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Pharmacological and formulation studies on growth hormone: *in-vivo* and *in-vitro*

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Abstract

Human growth hormone (HGH), also known as somatotropin, Growth hormone is indicated in many diseases, like Prader-Willi syndrome, chronic renal insufficiency, Tuner syndrome, AIDS-related wasting, idiopathic short stature in children, and the accumulation of fat in adults with lipodystrophy. A number of carriers have been utilized to carry drugs to target tissues, which include immunoglobulins, serum proteins, synthetic polymers, lipid vesicles, microspheres, niosomes, etc. In this study, in-vivo somatotropin was estimated by ultraviolet spectroscopy, and blood plasma showed an absorption maximum at 214 nm. In-vitro preparation of niosomes by hand shaking method, reverse phase evaporation method, and ether injection method. The niosomal formulation of somatotropin has proven to be highly effective in inducing growth as compared to the existing marketed formulations of somatotropin. As a result, growth hormone can now be synthesized by Escherichia coli bacteria as a result of the successful application of recombinant DNA technology. Therefore, this hormone is now beginning to become available in sufficient quantities for treatment purposes. The niosomal formulation of somatotropin has proven to be highly effective in inducing growth as compared to the existing marketed formulations of somatotropin.

Keywords: Growth hormone, Niosomes, Noisome, Pharmacokinetic data, Somatotropin.

Introduction

Hormone is a substance of intense biological activity that is produced by specific cells in the body and transported through circulation to act on its target cells. Hormones regulate body functions to bring about a programmed pattern of life events and maintain homeostasis in the face of a markedly variable external and internal environment (Brahmanakar and Jaiswal, 2018).

Growth hormone

It is a 191 amino acid, single-chain peptide with a molecular weight of 22000. Growth hormone (GH) promotes the growth of all

organs by inducing hyperplasia. There is a proportionate increase in the size and mass of all parts, but in the absence of gonadotropins, sexual maturation does not take place. The growth of the brain and eye is independent of growth hormone (Rebenson and Lee, 2009). It promotes the retention of nitrogen and other tissue constituents; more protoplasm is formed. Positive nitrogen balance results from increased uptake of amino acids by tissues and their synthesis into proteins. Growth hormone promotes the utilization of fat and spares carbohydrates. The uptake of glucose by muscles is reduced while its output from the liver is enhanced and fat is broken down. Excess production of growth hormone is responsible for gigantism in childhood and acromegaly in adults. Hyposecretion of growth hormone in children results in pituitary dwarfism (Toadd et al., 2007). It has also been tried in children with constitutionally short stature with encouraging results. Commercial interests are promoting it for accelerating growth in children without growth hormone deficiency.

An ideal dosage form of drug therapy for any disease is the one that immediately attains the desired therapeutic concentration of drug in plasma at the site of action and maintains constant concentration for the entire duration of treatment. The frequency of administration of the dose of any drug depends upon its half-life, or mean residence time (MRI), and its therapeutic index. In most cases, the dosing interval is much shorter than the half-life of the drug, resulting in a number of limitations associated with such a conventional dosage form (Vyas and Dixit, 2017).

1. Poor patient compliance increases the chances of missing the dose of a drug with a short half-life, for which frequent administration is necessary.
2. A typical peak-valley plasma concentration-time profile is obtained, which makes attainment of a steady-state condition difficult.
3. The unavoidable fluctuations in the drug concentration may lead to under-medication or over-medication as the C_{35} values fall or rise beyond the therapeutic range. 4. The fluctuating drug levels may lead to the precipitation of adverse effects, especially for a drug with a small therapeutic index, whenever over-medication occurs (Khandare et al., 2016).

There are two ways to overcome such a situation:

1. Development of new, better, and safer drugs with long half-lives and large therapeutic indexes.
2. Effective and safer use of existing drugs through concepts and techniques of controlled and targeted drug delivery systems.

Design of controlled drug delivery systems

In recent years, research has focused on the development of new drug delivery systems (Rothstein, 2010).

- Sustain drug action.
- Localize drug action.
- Target drug action

Carrier Systems for Drug Delivery

A number of carriers have been utilized to carry drugs to the target organ or tissue, which include immunoglobulins, serum proteins, synthetic polymers, lipid vesicles, microspheres, erythrocyte reversible

micelles,pharmacosomes, niosomes, etc. (Kiwada et al., 2008).

1. **Cellular Carriers**: resealed erythrocytes, serum albumin, antibodies, platelets, and leukocytes.
2. **Polymer-based systems:** signal-sensitive, mucoadhesive, bindable, soluble synthetic polymer carriers, dendrimer
3. **Macromolecular carriers:** Proteins, serum albumin, glycoproteins, neoglyeoproteins, and artificial viral envelopes. Glycosylated water-soluble polyamines poly-lysine. Monoclonal antibodies Immunological Fab fragments, antibody-enzyme complex
4. **Colloidal carriers:** Vesicular system: liposomes, pharmacosomes, virosomes, and immunoliposomes. Microparticulate system Microparticles, nanoparticles, nanocapsuies, nanospheres, and solid lipid nanoparticles.

Rothstein et al. concluded that a controlled-release formulation of somatotropin has been attempted to reduce the necessity of the multiple injections given to the patients. It has been generally known that protein molecules dissolved in an aqueous medium are precipitated upon contact with a water-miscible organic solvent (Stafford et al., 2016).

Material and Methodology

Quantitation of Somatotropin: Somatotropin was estimated in vivo by ultra violet spectroscopy, and in blood plasma, it showed an absorption maximum at 214 nm. Standard curves were prepared for the concentration range of 2–20 ug/mL in PBS pH 7.4 buffer. Standard curves were also prepared in blood (plasma), and urine observations were reproducible, and the curves showed excellent linearity.

Preparation and in-vitro characterization: Niosomes were prepared by the hand shaking method, the reverse phase evaporation method, and the ether injection method. The prepared materials were evaluated for size distribution, entrapment efficiency, and release characteristics. The effect of type of lipid and leakage on storage was investigated. Among the methods used, the ether injection method showed higher entrapment efficiency for all types of tweens and spans used, while among the tweens used, the tween showed the highest entrapment, probably due to the higher encapsulated aqueous niosomes available for the drug. The size distribution of niosomes prepared by the handshaking method showed a regular increase in the mean size of vesicles as the surfactants of higher HLB were used. Niosomes prepared using tween 80 (H.L.B., 4.3) were 347 micrometers and tween 20 (H.LB. 86) were 7.36 um. The in-vitro release was studied using dialysis. The release of drugs from vesicles was sustained and prepared into a free drug solution. Slower release was observed in niosomes prepared using tween 40 and tween 60 as compared to those prepared using tween 20 and tween 80, which may be due to their high transition temperature and thus supposed to be less permeable using tween 40 as surfactant. The effect of lipid composition and lipid concentration was investigated on the in-vitro characteristics of niosomes prepared by hand. Niosomes prepared using pure surfactant showed higher entrapment and greater mean vesicle size (8.1 um); the interaction of cholesterol reduced both entrapment efficiency (12.3%) and mean vesicle size (4.8 um). The incorporation of dicetyl phosphate

further reduced the entrapment efficiency to 10.9% and the mean vesicle size to 3.87 um. Release rate studies revealed pure surfactant vesicles to be most permeable; intercalation of cholesterol is suppressed by its membrane stabilizing effect. Incorporation of DCP further reduced the release, and an almost-near correlation was observed between the concentration of lipid and entrapment efficiency. Drug leakage was studied in niosomes stored at room temperature and in cold places. Niosomes prepared by the injection method showed lower leakage as compared to those prepared by the shaking method and the reverse phase evaporation method.

***In-vivo* Evaluation:** Based on their promising in vitro characteristics, niosomes prepared by the hand shaking method using tween 40, cholesterol, and a D.C.P. ratio of 1:3:5 was selected to investigate their *in vivo* behavior. The plasma drug profile of noisome-entrapped somatotropins displayed higher and sustained plasma mg levels as compared to the free drug solution. Half-life was increased from 30 min to 96 min, AUC from 5.62 to 77.94 ughrs-' ml, while the volume of distribution decreased from 961.5 ml to 423.7 ml and total body clearance from 3.3316 liters to 0.1835 liters hrs."

Table 1. Concentration of Somatotropins injection and optimized formulation in blood.

Sl. No.	Time (hr)	Concentration of somatotropin in blood			
		Somatotrophin injection		Optimized formulation	
		ng/ml	Log conc.	ng/ml	Log conc.
1.	0.85	11.2	1.048	10.8	1.542
2.	0.28	6.9	0.8426	41.3	1.618
3.	0.50	4.4	0.6327	30.8	1.4788
4.	0.75	1.1	0.0647	21.6	1.3440
5.	1.2	1.4	0.0787	18.9	1.2852
6.	2.0	1.3	0.0765	13.6	1.2138
7.	3.0	1.6	1.021	7.8	0.8999
8.	5.0	1.8	0.0952	3.8	0.5486

Stability studies

The leakage of somatotropin out of vesicles on storage was determined, and the suspension was prepared using surfactant, cholesterol, and D.C.P. in ratios of 1:3:5 (FD_8) Niosomes prepared by the hand shaking method, the ether injection method, and the reverse phase evaporation method were stored for drug studies. Niosomes prepared by the above methods were dialyzed and then stored in capped vials at

a. Room temperature (25.2°C)
b. in the refrigerator (4-6°C)

Results

The spectrophotometric method was selected for in vitro analysis of somatotropin in a phosphate-buffered saline (pH 7.4) solution. It was selected because of its specificity, sensitivity, reproducibility, feasibility, simplicity, rapidity, and accuracy. The test for identification of the drug was found to be

positive. Scanning in the UV range of 200–300 nm showed an absorption maximum at 214 nm of the drug solution in P.B.S. (7.4). The curve was found to obey Beer's law in the concentration range studied (2–20 ng/mL). Table 1 shows the standard curve of somatotropin in P.B.S. (pH 7.4) buffer by spectrophotometry. The calibration curves for quantitation of somatotropin in blood (plasma) and urine were prepared by spectrophotometry to be used in *in-vivo* studies.

Table 2. Pharmacokinetic data of somatotrophin injection and optimized formulation.

Sl. No	Parameter	Somatotrophin injection	Optimized formulation
1.	T1/2 (Min)	30.0	96.0
2.	Ks (Hrs)	2.31	0.433
3.	Vd (ML)	961.5	423.7
4.	AUC (µghrs ml^{-1})	5.84	80.96
5.	CLT (liter hrs^{-1})	3.3316	0.1835

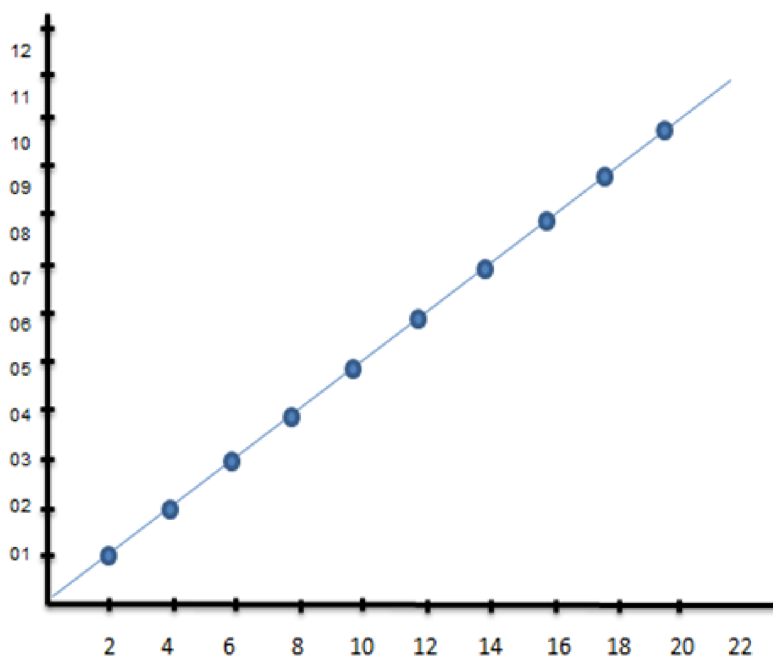

Fig 1. Standard Curve of Somatotropin in P.B.S (PH-7.4) Buffer.

Discussion

Niosomes were repaired by the hand shaking method (HSM), reverse phase. The evaporation method (R.E.M.) and ether injection method (E.I.M.) in the hand shaking method (Malhotra and Jain, 2016) by dried lipid film with an aqueous phase result in the formulation of niosomal dispersion. In the reverse phase evaporation method, water in an ether emulsion is sonicated; the evaporation of

ether causes reversal of the emulsion phase and, through the intermediate gel state, the formation of niosomal dispersions (Chauhan and Lawrence, 2018). In the ether injection method, lipids dissolved in ether are injected into a hot aqueous solution, where the evaporation of ether leaves behind a lipid bilayer encapsulating the aqueous phase. In the lipid mixture, tween and spans were used as nonionic surfactants (Baillie et al., 2015). Since the non-ionic surfactant tween 80 and span 60 have the highest phase transition temperature of 50 °C, all vesicle preparations were carried out above 50°C in the ether injection method; the aqueous phase was maintained at 40+2 °C; and in the hand shaking method, hydration of film was carried out at 40+2°C (Roberts and Azain, 2015). Vesicles were prepared from 250/mol of lipid containing surfactant, cholesterol, and dicetyl phosphate (D.C.P.) in the molar ratio of 1:3:5 (FD_8) (Allen et al., 2011). This ratio was chosen because it was found to have better *in-vivo* performance if non-ionic surfactant, cholesterol, and D.C.P. were used in the ratio 1:3:5 (FD_8) than any other composition (British Pharmacopoeia, 2011). D.C.P. was included in bilayer composition to impart a negative stage because negatively charged niosomes have been reported to be more efficient for drug delivery and have shown better *in-vivo* growth activity than positively charged or neutral niosomes (Resolw and Willard, 2009). The niosomes prepared by ether injection and reverse-phase evaporation methods exhibit higher entrapment efficiency than the hand shaking method. The difference is presumably due to the type of vesicles formed by each method. The uniflagellar structures formed by the ether injection method and the reverse phase evaporation method represent a more efficient surfactant than the multilamellar structures formed by the hand shaking method. Among the types of surfactants used, tween60 always showed the highest entrapment in niosomes prepared by any method. The physicochemical properties of the drug might be well correlating with the H.L.B. value of tween 60; entrapment efficiency depends upon the encapsulated volume, which was high in niosomes prepared using tween 60 (Sesalet and George, 2007).

In-vivo: The performance of a drug delivery system is the most important criteria in its development as a clinically acceptable dosage form. Preliminary in-vivo studies are carried out on laboratory animals such as rats, mice, hamsters, monkeys, etc. Observing the pharmacological response by analyzing the drug in the body can lead to in-vivo evaluations. Drugs can be analyzed in the organs to ascertain the ability of a system to achieve compartmental utilization of the drug in target tissue, which will reduce the concentration in non-targeted healthy tissues (Cook et al., 2016). While determining the blood level would ascertain the ability of the system to prolong the action of the drug, niosomes prepared by the hand shaking method using span, cholesterol, and D.C.P. in the molar ratio 1:3:5 (FD_8) was selected for *in-vivo* studies on the basis of their promising *in-vitro* performance. The blood levels of niosome-entrapped drugs after administration of a bolus injection were compared with those obtained from a plain drug solution (Hansen et al., 2006). The drug was analyzed in the blood by spectrophotometry. Albino rats (Sprague-Dawle strain) of either sex weighing 40–50 g was used in the study. The rats were procured, conditioned, and maintained on laboratory rat feed (Eppard et al., 2016).

Blood somatotropin level: blood samples were analyzed by spectrophotometry. The blood samples were centrifuged, plasma collected, mixed with 0.1 ml of 0.25 NaH_2PO_4 buffer, and extracted with 6.0 ml of ethyl acetate. After centrifugation at 5000 rpm for 5 min, 3 ml of the organic layer was evaporated at 55°C. The residue was redissolved in water (triple distilled) and estimated spectrophotometrically (Moallem et al., 2015). The optimized formulation of somatotropin increased its life from 30 minutes to 96 minutes. Area under the curve (AUC) from 5.84 to 80.96 ml-1 to 423.7 ml and total body clearance (LT) from 3.3316 liters to 6.1865 liters increase in half-life and decrease in clearance blisher the efficiency of niosomes in sustaining the action of drugs (Polidori et al., 2018).

The control group shows a moderate growth rate, while the animals with the marketed formulation of somatotropin showed a sharp growth rate, as evident from their weight gain. Groups administered with niosomal formulations exhibit a tremendous growth rate, which may be attributed to the fact that niosomal formulations are able to deliver the drug in a controlled manner over a prolonged period of time. Thus, the niosomal formulation of Somatotropin has proven to be highly effective in inducing growth as compared to the existing marketed formulations of Somatotropin (Fernandez et al., 2019).

Conclusion

In the past, it has been difficult to obtain sufficient quantities of human growth hormone to treat patients with growth hormone deficiency, except on an experimental basis, because it has had to be prepared from the human pituitary gland. Human growth hormone can now be synthesized by Escherichia coli bacteria as a result of the successful application of recombinant DNA technology. Therefore, this hormone is now beginning to become available in sufficient quantities for treatment purposes. Dwarfs who have a pure growth hormone deficiency can be completely cured. Also, human growth hormone might prove beneficial in other metabolic disorders because of its widespread metabolic functions.

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