Sustained delivery of human growth hormone from a novel gel system: SABER™

Franklin W. Okumua, Le N. Dao, Paul J. Fielder, Noel Dybdal, Dennis Brooks, Samir Sane, Jeffrey L. Cleland

Abstract

Purpose: The purpose of this study was to evaluate the release of recombinant human growth hormone (rhGH) from a novel non-polymeric sustained release system, SABER™.

Methods: The SABER system consists of sucrose acetate isobutyrate, a solvent and a polymeric release modifier. Spray dried formulations of zinc complexed rhGH in sodium bicarbonate containing sucrose and polysorbate 20 were homogenized with various SABER systems (10% w/v rhGH) and assessed in vitro and in vivo (rat studies). The effect of protein to sucrose ratio in the spray dried formulation and a release modifier, poly-d,l-lactic acid (PLA), in the SABER system, on the initial release was investigated along with the effect of dose volume.

Results: The in vitro release studies with rhGH SABER suspensions indicate that increasing the sucrose content from 2 to 5 mg/ml in the rhGH formulations increased the initial release (24 h) from 78.0% to 93.5%. When the protein formulation was held constant and 1.0% w/w PLA was added to the solvent phase, the initial release was reduced from 78.0% to less than 5.0%. The initial release in vivo after subcutaneous administration (SC) in rats (15 mg/kg rhGH) decreased with increasing PLA content (1.0% w/w PLA, $C_{\text{max}} = 342.8$ ng/ml; 10% w/w PLA, $C_{\text{max}} = 35.4$ ng/ml), while increased sucrose content increased both the initial release (AUC$_{0-2}$ days) and persistence (AUC$_{2-7}$ days) over the 7 days from 64.2 to 228.4 ng day/ml (total AUC). A linear dose response (rhGH serum levels) was observed after SC administration of different rhGH SABER volumes greater than 100 μl. Histological examination of the injection sites indicated a mild inflammatory response similar to that observed after injection of PLA microspheres.

Conclusions: The addition of PLA reduced the initial release rate of protein release from SABER, while increasing the sucrose content of the protein formulation yielded increased rhGH persistence. These results demonstrate that the SABER delivery system allows weight-based dosing at volumes greater than 100 μl to achieve sustained release of intact rhGH in vivo for at least 7 days.

Keywords: Drug delivery; SAIB; Sustained release; Biodegradable; Human growth hormone

1. Introduction

Recent advances have broadened the acceptance of depot systems for use in humans [1–5]; however, commercial use of these systems can be hindered by the complexity and cost of the manufacturing process used to produce the delivery matrices [6,7]. Sucrose acetate isobutyrate (SAIB, Fig. 1) extended release (SABER) is a novel biodegradable extended release system based on a high viscosity non-polymeric scaffold [8–11]. One characteristic of the system is that a SAIB/solvent mixture has a low viscosity, but upon injection the viscosity increases dramatically as the solvent diffuses away from the SAIB. The main advantages of this system are the ease of use (SC injection of a liquid) and sustained delivery of active agent for the desired period of time. SABER systems are currently being developed to deliver synthetic small molecules and peptides over a period of 1–4 weeks [12]. Considerable effort has been put into developing PLGA based sustained release systems for proteins and polypeptides.
over the last 10 years. The primary challenges have been stabilizing the protein prior to and during the encapsulation process as well as in vivo release of bioactive protein [13–16]. In 1999 the US FDA approved a PLGA microsphere formulation, Nutropin Depot, as a once a month alternative to daily injections of human growth hormone. The pharmacokinetic (PK) profile after SC administration of Nutropin Depot microspheres can be described by an early release phase (0–2 days) during which 20% of the total bioavailable growth hormone is released, followed by a sustained release phase (14–25 days) where the remaining growth hormone is released [17]. The SABER system distinguishes itself from PLGA microsphere systems in that the injectable liquid matrix controls the release of the entrapped drug. This feature was desirable for two reasons: higher concentrations of drug can be achieved in each injection (50–100 mg/ml) and the ability to administer these high doses via conventional syringe and needle (25 Ga). Based on these properties and our interest in developing systems suitable for extended delivery of large hydrophilic proteins, we designed this study to examine the feasibility of using SABER for sustained delivery of rhGH. The following factors were examined: the protein formulation, the effect of polymeric additives on the in vitro initial release, and the in vivo pharmacokinetics.

2. Materials and methods

2.1. Materials

SAIB was provided by Southern BioSystems Inc., a subsidiary of DURECT Corporation (Birmingham, AL). Poly-D,L-lactic acid (0.18 dl/g) was obtained from Boehringer Ingelheim (Ingelheim, Germany). *E. coli* derived recombinant human growth hormone (rhGH) was produced and purified by Genentech Inc. (S. San Francisco, CA). Sucrose (USP/NF grade) was obtained from Pfanstiehl (Waukegan, IL). Solvents used were USP/NF grade unless otherwise stated. All buffers were prepared using reagent grade materials unless otherwise stated.

2.2. Preparation of protein formulations

Protein formulations (Table 1) were prepared by adding zinc using a 100 mM zinc acetate to yield a 10:1 M ratio of Zn:rhGH followed by adding sucrose and polysorbate 20 (10% w/v stock solution) to a 20 mg/ml solution (0.22 μm filtered) of rhGH formulated in 25 mM sodium bicarbonate buffer at pH 7.5. The resulting suspensions were maintained via magnetic mixing prior to spray drying.

2.3. Spray drying

Spray dried powders were prepared using a Büchi Model 190 mini spray dryer (Büchi Labortechnik AG, Flawil, Switzerland). The protein formulations were atomized using compressed air from an in-house supply coupled to an internal mixing two-fluid nozzle (0.5 mm). The following operating conditions were used: inlet temperature of 90°C, a drying air flow rate of 0.6 m³/min, an atomizing air flow rate of 0.9 m³/h, and a liquid feed of 5.0 ml/min. Operation under these conditions lead to an outlet temperature of approximately 50°C.

2.4. Residual moisture content

The residual moisture content of the rhGH powders was determined by Karl Fischer titration (Metrohm, Herisau, Switzerland) [18].

2.5. Preparation of SABER rhGH suspensions

SABER rhGH suspensions were prepared by mixing rhGH powder with SABER solutions (Table 2) followed by homogenization for 2 min at 8000 rpm (5 mm microfine shear homogenizer, Virtis, Gardner, NJ). The SAIB/solvent ratio for SABER solutions was selected to produce mixtures with the desired viscosity. PLA/solvent was added as a release modifier prior to protein addition (Fig. 2).

2.6. In vitro release

SABER rhGH suspensions (100 μl ca.) were placed in 1.5 ml of release buffer (50 mM HEPES, 5 mM EDTA, 0.01% w/v NaN₃, pH 8.0) and incubated at 37°C. The release buffer was sampled and replaced after incubation for 1 and 24 h. The monomeric content and the amount of rhGH released were determined by size exclusion chromatography (SEC).
2.7. Protein analysis

SEC was used to determine the amount of rhGH monomer present in the samples. This assay was run using a 7.8/300 mm2 TSK 2000SWXL column held at room temperature. The mobile phase used was 50 mM NaH2PO4, 150 mM NaCl, pH 7.2 with a flow rate of 1.0 ml/min and a run time of 20 min. Twenty-five microliters of each sample was injected and the eluent monitored for absorbance at 214 nm. The amount of protein present in each formulation was determined by dissolving weighed amounts of spray dried rhGH in release buffer and determining the protein content by UV spectrophotometric analysis.

2.8. UV spectrophotometric analysis

Protein concentrations were measured at 277 nm (extinction coefficient 0.81 ml/mg cm) with a HP8453 spectrophotometer (Agilent Technologies, Mountain View, CA) and 1.0 cm quartz cuvette (Hellma, Plainview, NY).

2.9. In vivo studies

Pharmacokinetics of rhGH were determined after subcutaneous (SC) injections of SABER rhGH suspension (dose = 15–90 mg/kg) depots in Sprague Dawley (SD) rats (Charles River, Wilmington, MA) with six animals per dose group. rhGH serum levels were determined by ELISA (Genentech Bioassay group) with an assay detection limit of 0.1 ng/ml. Injection sites were collected after necropsy and stored in formalin prior to routine histological evaluation. PK results were generated using non-compartmental analysis.

3. Results and discussion

Powders produced by spray drying zinc complexed rhGH maintained high monomeric contents and produced clear solutions when dissolved in release buffer (Table 1). Both powders exhibited low residual moisture levels (<5%). Formulations A and B contained 60% and 50% rhGH by weight, respectively. The decrease in protein mass fraction observed with Formulation B is likely due to the higher sucrose content present in this formulation. After reconstitution, rhGH from Formulations A and B contained greater than 99.0% monomer (SEC) indicating that the formulation excipients used
were able to protect the native structure of rhGH during the spray drying process. Significant loss of rhGH monomer was observed after 24 h incubation of SABER rhGH formulations in release buffer at 37°C (Table 3). This loss of monomer may be due to prolonged exposure of dilute rhGH to high organic solvent concentrations in the aqueous release buffer at elevated temperatures, because rhGH tends to denature at water–organic interfaces. [19] The initial in vitro release of rhGH was heavily dependent on the amount of PLA present in each formulation and decreased from 78.0–0.2% (Formulation A) and 93.5–0.5% (Formulation B) when 10% (w/w) PLA was added to SABER formulations (Table 3). The presence of PLA in the SABER formulations likely modulated the initial release of rhGH by forming a diffusional barrier (skin) around the depot after contact with the aqueous buffer. This skin is formed when the solvent composition at the aqueous interface changes as the organic solvents diffuse away from the depot and water infuses in, causing the dissolved PLA to precipitate. Measurement of in vitro sustained release was not possible in these studies due to the lack of a system to degrade the SAIB.

To evaluate the sustained release phase and effects of PLA and sucrose content on the overall release, SABER rhGH formulations were administered subcutaneously to rats (Fig. 3). The C_max and AUC_0–2 days decreased when the PLA content was increased from 1.0% to 10% w/w (Table 4). An increase in C_max and AUC_0–2 days was observed when formulation B was used in conjunction with 10% PLA. While the additional sucrose in formulation B facilitates more rapid initial protein release, it also appears to increase the amount released during the sustained release phase (higher AUC_2–7 days). This persistent higher level of released protein may be due to the presence of a higher sucrose level, which further stabilizes the native rhGH structure inside the eroding depot. The presence of additional dissolved sucrose could also lead to higher influx of water into the hydrophobic regions of the depot, where it could increase the dissolution rate and subsequent release of native rhGH from the depot.

With the release of rhGH being controlled in part by erosion of SAIB and PLA at the depot surface, it is possible that the tissue exposed surface area of each depot could ultimately determine the protein release rate, making weight-based dosing difficult. Currently, rhGH is administered to patients based on their body weight (mg/kg). In the case of daily SC injections, weight-based dosing is accomplished by injecting larger volumes of a fixed protein concentration solution as the dose increases with weight. Non-linear PK has been observed after IM administration of small organic molecules formulated in oils [20]. To determine if weight-based dosing of a SABER rhGH formulation provides linear PK over the desired dose range, three different volumes of rhGH SABER depots (80, 241 and 481 μl) were administered to rats (Fig. 4). The lowest dose did not correlate to a linear extrapolation of the two higher doses suggesting that the surface area to

Table 3

<table>
<thead>
<tr>
<th>SABER formulation</th>
<th>Protein formulation</th>
<th>Initial release (%)</th>
<th>Monomer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A</td>
<td>78.0</td>
<td>95.0</td>
</tr>
<tr>
<td>II</td>
<td>A</td>
<td>2.0</td>
<td>67.0</td>
</tr>
<tr>
<td>III</td>
<td>A</td>
<td>0.2</td>
<td>97.4</td>
</tr>
<tr>
<td>I</td>
<td>B</td>
<td>93.5</td>
<td>89.0</td>
</tr>
<tr>
<td>II</td>
<td>B</td>
<td>3.4</td>
<td>65.0</td>
</tr>
<tr>
<td>III</td>
<td>B</td>
<td>0.5</td>
<td>59.0</td>
</tr>
</tbody>
</table>

*a Percent of total rhGH contained in depot that is released into buffer over 24 h.

Table 4

<table>
<thead>
<tr>
<th>SABER formulation</th>
<th>Protein formulation</th>
<th>Dose (mg/kg)</th>
<th>AUC_0–2 days (ng day/ml)</th>
<th>AUC_2–7 days (ng day/ml)</th>
<th>C_max (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A</td>
<td>15.0</td>
<td>268.9</td>
<td>88.6</td>
<td>342.8</td>
</tr>
<tr>
<td>II</td>
<td>A</td>
<td>15.0</td>
<td>38.3</td>
<td>25.9</td>
<td>35.4</td>
</tr>
<tr>
<td>III</td>
<td>B</td>
<td>15.0</td>
<td>142.9</td>
<td>85.5</td>
<td>159.0</td>
</tr>
</tbody>
</table>

*a SD rats.

*b rhGH SABER suspensions were prepared to contain 10% (w/v).
volume ratio of the injected gel may have an impact on
the release kinetics for small volumes (e.g. <100 μl). The
lower dose also had a greater standard deviation
suggesting that small volumes may also provide more
variability in release rates. However, a fairly linear
relationship between dose and serum rhGH levels
existed for the higher doses (241 and 481 μl). This
linearity may permit weight based dosing (mg/kg) by
adjustment of the rhGH SABER depot injection
volume, but may be restricted by a minimum volume
requirement. These data also suggested that the presence
of PLA at the surface of the SABER rhGH depot may
reduce the accessibility of the protein to the depot/water
interface causing release governed by protein diffusion
and degradation of SAIB leading to a continuous release
of native rhGH with minimal initial release [21,22].

The biocompatibility and safety of SAIB for use as a
food additive has been established for daily intake of up
to 20 mg/kg body weight, and the US FDA has recently
approved SAIB for use as a food additive [11]. The use
of SAIB as a drug delivery matrix, however, would
require compatibility of the solvents and any added
excipients with the encapsulated drug and the local
injection site. The biocompatibility and local tolerance
of SAIB in combination with benzyl benzoate, ethanol
and benzyl alcohol was evaluated in SD rats 10 days
after SC injection of SABER formulations (0.3 ml/kg).
After injection the SABER formulations were found
distributed in multiple small droplets around a central
mass. Mild inflammation was observed at the injection
sites and all SAIB solvent combinations were well
tolerated by the animals.

Because all three solvents were well tolerated, further
evaluation of tissue compatibility was based upon the
better protein compatibility and lower initial release rate
from formulations containing ethanol and benzyl
alcohol as compared to formulation with benzyl
benzoate [23]. Histological examination of the injection
sites after administration of ethanol/benzyl alcohol
formulations indicated the presence of a localized,
cavitated inflammatory process associated with rhGH
SABER depots 7 days after injection (end of study). The
walls of the cavitated lesions were composed of
immature fibrovascular reaction (granulation tissue)
admixed with lymphocytes, plasma cells and histiocytes
(Fig. 5). Cellular and formulation debris were also
present but were not associated with any apparent
increased inflammation or necrosis. The local tissue
response to rhGH SABER depots appeared to be very
similar to that observed after subcutaneous injection of
PLGA and PLA microspheres [24,25]. Further studies
are required to fully characterize the biodegradation and
biocompatibility of rhGH SABER depots and the
impact of repeat administration. However, these pre-
liminary findings indicated that SABER formulations

![Pharmacokinetics after SC injection of 50 mg/ml rhGH SABER Depot (SABER III w/Formulation B) at 15 mg/kg (●), 45 mg/kg (□) and 90 mg/kg (■) (data are normalized to 15 mg/kg rhGH).](image1)

![Representative histology of injection sites 7 days after administration of SABER rhGH depot (SABER III w/Formulation B).](image2)
containing PLA and rhGH are biocompatible and well tolerated.

4. Conclusions

An injectable gel formulation of rhGH as a sustained release depot preparation was developed using the SABER system. Addition of a non-water soluble polymer additive, PLA, may reduce the amount of protein available for rapid dissolution at the surface of the SABER rhGH depots. The use of disaccharides such as sucrose in the protein formulation may protect the protein from degradation both during spray drying and exposure to organic solvents, leading to an enhanced protein available for rapid dissolution at the surface of the depot. These initial in vivo studies suggest that SABER rhGH depots may have utility as a sustained delivery system to allow administration to growth hormone deficient patients on a weekly or less frequent basis.

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References