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Sustained release hGH microsphere formulation produced by a novel supercritical fluid technology: *In vivo* studies

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ABSTRACT

Novel sustained release formulations of hGH prepared by supercritical fluid processing of PLGA/PLA (the CriticalMix™ process) were produced in the form of microparticles for subcutaneous injection. The basis of the process is that PLGA/PLA polymers liquefy when exposed to supercritical CO_2, thereby allowing the hGH to be mixed efficiently into the polymers at an ambient temperature and in the absence of solvents. The CO_2 was removed from the mixture by depressurisation through a nozzle, resulting in the production of microparticles containing the hGH, which were collected in a cyclone. The best microparticle formulations showed an initial *in vitro* burst of around 35% and a sustained release over 14 days. When tested in the rat model, which displays a faster clearance rate of hGH than other animal models, two formulations showed prolonged release over 2–3 days with sustained plasma levels at 1–5 ng/ml whereas the soluble hGH formulation was cleared within 24 h. Two selected sustained release formulations were tested in cynomolgus monkeys and compared to a single injection of soluble hGH. The burst release from the sustained release formulations was similar in magnitude to a daily dose of hGH and serum hGH levels were maintained for a seven day period. It is probable from the data that the sustained release would have continued for up to 14 days if sampling had been continued. The IGF-1 results showed there was no significant difference between the levels obtained for once daily injection of soluble hGH and the two sustained release formulations.

1. Introduction

Human growth hormone (hGH) is a 22 kDa protein, that stimulates growth and cell reproduction in humans and other animals. In healthy individuals it is synthesized and stored in the anterior pituitary gland and secreted into the circulation in a pulsatile pattern. Recombinant hGH is currently used therapeutically to treat a number of conditions including growth hormone deficiency in children with hypopituitary disorders, growth failure due to chronic renal insufficiency, Turners syndrome and growth hormone deficiency in adults. hGH is poorly absorbed orally and therefore is normally administered by daily injections for a period of several years [1,2]. This treatment regimen has a considerable impact upon patients' lives, and can affect patient compliance. A sustained release formulation of hGH to be administered twice a month would therefore provide a significant therapeutic advantage over current treatments. Clinical studies have shown that a continuous infusion of hGH via a pump is as efficacious as daily administration with equivalent growth velocity and IGF-I levels, the principle mediator of hGH effects [3–6]. No significant difference in safety profile between the two modes of delivery was shown [3–6]. A sustained release formulation of hGH can be achieved by encapsulation of the drug into injectable microspheres of biodegradable and biocompatible polymers such as PLGA or PLA. The hGH is slowly released from the microspheres by diffusion and by degradation of the polymer to lactic or glycolic acid. Such a formulation, Nutropin Depot, manufactured using Alkermes' Prolease microsphere system, was approved by the FDA and marketed in 1999 by Genentech for treatment of pediatric growth hormone deficiency. The hGH was stabilized by forming an insoluble complex with zinc and encapsulated into PLGA microspheres using a non-aqueous cryogenic method [7,8]. The product was removed from the market in 2004 due to its non-competitiveness with the daily injection formulation. The reasons for this were mainly the high costs associated with the lengthy production process in which it took two weeks to manufacture a batch [9].

Jostel et al. [10] has described a sustained release hGH microsphere formulation based on encapsulation of hGH in amylopectin microspheres coated with PLGA that gave sustained release in human volunteers over 14 days. A similar hGH formulation based on hydroxyethyl methacrylated dextran microspheres showed sustained release over 7 days in human volunteers [11]. For these different microsphere preparations, processing methods such as encapsulation by emulsification processes was used, which require multiple steps, increasing production time and cost.

DeBenedetti et al. (1993) applied a Rapid Expansion of Supercritical Solvents (RESS) technique to encapsulate drugs into polymers for

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sustained release applications. In this technique both the drug and the polymer need to be soluble in supercritical CO_2 ($scCO_2$), which often has a co-solvent such as acetone added to it. Hence, this technique is only applicable to very low molecular weight polymers (around 1000 Da) and small molecular weight drugs [25].

The PGSS (Particles from Gas Saturated Solutions) production process used here to produce the present hGH microsphere formulations was a simple, one-step process based on a supercritical fluid production technology (CriticalMix™). This technology has significant advantages over conventional production methods, as no organic solvents are required for the process and therefore there is no potential for residual solvents in the final product. Furthermore, the method results in near 100% encapsulation of the protein, with no structural changes to the protein during processing and drug loading as high as 20–30% dependent on the drug [12,13]. When amorphous polyesters, such as PLGA or PLA (even at high molecular weights) are exposed to supercritical carbon dioxide ($scCO_2$) the $scCO_2$ dissolves into the polymer, and acts as a molecular lubricant liquefying the polymer at temperatures significantly below its glass transition temperature. Therefore, PLGA or PLA exposed to $scCO_2$ in a pressure vessel, will liquefy allowing the API, here hGH, in the dry state to be mixed efficiently into the polymer at an ambient temperature and in the absence of solvents. Following mixing, the mixture is depressurised through a nozzle whereby the CO_2 returns to a gaseous state and evaporates solidifying the polymer around the hGH, and resulting in the production of microparticles containing the hGH [12,13].

The present study evaluated the effect of process variables of the supercritical fluid microparticle technology such as polymer composition and addition of different excipients on the *in vitro* release of the entrapped hGH. Promising sustained release hGH formulations were further evaluated *in vivo* in two selected animal models i.e. the rat and the monkey and compared to a daily injection of soluble hGH.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA) with an inherent viscosity of 0.16–0.24 dl/g and a ratio of lactide to glycolide of 50:50 and Poly(D,L-lactide) (PLA) with an inherent viscosity of 0.16–0.24 dl/g were purchased from Boehringer Ingelheim GmbH (Ingelheim, Germany). Solutol HS 15, Poloxamer 407 (Lutrol® F127) and Poloxamer 188 (Lutrol® F68) were obtained from BASF (Ludwigshafen, Germany). hGH was kindly donated by Bioker (Sardinia, Italy). HEPES, acetone and dichloromethane (DCM) were purchased from Fisher Scientific (Leicestershire, UK). Carbon dioxide was purchased from BOC (Surrey, UK).

2.2. Manufacture of hGH microparticle formulations

A number of formulations of hGH loaded PLGA/PLA microparticles were prepared using a CriticalMix™ process described previously [12]. During this process the protein remains in its solid state and therefore requires micronisation before encapsulation. Two methods to micronise the hGH were used — spray drying and zinc precipitation. Spray drying was performed by Upperton Ltd (Nottingham, UK) using a Buchi B-191 spray dryer equipped with a Schlik 0.5 mm Nozzle and an inlet temperature of 85 °C. 0.952 g of hGH was dissolved in 48 ml of 5 mM phosphate buffer containing 20% v/v Tween-20, and zinc added at a 2:1 ratio Zn:hGH. This produced free flowing hGH particles with a mean diameter of approximately 2–5 μm as measured by laser diffraction (Sympatec, Germany). As an alternative to spray drying, hGH was precipitated into nanoparticles by complexation with zinc at a molar ratio of 5:1 zinc:hGH. 10 mM zinc acetate was added dropwise to a 9.1 mg/ml solution of hGH in a quartz cuvette, whilst stirring at 1200 rpm using a magnetic stirrer. The resulting suspension was freeze dried before encapsulation.

The $scCO_2$ microparticle manufacturing process used was described in detail by Whitaker et al. [12]. The process parameters were kept constant for all microparticle batches but the excipients and polymer ratios were altered to determine their effect on release kinetics, particle size distribution and morphology. The selection of polymers, the ratio of these and the processing aids were chosen based on a range of preliminary *in vitro* release studies and particle size measurements. It was found that neither the use of PLGA alone nor PLA gave the desired properties for obtaining a sufficiently small particle size, low burst and sustained release characteristics. Briefly, micronised hGH (10% w/w of the formulation), suitable quantities of PLGA and PLA in different ratios (80% w/w of the formulation) and GRAS excipients consisting of poloxamer 188, poloxamer 407 or Solutol HS15 (alone or in combination to make up the remaining 10% w/w of the formulation) were loaded into a pressure vessel which was sealed. CO_2 was introduced into the pressure vessel and the temperature and pressure were increased to above 32 °C and 76 bar, respectively. The $scCO_2$ dissolved into the polymers which became liquefied. The liquefied polymer, micronised hGH and excipients were then mixed in the pressure vessel using a stirrer at 150 rpm to produce a homogenous mixture. Microparticles were formed upon atomisation and depressurisation of the mixture through a nozzle into a lower pressure environment. All batches were made on a laboratory scale apparatus with a 2 g batch size and in triplicate. A total of five formulations were prepared and details of the compositions are given in Table 1.

The microparticles were characterised in terms of encapsulation efficiency, hGH loading, morphology and particle size distribution. To determine the encapsulation efficiency and loading of the formulations, hGH was extracted from a known mass of microparticles by adding 1 ml of a 2:1 mixture of DCM: acetone to dissolve the polymer component. The tubes were centrifuged at $4355 \times g$ to pellet the protein and the supernatant discarded. The pellet was resuspended in 2:1 DCM: acetone and the extraction procedure repeated a further two times. After the final wash and centrifugation the supernatant was discarded and the pellet collected. The pellet was dried to remove any residual solvent, dissolved in 0.063 M phosphate buffer and assayed for hGH content by size exclusion high performance liquid chromatography (SEC-HPLC) following the method described in the European Pharmacopoeia (E.P.). Determination of drug in several samples of microparticles showed the homogeneity of the drug distribution in the batch. The encapsulation efficiency of the hGH microparticles was determined by calculating the percentage of the measured drug loading in relation to the theoretical drug loading.

Scanning electron microscopy (SEM) of the microparticles was carried out using a JEOL JSM-6060LV (Tokyo, Japan). The particles were coated with gold in an argon atmosphere using a Balzers Sputter Coater (AG, Liechtenstein) for 4 min before analysis using the SEM.

hGH was assayed and soluble aggregate formation quantitated using SEC-HPLC as described in the European Pharmacopoeia. Briefly, an Agilent 1100 HPLC system was fitted with a TSKgel G2000SWXL column (TOSOH Bioscience, Japan), and the column was equilibrated with a mobile phaseof 97%v/v 0.063 M phosphate buffer pH 7 and 3% v/v 2-propanol at a flow rate of 0.6 ml/min before injection of 20 μl of

Table 1
Composition of the hGH microparticle formulations.

	PLGA:PLA ratio	hGH (% w/w)	Other excipients (% w/w)
Formulation A	90:10	10% Spray dried hGH	10% Poloxamer 407
Formulation B	85:15	10% Spray dried hGH	10% Poloxamer 188
Formulation C	87.5:12.5	10% Spray dried hGH	9.5% Poloxamer 188 0.5% Solutol HS15
Formulation D	90:10	10% Spray dried hGH	9.5% Poloxamer 188 0.5% Solutol HS15
Formulation E	85:15	10% Zn:hGH precipitate	10% Poloxamer 407