Development of prolonged release somatropin (growth hormone) liposomes

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INTRODUCTION

Growth Hormone Deficiency (GHD) is a medical condition in which the body does not produce enough growth hormone (GH). Growth hormone, also called somatropin, is a polypeptide hormone which stimulates growth and cell reproduction. GH deficiency can be congenital or acquired in childhood or adult life. Severe GH deficiency in early childhood also results in slower muscular development, so that gross motor milestones such as standing, walking, and jumping may be delayed. Body composition (i.e., the relative amounts of bone, muscle, and fat) is affected in many children with severe deficiency, so that mild to moderate chubbiness is common (though GH deficiency alone rarely causes severe obesity). Some severely GH-deficient children have recognizable, cherubic facial features characterized by maxillary hypoplasia and forehead prominence. Deficiency in adults is rare, but may feature diminished lean body mass, poor bone density, and a number of physical and psychological symptoms.

Current therapeutic use of GH is limited by its short half life, renal toxicity, rapid clearance, and the need for multiple injections. Thus, a prolonged release formulation of GH administered subcutaneously could provide several advantages. The short in vivo half-life, the physical and chemical instability, and the low oral bioavailability of proteins currently necessitate their administration by frequent injections of protein solutions. This problem can be overcome by use of injectable depot formulations in which the protein is encapsulated in, and released slowly from liposomes. In the past two decades the potential usefulness of liposomes as drug carriers has attracted considerable interest. These phospholipid vesicles are capable of encapsulating both hydrophobic and hydrophilic drugs; they are biodegradable and are non toxic in vivo.

OBJECTIVE

The study aims at the formulation and evaluation of controlled release injectable formulation of somatropin. The FDA-approved injectable formulations are available as liquid preparations, or as powder with a diluent for reconstitution. Recombinant human growth hormone (rhGH), used mainly for the treatment of growth hormone deficiency in children, requires daily subcutaneous injections for longer periods. The use of controlled release liposomal formulations with appropriate rhGH release kinetics reduces the frequency of medication, improving patient compliance and quality of life. Currently, rhGH requires daily injections over a period of several years, causing the patient discomfort with risk of dosing errors and noncompliance. Reduced frequency of medication would greatly improve patient compliance and increase patient convenience in terms of quality of life.

EXPERIMENTAL METHODS

Conventional liposomes were prepared with different methods such as thin film hydration method, freeze thawing method and dehydration-rehydration method by varying the concentration of lipid molar ratio to drug (5mg). Pegylated liposmes were also prepared with the MPEG-DSPE (6mg) with varying concentrations of lipid molar ratio to drug (5mg). In the preparation, Initially lipids were dissolved in chloroform, transferred to 100ml rotor flask. The rotor flask was attached to rotor evaporator for complete evaporation of the solvent to form a thin film. The lipid film was flushed with nitrogen for at least 30 min. The thin layer formed was hydrated with 5ml of phosphate buffer saline pH 7.4 containing drug and sonicated for 5min using ice water bath with probe sonicator. The liposomal suspension obtained was separated by sephdexG-25 column to obtain entrapped liposomes and is stored at 2-8 ºC until use. Encapsulation efficiency was determined by adding 0.1ml of triton-X (10%w/v) followed by 0.2ml of methanol and mobile phase mixture of trifluoroacetic acid (0.1%v/v): acetonitrile (50:50). The solution was filtered through 0.22µ membrane filter and after suitable dilution the drug content was determined by HPLC (column:phenomenox C4 (250×4.6mm) 5µ, flow rate: 1ml/min, injection volume:50 µl R_t = 20.0 ± 0.5min at 210nm). Particle size, polydispersibility index (PDI) and Zeta potential of liposomes were determined by using Zeta sizer (Nano series) (Malvern, UK). The shape and surface morphology of the liposomes was examined using transmission electron microscope. I*n vitro* release of liposomes was carried out by vial method containing equivalent amount of 0.66mg of somatropin introduced into a vial containing 10ml phosphate buffer saline pH 7.4 and stirred using magnetic stirrer. Drug release was assessed by intermittently sampling the receptor media (1 ml) at predetermined time intervals. The amount of somatropin released in the buffer solution was analyzed by HPLC. Stability studies of the optimized formulation were carried out at accelerated conditions (25±2^{o}C, 60±5%RH) and at storage condition of 2-8 ^{o}C for a period of 2 months. The samples were analyzed for percentage encapsulation efficiency and particle size analysis as a function of time.

RESULTS AND DISCUSSION

Compatibility studies analyzed by validated HPLC method revealed the lipids used in formulation were compatible with GH. The liposomes were in the form of multilamellar and unilamellar vesicles. The encapsulation efficiency of different formulations varied from 15.61±0.39% to 83.56±0.92%. The pegylated liposomes were prepared with rehydration method showed maximum encapsulation efficiency (83.56±0.92%). The mean diameter and particle size distribution of all formulations was found to be in the range of 229.1- 434.1nm. The zeta potential of conventional liposomes prepared was found to be -42.0 to -69.4. Similarly zeta potential of pegylated liposomes prepared was found to be -74.6 to -79.5. The surface of the liposomes was found to be smooth and regular. The shape of the liposomes was found to be spherical in nature as shown in figure 1. The release of GH from conventional liposomes was rapid up to 2h, followed by slow release of drug from the formulation for a period of 24h and for pegylated systems it was shown up to 48h. The initial drug release of conventional liposomes (lipid molar ratio- 9:1) was 21.29±0.5% in first 2h followed by 95.67±2.1% at the end of 24h and for pegylated liposomes initial drug release was 24.60±0.9 in 2h followed by 78.89±1.4% at the end of 48h. The results of in vitro release profile of conventional and pegylated liposomes (lipid molar ratio- 9:1) are shown in figure 2. Stability study was carried out at accelerated condition of 25±2^{o}C, 60±5%RH. Liposomes formulation was found to be stable at storage temperature of 2-8 ^{o}C for a period of 2 months.

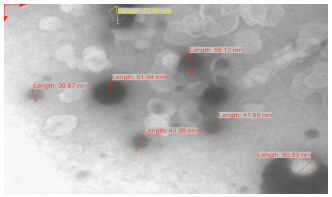

Figure 1. TEM analysis of liposomes

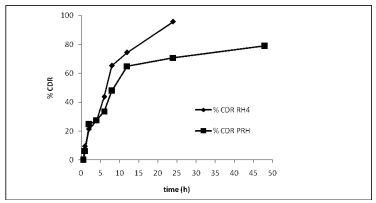

Figure 2. In vitro release profile conventional and pegylated liposomes

CONCLUSION

Prolonged release conventional and pegylated liposomal formulation of somatropin was successfully formulated by rehydration technique. The optimized formulations of conventional and pegylated somatropin liposomes release the drug for a period of 24 and 48 hours and were found to be stable at 2-8^{o}C

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