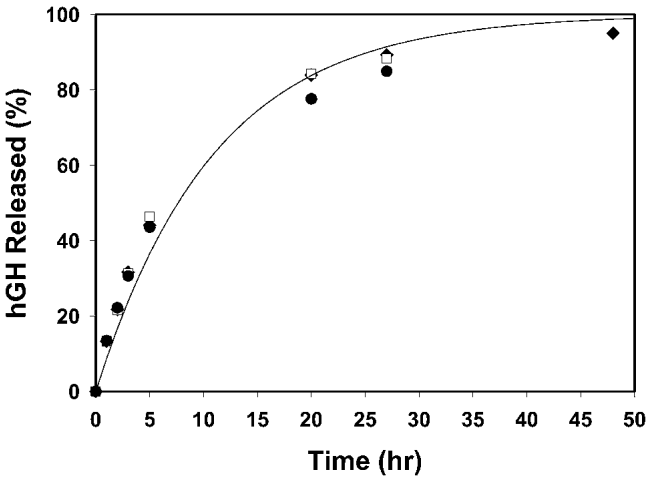

Fig. 6. *In vitro* release profile of hGH from SR-hGH microparticles: (♦) batch no. 1, (□) batch no. 2, and (●) batch no. 3. The data represent mean values of triplicate experiments with standard deviations less than 2.0%. Solid line represents curve fitting assuming first-order release kinetics.

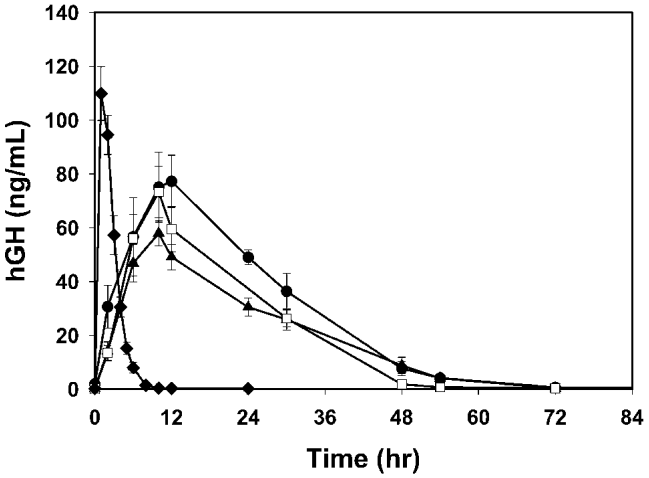

Fig. 7. The average serum hGH concentration of beagle dogs (n = 6) treated with an aqueous hGH formulation (0.15 mg hGH/kg, ♦) and SR-hGH microparticles (1.0 mg/kg): (●) batch no. 1, (□) batch no. 2, and (▲) batch no. 3. The error bars stand for the standard deviations.

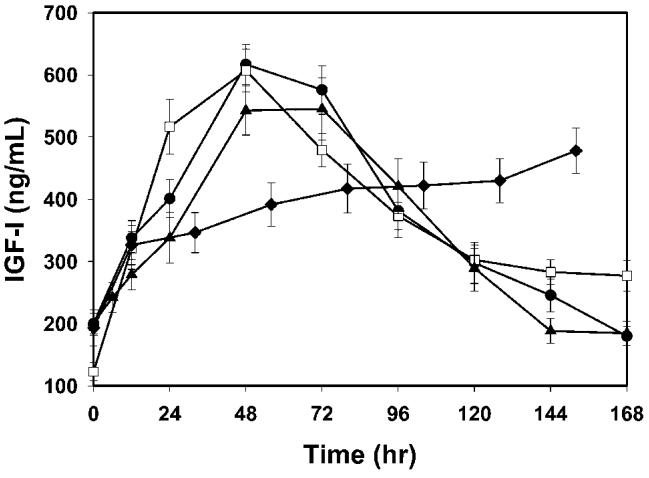

Fig. 8. The average serum IGF-I concentration of beagle dogs (n = 6) treated with an aqueous hGH formulation (0.15 mg hGH/kg, ♦) and SR-hGH microparticles (1.0 mg/kg): (●) batch no. 1, (□) batch no. 2, and (▲) batch no. 3. The error bars stand for the standard deviations.

with the equivalent dose. To state, there was no initial burst from SR-hGH. The AUCs for three batches of SR-hGH with the same compositions were slightly different from one another, which might be due to both the small differences in loaded hGH contents and the interanimal variations (Table II). Considering the AUC/dose, the bioavailability of SR-hGH was thought to be comparable to that of the daily injection formulation (Table II). Although a direct correlation between *in vitro* and *in vivo* tests was not observed, the results in the *in vitro* release test could be supported by the pharmacokinetic study in beagle dogs.

IGF-I, also known as somatomedin C, mediates the actions of growth hormone for tissue growth and metabolism and reflects the amount of hGH delivered. The daily administration of Eutropin bulk for a week resulted in a steady increase of IGF-I level with a final value of 478 ± 90.2 ng/ml as shown in Fig. 8. A single administration of SR-hGH induced continuous elevation of serum IGF-I level for 6 days with a maximum value higher than the predose level by ca. 350 ng/ml between 48 and 72 h (Fig. 8). After 6 days, IGF-I level returned to the initial baseline level. The IGF-I release profile was in the same pattern with that of Nutropin Depot using PLGA (13). Continuous induction of IGF-I by a single administration of SR-hGH demonstrated the bioactivity of hGH released from SR-hGH and supported the administration interval of a week.

There were no unscheduled deaths during the study. No clinical signs or effects on body weights and no abnormalities in ophthalmic examinations were observed after the administration of SR-hGH. Blood chemistry and hematological investigations revealed no effects related to the administration of SR-hGH. From the results, it was concluded that SR-hGH caused no adverse effects in beagle dogs.

HA is a biodegradable, biocompatible, nonimmunogenic, and noninflammatory polysaccharide that can be used as a good candidate for sustained delivery of protein drugs (20,21). The spray-dried HA solid microparticle with a high molecular weight worked as an excellent protein drug reservoir. Though conventional drug delivery systems using PLGA exhibited protein denaturation due to the hydrophobic interaction (12), denaturation of hGH in the presence of sodium hyaluronate was not observed both in aqueous phase and in solid state during and after the formulation of SR-hGH. There appeared to be no interaction between hGH and sodium hyaluronate, which was supported by the complete release of hGH as demonstrated in *in vitro* release test. In addition, beagle dog test did not exhibit a big initial burst, which was another disadvantage of PLGA systems (15). Compared

Table II. Pharmacokinetic Parameters of SR-hGH and Eutropin

Sample	Eutropin	SR-hGH (1)	SR-hGH (2)	SR-hGH (3)
Dose (mg/kg)	0.15	1.0	1.0	1.0
C_{max} (ng/ml)[a]	109.9 ± 10.0	77.2 ± 9.7	57.9 ± 4.7	73.3 ± 9.5
T_{max} (h)[b]	1	12	10	10
AUC (h · ng/ml)[c]	326	2158	1505	1600
AUC/dose (min · kg/ml)	0.130	0.129	0.090	0.096

SR-hGH, sustained-release human growth hormone; AUC, area under the curve.

[a] Average maximum serum hGH concentrations. All values are mean ± standard deviation.

[b] T_{max} was determined from the average serum hGH concentrations for six beagle dogs at the predetermined sampling time.

[c] AUC for Eutropin was determined from the curve area of average serum hGH concentrations between 0 and 24 h and AUC for SR-HGH between 0 and 168 h.

to the hydrogels prepared by radical polymerization (11) and specific cross-linking reaction by Michael-type addition (12) in the presence of protein drugs, SR-hGH was prepared by spray-drying method without a chemical reaction excluding any possible protein denaturation. SR-hGH is unique in safety issues and may successfully be used for clinical development.

In summary, SR-hGH has many distinctive advantages. A single administration of SR-hGH induced continuous elevation of serum IGF-I for 6 days, which supported the possibility of once-a-week injection formulation of hGH. With a small particle size and properly modified surface properties, SR-hGH showed excellent dispersability in MCT. The dispersion systems could easily be injected through a 26-gauge needle, which will significantly contribute to the patient compliance. No adverse effect was observed in beagle dog tests.

CONCLUSIONS

Aiming at once-a-week injection, a sustained-release formulation of human growth hormone was successfully developed using spray-drying method. Structural integrity of hGH was maintained during the formulation and storage according to the physicochemical analysis by SEC, RP-HPLC, and SDS-PAGE. The continuous and monotonous release profile observed in *in vitro* release test was supported by pharmacokinetic study in beagle dogs with C_{max} of 69.5 ± 8.0 ng/ml and T_{max} between 10 and 12 h. Bioavailability of SR-hGH was comparable to that of conventional aqueous daily injection formulation with an equivalent dose. The administration of SR-hGH induced elevation of serum IGF-I level for 6 days with a maximum value higher than the predose level by ca. 350 ng/ml between 48 and 72 h. After 6 days, IGF-I level returned to the initial baseline level. No adverse effect was observed during and after the beagle dog tests.

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