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Controlled release of human growth hormone following subcutaneous administration in dogs

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Abstract

Recombinant human growth hormone (rhGH) formulated in poloxamer-407 gel was administered subcutaneously in mixed breed dogs. To calculate pharmacokinetic parameters, all dogs were first administered 0.2 mg/kg of rhGH by an intravenous (i.v.) bolus injection. The i.v. dose was completely cleared in 3 h, with an elimination $t_{1/2}\beta$ of 0.574 h and $t_{1/2}\alpha$ of 0.068 h. After a wash out period of 7 days, all dogs received 0.7 mg/kg of rhGH subcutaneously as a gel formulation. Blood samples were drawn at frequent intervals for 132 h. Subcutaneous administration of the gel resulted in a peak maxima, 9 h after injection and concentrations of rhGH remained in the therapeutic window for 132 h. As compared to rhGH solutions, rhGH formulated in poloxamer gel resulted in a decrease in the maximum blood concentration and increased the time required to achieve maximum blood concentration. Hence, this approach may be promising for controlled release of rhGH, but needs further investigation to avoid a initial burst effect that was seen and to account for an observed loss of a portion of the administered dose. © 1997 Elsevier Science B.V.

Keywords: Human growth hormone; Controlled release; Poloxamers; Dog study

1. Introduction

Deficiency of growth hormone in children has been treated with pituitary derived human growth hormone for several decades, but it was only in mid-1980s that recombinant human growth hormone (rhGH) was marketed for this purpose. Pituitary derived human growth hormone (potency 2 IU/mg) is typically administered intramuscularly at a dose of 0.06–0.10 IU/kg three times a week (Frasier, 1983). After the introduction of rhGH, a potentially unlimited supply of the hormone free of pituitary contaminants became available (Pearlman and Bewley, 1993). Since then, efforts have been directed towards refining growth hormone treatment schedule to improve therapy. Switching from thrice weekly intramuscular (i.m.)

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injections to daily subcutaneous (s.c.) injections at bed time significantly improved patient compliance and resulted in an increased growth response (Kastrup et al., 1983; Hermanussen et al., 1985). In another report, better growth rates in children were observed by increasing the number of injections of rhGH per week (Groesbeck and Parlow, 1987). In addition to these considerations, current therapeutic use of rhGH is limited by its short half life, renal toxicity, rapid clearance, and the need for multiple injections. Thus, a controlled release formulation of rhGH administered subcutaneously could provide several advantages. Very recently, a microsphere formulation for rhGH has been reported, which provided elevated serum levels of rhGH for more than 1 month following a single injection in monkeys (Johnson et al., 1996).

Poloxamers are nonionic polyoxyethylene–polyoxypropylene copolymers, differing in the relative amounts of (hydrophobic) polyoxypropylene and (hydrophilic) polyoxyethylene segments (Wade and Weller, 1994). Poloxamers are commercially available as a series of polymers with varying surface properties (BASF, 1975). Some exhibit the property of reverse thermal gelation. These polymers exist as a mobile viscous liquid at low temperatures, but form a rigid semisolid gel at higher temperatures. Thus, it is possible to use these polymers to design a formulation which is liquid at room temperature or at lower temperatures and below, but gels once injected, thus providing a depot of drug at the injection site. The most promising of these polymers is Poloxamer 407 (Pluronic F 127), which has been evaluated for its toxicity potential following i.m. injection in rabbits (Johnston and Miller, 1985). Poloxamer 407 has been used for the sustained delivery of interleukin-2 (Johnston et al., 1992) and urease (Fults and Johnston, 1990) from a gel matrix. Since rhGH is rapidly cleared from the body, efforts to develop a sustained release version are currently underway (F-D-C Reports The Pink Sheet®, 1995, 6 February). In an effort to achieve sustained release, conjugation of growth hormone with albumin has been reported to alter the pharmacokinetics of the hormone (Poznansky et al., 1988). Poloxamer 407 enhances the stability of urease and interleukin-2 (Wang and Johnston, 1995) and we have previously shown that another poloxamer enhances the stability of rhGH (Katakam et al., 1995). Use of poloxamer gels to develop such a product would not only provide a sustained release formulation, but also enhance the physical stability of the hormone. Administering the gel as a solution eliminates the need for any surgical techniques, which may be required for some other sustained release formulations such as implants.

In the present study, rhGH was administered s.c. into dogs as a controlled release formulation using poloxamer-407 gel. The pharmacokinetic profile of rhGH administered i.v. was also studied. The peak pattern and duration of release of rhGH from gels were evaluated.

2. Materials and methods

2.1. Materials

Freeze dried recombinant human growth hormone was obtained as a gift from Eli Lilly and Company (Lilly Corporate Center, Indianapolis, IN). Poloxamers were gift samples from BASF Wyandotte (Parsippany, NJ) and were used as received. Sodium phosphate monobasic and sodium phosphate dibasic heptahydrate were obtained from Fisher Scientific (Fairlawn, NJ).

2.2. Animals

Four mixed breed dogs weighing 10–20 kgs were used. They were vaccinated and evaluated by physical examination and clinical laboratory tests. Animals were acclimated 1 week at the College of Veterinary Medicine Animal Facility prior to the study.

2.3. Preparation of rhGH solutions and poloxamer gels

Solutions of rhGH were prepared in phosphate buffer (20 mM). Phosphate buffer was prepared in double distilled filtered (0.22 μ) water and its pH was adjusted to 7.4 ± 0.02 with sodium

hydroxide solution. Final concentration of gel injected into dogs ranged from 23 to 46 mg/ml, depending on the animal weight, so as to allow us to inject a constant volume (0.3 ml) to all dogs. Calculated amount of rhGH required for injection was dissolved in phosphate buffer and sterilized by filtering through 0.22 μ sterile filter. Solutions of rhGH were prepared immediately prior to use. Poloxamer gels were prepared with 36% w/w poloxamer 407. All gels were prepared on a weight percentage using the cold method of preparation (BASF, 1975). Poloxamer was added to a tube containing the solution of rhGH in phosphate buffer. These tubes were placed at 4°C overnight to facilitate dissolution of the polymer. After staying overnight at 4°C, the tubes were placed in ice bath and swirled at 25 rpm to ensure a homogenous solution.

2.4. In-vivo study in dogs

Recombinant human growth hormone was administered twice to dogs: first as an i.v. dose of a 0.2 mg/kg solution and then (after a washout period) as a 0.7 mg/kg s.c. dose of the poloxamer delivery system. This dose was based on in-vitro release studies conducted from the poloxamer gel (data not shown). For i.v. studies, a jugular vein catheter was placed prior to dosing. Hormone solution was administered via the cephalic vein followed by drawing 15 blood samples (2 ml each) over a period of 3 h via the jugular catheter. After a 1 week wash out period, dogs received a s.c. dose of rhGH in poloxamer gel injected over the cranial thoracic vertebra. The volume of injected gel was from 0.3 to 0.5 ml depending on the animal's weight. A total of 14 blood samples (2 ml each) were drawn over a period of 5 days by venous puncture of alternate jugular veins.

2.5. Quantitation of rhGH release

Serum samples were analyzed for rhGH by a commercially available Tandem®-R HGH immunoradiometric assay (Hybritech, San Diego, CA). This is a solid phase two-site immunoradiometric assay, where a sandwich of solid phase/hGH/labeled antibody is formed in the presence of rhGH in the sample.

2.6. Pharmacokinetic calculations

The pharmacokinetics of rhGH was obtained by modeling each subject intravenous data to both a two and three-compartment model by nonlinear least squares procedures (PCNONLIN, Statistical Consultants, Lexington, KY). Based on residual plots and improvement in the weighed $(1/y)$ sum of squares a two-compartment model best described the plasma concentration profile. From coefficients and exponents, rate constants, volumes and clearance were calculated by traditional methods.

The percentage of the rhGH dose remaining to be absorbed as a function of time was obtained in each dog following administration of the poloxamer gel formulation. Utilizing the i.v. kinetic parameters, the percentage absorbed at each time period was estimated by a method described by Wagner (Wagner, 1983). This method predicts the amount that has been absorbed at each sampling based on mass balance and is applicable to agents displaying multicompartment features following i.v. administration. By predicting the amount of drug that is in the central and peripheral compartments and has been eliminated at each sample time, the amount of drug which has been absorbed is calculated. Drug amounts are estimated from microrate constants of the i.v. two compartment model for each dog and the cumulative area under the curve for each dog following s.c. administration of the rhGH poloxamer gel.

3. Results and discussion

There are only a few reports on the controlled release of rhGH (Poznansky et al., 1988) and none with the use of poloxamers. With a 22 000 Da molecular weight, rhGH is a complex protein with formulation problems in terms of maintaining activity. A poloxamer is a promising agent to stabilize rhGH against processing stresses (Katakam et al., 1995). The poloxamer-407 used in the present study was also shown to be promising in stabilizing against processing stress such as shaking (data not shown). Hence, using poloxamer in the present study served the dual purpose

of stabilizing rhGH as well as controlling its release. Also, there are no reports in the literature on the i.v. bolus profile of rhGH in dogs. A study to determine necessary pharmacokinetic parameters was conducted by administering rhGH as an i.v. bolus thus, allowing the calculations of the percentage of the dose absorbed.

All plasma samples were analyzed by immunoradiometric monoclonal antibody assay (Schalch and Parker, 1964; Pearlman and Bewley, 1993), where there was no interference of canine growth hormone. This assay is considered as very sensitive and selective towards detecting bioactive protein in the plasma. It is widely used clinically to estimate endogenous rhGH levels in children (Hybritech Product Information).

The plasma concentration time curve following i.v. administration of rhGH (0.2 mg/kg) is shown in Fig. 1. The decline of plasma levels was biphasic and reached to undetectable levels after 3 h. The $t_{1/2}\alpha$, $t_{1/2}\beta$ and MRT are listed in Table 1. The terminal half life of 0.574 h is also similar to reported value in humans (Jorgensen, 1991). Elimination of rhGH in humans follows first order kinetics with serum $t_{1/2}$ ranging from 9–28 min after i.v. bolus injections. This wide range is dependent on the duration of the initial distribution phase. This distribution phase reflects both a redistribution of growth hormone to the peripheral compartment and true elimination from the central compartment. The plasma concentration time curve following s.c. administration of the controlled release poloxamer gel formulation of rhGH is shown in Fig. 2. The percentage of rhGH remaining to be absorbed is displayed in Fig. 3. From both graphs, an initial burst effect was briefly observed. This may be due to rapid absorption during the lag time which was required for the injected solution to get into gel form. The formulation was stored at 2–8°C until ready to administer. Once the formulation was bought to room temperature, it gelled within a few minutes. The problem of burst release during this time can possibly be adjusted by formulation changes in further studies, such as by increasing the concentration of poloxamer in the gel. The mechanism of release from the gel seems to be a combination of gel erosion and diffusion. In-vitro release of

Table 1
Pharmacokinetic parameters of rhGH in dogs ($n = 4$) following a 0.2 mg/kg i.v. bolus dose

Parameter	Mean	S.D.
$t_{1/2}$-β	0.574 (h)	0.257
$t_{1/2}$-α	0.0687(h)	0.356
MRT	0.345 (h)	0.120
Vc	0.0291 (l/kg)	0.014
Vss	0.0670 (l/kg)	0.027
*k*12	4.29 (h−1)	4.060
*k*21	2.57 (h−1)	1.770
*k*10	7.78 (h−1)	3.980
Cl	0.194 (l/h per kg)	0.038
r	0.986	0.008

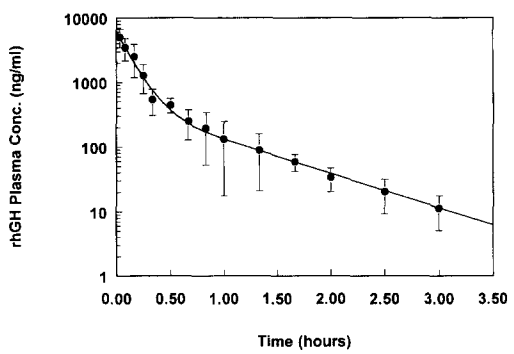

Fig. 1. Pharmacokinetics of human growth hormone following an intravenous dose (0.2 mg/kg) in dogs. Each point represents the mean (± S.D.) of the values from four dogs.

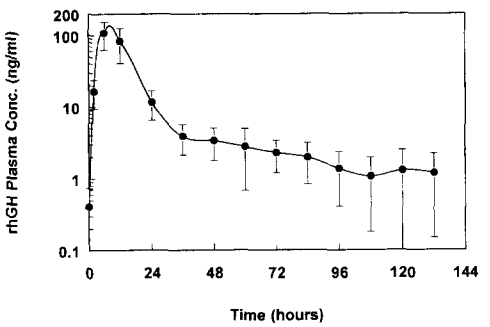

Fig. 2. Controlled release of human growth hormone (after a burst effect) from poloxamer gel following subcutaneous administration in dogs (0.7 mg/kg). Each point represents the mean (± S.D.) of the values from four dogs.

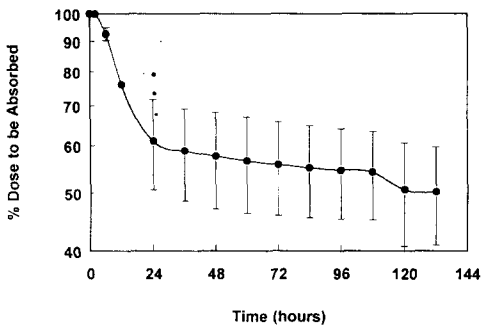

Fig. 3. Human growth hormone remaining to be absorbed from poloxamer gel following subcutaneous administration in dogs (0.7 mg/kg). Each point represents the mean (± S.D.) of the values from four dogs.

rhGH from this gel has been described by us recently (Katakam et al., 1997). The therapeutic range for rhGH in blood is 5–10 ng/ml (Eddy et al., 1974; Tandem®-R HGH Immunoradiometric assay product sheet, Hybritech, San Diego, CA). Blood levels were maintained in or close to this therapeutic range for 132 h. The C_{max}, t_p and AUC at 132 h (AUC_{132h}) are listed in Table 2. Earlier investigations in humans, as well as in rats, have indicated that after s.c. administration t_p was 3–4 h and time for complete disappearance was less than 24 h (Sandahl et al., 1983; Jorgensen et al., 1987, 1988; Hedin et al., 1993). When compared to these, the poloxamer gel formulation of rhGH administered s.c. is prolonging delivery. Before these dog studies, we found that s.c. injection of poloxamer gel to rats resulted in a prolonged release as compared to s.c. injection of control solutions (data not shown).

As shown in Fig. 3, a significant amount of administered rhGH seems to be unabsorbed or unaccounted for. This may be due to degradation at the site of injection. It has been shown by (Jorgensen et al., 1988) that there is a significant amount of rhGH degradation at the site of s.c. injection. The poloxamer system may protect the protein against local proteolytic enzymes as long as the protein is entrapped in the gel and not after passing from the gel to the tissue and fluids in the injection site.

Table 2
Pharmacokinetics of rhGH in dogs following a 700 μg/kg subcutaneous dose of gel

Parameter	Mean	S.D.
C_{max}	131.59 (ng/ml)	9.12
t_p	9.0 (h)	3.46
AUC_{132h}	31228.55 (ng/h per ml)	6858.2

In this study our results also confirm the earlier reports of first order elimination of rhGH. Also, the total body clearance (Cl) of rhGH (0.194 l/h per kg) in dogs is reported to be in between that for humans (0.124 l/h per kg) and rats (0.959 l/h per kg) (Mordenti et al., 1991). Therefore, because of low clearance values in humans than in dogs, humans would require a lower dose (per kg) as compared to dogs.

To summarize, the present study in dogs has demonstrated the in-vivo release of rhGH from a poloxamer parenteral delivery system following s.c. administration. The plasma concentration profiles of rhGH resulting from the delivery system were evaluated to estimate the in-vivo release rate of immunoreactive rhGH. A controlled release formulation of rhGH can improve patient compliance and therapy, though further studies are needed to optimize the formulation and eliminate the initial burst effect.

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