ABSTRACT

Growth hormone (GH) treatment has many indications including the treatment of growth hormone deficiency (GHD), Prader–Willi syndrome, chronic renal insufficiency, Turner syndrome, AIDS-related wasting, idiopathic short stature in children, and accumulation of fat which related in adults with lipodystrophy. Conventional therapy was achieved using daily intramuscular (IM) and subcutaneous (SC) injections. However, patient’s noncompliance was very high. To solve this; several studies have been attempted to prepare long-acting formulations and to make prolonged half-life derivatives of GH. New delivery systems were also used such as needle-free and auto-injector devices to enhance the conveniences and adjustability. Furthermore, alternative routes of administration, such as intranasal, pulmonary, transdermal and oral are other strategies used to deliver the GH in a noninvasive way. In this review, we present a summary of various sustained-release formulations and technologies via injection. Noninvasive routes of GH delivery will be also reviewed with the principles of key points, selected additives, and limitations.

Keywords: Growth Hormone (GH), Technologies, Delivery, Formulations, Long-acting, Alternative Routes
stimulating effects on a wide variety of tissues. Another stimulation effect of IGF-1 to promote bone growth is in osteoblast and chondrocyte activity. Moreover, there are some indications that GH may also be involved such as the regulation of the mental well-being, immune function, and aging process.

Every year, approximately 1 in 4000 children are born with growth hormone deficiency (GHD). GHD causes less growth velocity, short stature, high subcutaneous fat and delayed skeletal maturation [9], which have a considerable effect on psychological and physical functioning. Cardiovascular risk has increased in adults with untreated GHD [10].

GH treatment has many indications including the treatment of GHD, prader–willi syndrome, chronic renal insufficiency, Turner syndrome, AIDS-related wasting, idopathic short stature in children, and accumulation of fat which associated with lipodystrophy in adults.

In the past, GH extracted from human pituitary gland was used for the treatment of children with GHD. Unfortunately, in 1985 the process was limited by the risk of transmitting viral infections such as Creutzfeldt-Jakob disease. This risk has led to the production of biosynthetic GH, or recombinant human growth hormone (rhGH), which replaced the extracted human GH for therapeutic uses. By using genetically engineered bacterial cells (Escherichia coli), the rhGH was produced. Recombinant technology solved the problems of disease transmission and availability.

In the beginning, rhGH was injected intramuscularly; however, the SC injection was shown to be more efficient. Generally, GH treatment has acceptable safety in term of its benefits when used in the approved way. Some side effects of GH therapy are common such as injection-site reaction. Others are rare, for instance, patients can experience joint swelling, joint pain, visual problems, nausea, vomiting, fluid retention, and headache.

There is increasing demand for new technologies and dosage forms for the delivery of GH, which can improve patient compliance and decrease side effects. Several dosage forms, formulations, and routes of administration are presented in this review including conventional administration of GH such as IM and SC and other alternative routes such as intranasal, pulmonary, transdermal and oral. Technologies that are used to improve the formulation of GH are also discussed.

**Conventional administration of GH via injection**

Conventional human GH therapy is achieved by parenteral administration. Frequent dosing of GH is required for parenteral routes due to the short serum half-life of GH. A daily injection of GH, or a minimum of three times a week, is required. It can be administered by SC and IM injection, with the SC being more attractive, enabling self-administration, shorter needles, and reduced pain.

Many studies have been performed concerning the pharmacokinetics (PK) and pharmacodynamics (PD) parameters of GH. By comparing the PK parameters between IM and SC administration of a highly-purified GH (nanormon®). SC injection resulted in a peak in rhGH concentration at 4 hours which returned to the baseline after 18 hours following injection. While IM injection reached a peak level at 2 hours; it returns to the baseline 8-10 hours after injection. The results indicated that the absorption of an IM injection is far too short to give a physiological diurnal profile of GH in plasma even by the daily administration. Daily SC injection would, if provided in the evening, imitate typical nocturnal human GH profile [11]. Another study concerning GH (Humatrope, 0.1 mg/kg in men and women), which showed that both maximum plasma concentration ([Cmax]) and area under the curve (AUC) were higher after IM injection though the bioavailability and half-life were greater following SCadministration [12]. It is crucial to correlate GH PK and PD to age, sex, and body composition. It is reported that GH secretion declines with age and males secrete less GH than females. Furthermore, the half-life of GH is reduced with age and obesity [13].

In 1997, an observational study was conducted for the association of GH PK with age, sex, and body composition. It showed that the AUC of GH after IV infusion was much lower in older subjects than in young, whereas the clearance and volume of distribution were less in the young group. The results also suggested that the lipolytic response was higher in young subjects, which was positively associated with a less fat mass in the young group. Therefore, it could be speculated that GH is distributed in and cleared by adipose tissue, which is more in the elderly [13]. Patterns of fat distribution in male and female were also proved to differ substantially and could potentially be attributed to differences in absorption of rhGH from the injection [14].

Maybe it is inconvenient for patients to use the daily schedule of GH, possibly causing non-adherence with the regimen of the treatment or premature termination of therapy. Additionally, patients could face the risks of dosing errors and increased supply costs as well. Because patient's compliance with daily injections might be limited during lifelong therapy, many studies have been focused on lowering the injections frequency by the development of extended release formulations.

**Long-acting formulations of GH via injection**

Long-acting formulations can give higher efficacy and safety. Also, patient compliance will improve. Several GH formulations were examined in humans or experimental animals including microspheres, benzyl benzoate/poly-D, L-lactic-co-glycolic acid PLGA depot solution, PEGylated GH, prodrugs, fusion with other proteins, hydrogels, and PLGA implants. These formulations are discussed here based on the additives used to formulate them.

**Microspheres/microparticles formulations**

Microspheres have been applied to deliver proteins and peptides. Microspheres/micro particles are crucial in drug delivery due to several superior advantages including their small size and their ability to enhance efficacy and reduce toxicity [15]. Usually, the system is composed of biodegradable polymeric microspheres containing bioactive material that can be administered by injection through a narrow gauge needle. Microspheres from different polymeric systems were formulated and evaluated for the GH delivery. Main of which is discussed in (table 1).

The nutropin depot® is the first sustained release microsphere formulation of rhGH based on PLGA (FDA approved in December 1999). The most ordinary notified adverse effects were at injection site including pain, erythema, and nodules [16].

In 2004, nutropin depot® was withdrawn from the market due to many disadvantages including high burst release, the possibility of aggregation after administration, protein denaturation, and the high cost of manufacturing. Furthermore, when microspheres have to be injected, the injection steps may be more painful because of both a larger needle size and a larger volume have to be administered to achieve extended effect [16].

**Fig. 2: Plasma concentrations of rhGH in rats treated with different rhGH-loaded microspheres for 69 d and rhGH solution. The in vivo results clarified the advantages of PELA microspheres over poly (D, L-lactic acid) PLA and PLGA microspheres [17].**

Mohammed et al. Int J Pharm Pharm Sci, Vol 9, Issue 7, 1-12
Benzyl benzoate/PLGA solution depot
This approach was based on changing the solvent by the addition of non-solvent to it. In PLGA solution, when modifying the solvent affinity to PLGA, the dynamics of phase inversion would slow to control the release of the drug. Brodbeck et al. examined four different solvents of PLGA, with strong to mild solvent/nonsolvent (water) affinity and solution strength. Benzyl benzoate showed to have less affinity for water. Therefore, it was used as Benzyl benzoate/PLGA solution depot. This solution maintained sustained-release of hGH in rats for 28 d [24].

Pegylation of GH
This approach (pegylation) is based on the attachment of polyethylene glycol (PEG) to GH (PEG-GH) by a covalent bond. This attachment enhances the hydrodynamic size and prolongs residence time by increasing the absorption time and reducing renal clearance. It also generates less common dosage forms.

Many studies have been done in human and animal models [25, 26]. Table 2 displays the main pegylation formulation of GH.

Table 1: Microspheres/microparticles formulations of GH

| No | The name of the formulation | Formulation description | The results and outcomes | References |
|----|----------------------------|-------------------------|--------------------------|------------|
| 1  | Poly D, L-lactic-co-glycolic acid (PLGA)® microspheres (nutropin depot) | Microsphere formulation of rhGH based on PLGA | Twice a mo at 0.75 mg/kg or once a mo at 1.5 mg/kg in children. In adults, 0.25 mg/kg and 0.5 mg/kg, major enhancement in the concentration of GH reaching Cmax at 24 h after injection. Lower Cmax less initial burst and longer sustained plasma levels of rhGH during the first 2 h after a single SC administration. | [16], [19] |
| 2  | DA-3003 microspheres | Water-in-oil-in-water (w/o/w) double emulsion solvent evaporation with PLGA as a release modulator, zinc oxide as a stabilizer and hydroxypropyl-β-cyclodextrin as an aggregation-preventing agent. | The rhGH released at a higher rate and continuously for 28 d. The PELA microspheres prolong the rhGH release duration for up to 56 d. It administered only once every two mo. | [20] |
| 3  | Poly monomethoxy polyethylene glycol-co-D-L-lactide (PELA) microspheres | Double emulsion method with narrow size distribution combined with membrane emulsification technique without any using any stabilizing excipients. | Decreasing the dissolution rate of rhGH crystals. In vivo results in monkeys, the efficacy of SC when given once w. The rhGH blood concentration lasted for 72 h in dogs and for less than 30 h in monkeys. | [21] |
| 4  | Crystalline GH microspheres | Crystal coating of rhGH based on electrostatic interactions between crystal surface with a positive charge (polyarginine) Prepared by spray drying method. Lecithin to minimize the denaturation of the GH during spray drying | | |
| 5  | Sodium hyaluronate (NaHA) | | The rhGH blood concentration lasted for 72 h in dogs and for less than 30 h in monkeys. NaHA modulated the release of hGH since the release rate of rhGH was more prolonged as the amount of NaHA increased. AUC of LB03002 was 7-fold higher than daily GH. The administration of LB03002 doubled Cmax compared to daily GH. Use of once w. | [18] |
| 6  | Hyaluronic acid (HA)® (LB03002) | Microspheres preparation was dispersed in an oil base of medium-chain triglycerides. | | |
| 7  | Microspheres by Critical Mix® process | PLGA/PLA polymers were liquefied by exposing them to supercritical CO2. Then hGH was added in the dry state and mixed efficiently into polymers. | Initial burst release was 33-49 %. In vivo, sustained release for 14 d periods. In rats, the release extended for 3 d. In monkeys, prolonged release for 7 d | [23] |

---

*PLGA is a biocompatible and biodegradable copolymer that most repeatedly aimed in the controlled release of drugs
* nutropin depot® is the first sustained release microsphere formulation of rhGH based on PLGA (FDA approved in December 1999).
* PELA microspheres provided a stable microenvironment since the amphiphilic PELA blocks the contact of protein with the oil/water interface, so it enhances retention of the protein drug.
* Hyaluronic acid (HA) is a natural biocompatible and biodegradable material that is often used as a matrix in microparticle formulations. It is found in connective tissue such as skin and cartilage. As a step of the neutrally occurring process, HA is degraded by hyaluronidase.
* A novel supercritical fluid technology produced sustained release MGH microsphere formulation, which was evaluated in rats and monkeys. This process provides the advantages of the absence of residual solvents in the final product and 100% encapsulation efficacy of the GH with the stability of protein structure during processing in a single step process.

Fig. 3: Blood levels of GH after daily GH administration and the first (W1) and fifth (W5) w doses of LB03002 [18]
Hydrogels have many applications in pharmaceuticals and drug delivery. They are hydrophilic with three-dimensional networks, which allow them to take the high contents of water into large extent. They stimulate many physiological factors in the body such as temperature, pH and ionic strength. Many studies need to achieve a sustained release using hydrogel formulations of GH. Table 5 describes the hydrogel formulation of GH.

### Table 2: Pegylation formulations of GH.

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|------------------------|-------------------------|--------------------------|------------|
| 1  | PHA-794428            | Addition of branched 40 kD PEG to GH at the N-terminus. Used as a lyophilized solid afterward, resuspended in water for injection for SC administration. | 10-20-fold increase in AUC and half-life as compared with GH in human. The renal clearance is lowered because the CI/F declined from 9.6 to 0.11 h for GH and PHA-794428, respectively. Lipopolyaoxyethylene was developed at the site of injection so all more development was stopped [27]. | [28] |
| 2  | NNC126-0083          | "PEG residue attached to glutamine 141 of the rhGH". | In healthy male, 12.6 to 26.2 h is the average time that reaches to the Cmax. It nearly 4-5 times higher than that after a single SC rhGH injection. No injection-site reactions were reported. | [29] |
| 3  | ARX201                | GH analog PEGylated at amino acid 35' in which p-acetlyphenyl alanine substituted with the native tyrosine | Once a w. It prolonged the half-life and IGF-1 reach to its standard value. | [30] |
| 4  | PEGylated GH          | PEGylation approach in rats. | Proved to be well-tolerated. | [31] |
| 5  | PEG beta alanine-NHCD | PEGylation approach in monkeys and rats | GH half-life increased from 3.1 to 20.8 h in monkeys and from 0.8 to 8.3 h in rats. | [32] |

### Prodrug formulations

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|------------------------|-------------------------|--------------------------|------------|
| 1  | ACP-001                | The prodrug is linked to a carrier. It undergoes non-enzymatic cleavage solely to release unmodified GH depending on physiological pH and temperature. | Safe and absorbed into the blood rapidly. Released GH over an extended period of time. | [33] |
| 2  | NNC0195-0092          | Mutation in the GH backbone in only single-point to which attached non-covalent albumin-binding properties with a side chain of terminal fatty acid. | It has the potential for well-tolerated, effective and once w GH treatment. | [34] |

### GH Fusion protein technology

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|------------------------|-------------------------|--------------------------|------------|
| 1  | TV-1106 (Albutropin)   | Fusion of the N-terminus of rhGH with human serum albumin (HSA). | In rats, the SC route gave four times increase in serum half-life and the twofold decrease in clearance with albutropin compared to hGH. In monkeys, eight times slower clearance and six-fold longer terminal half-life. | [35] |
| 2  | VRS-317                | Fusing a specific hydrophilic amino acid called xten with rhGH at the C-terminus and N-terminus | It had a half-life of 110 h, with 100% bioavailability. Well tolerated | [36] |
| 3  | MOD-4023               | Fusion of rhGH with three copies of the "carboxy-terminal peptide of human chorionic gonadotropin" | No side effects after 14 w SC administrations. Administered weekly | [37] |
| 4  | GX-H9: Hybrid Fc fragment | A "hybrid Fc fragment containing partial Fc domains of human IgD and IgE" was fused with hGH | Raised the hydrodynamic diameter from 5 to 11 nm. The sustained release was achieved using nanoporous polymer membrane for one mo. | [38] |

**Note:**
- PHA-794428 is the first a PEGylated form of GH that was studied in GHD patient. The study based on comparison PK of PEG-GH to those of GH in adults.
- The clearance was also decreased by reducing the conjugate affinity for the receptor.
Table 5: Hydrogel formulations of GH.

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|-------------------------|-------------------------|-------------------------|------------|
| 1  | Poloxamer 407\(h\)      | Gel formulation of rhGH with Poloxamer 407 | Sustained the release of rhGH for 132 h. High initial burst showed greater than ten times of wanting therapeutic need. | [41]       |
| 2  | Polyethylene glycols PEGs modified with fluorocarbon\(i\) | PEGs modified with fluorocarbon able to transit injection liquid to the gel state by hydrophobic interaction of the end groups. Zn\(^{2+}\) was added to increase the stability of GH and to prevent aggregation. | In vitro properties of prolonged release of GH based on PEGs proved to be released, without an initial burst, for more than two w. | [42]       |
| 3  | Pluronic/chitosan hydrogel | Prepared in situ gelaions by employing acrylated chitosan and di-acrylated Pluronic for photo-cross-linkable and thermo-responsive. | The long cross-linking impeded the release of the hGH. Decreased Burst release of hGH from the hydrogels. Achieved 20 d sustained release in vitro. (fig. 4). | [43]       |
| 4  | PEG-poly (L-alanine-co-L-phenylalanine) (PEG–PAF) | At 16–34°C the PEG–PAF aqueous solution goes sol-to-gel transition. | Stable in water for one-m storage as an aqueous solution at room temperature and at pH (7.2–7.8) No cell damage or protein denaturation during exposure to the acidic environment. Once-per-w formulation. | [44]       |
| 5  | (Organo phosphazene) hydrogel polyelectrolyte complex (PEC) | A collective system of thermo-sensitive injectable and biodegradable PEC formed between positively charged poly-L-arginine (PLA) and negatively charged hGH by ionic interactions. | Stability and release behaviors of hGH have been affected by Polycations and zinc. The result showed hGH sustained release for 5 d. | [45]       |
| 6  | Nanobiohybrid hydrogels (PAEU/LDH–hGH) | The LDH–hGH complex was dispersed into injectable copolymer PEAU with a cationic temperature-and Ph-sensitive. | Reduced the initial burst release of hGH. Enhance the release in vitro for 13 d and for 5 in vivo | [46]       |
| 7  | Poly (ethylene glycol)-poly (amino carbonate urethane) (PEG-PACU). | Prepared in situ gelations in Sprague-Dawley (SD) rats. | Reduced the initial burst release of hGH. Sustained the release of hGH | [47]       |

Fig. 4: Serum concentrations of hGH after a single SC injection of microparticle formulations, compared to daily SC injections of soluble hGH. The degradation rates of the hydrogels were hindered by the chitosan contents in the hydrogels, due to the high degree of interconnected polymer networks between acrylated chitosan and acrylated Pluronic [43]

---

\(h\) Poloxamer 407 are nonionic polyethylene polyoxypropylene copolymers. Poloxamer 407 has reverse thermal gelation property, so it is liquid at room temperature whereas at high temperature thickens into the semisolid gel.

\(i\) In situ transformation was achieved using as N-methyl pyrroloidone, an organic solvent, which convert the formulation from gel to injectable state. After injection, liquid rapidly returns to the gel state.
Although hydrogels proved the efficiency and succeeded in some sustained release formulations; yet it suffered from hydrophilicity property which causes limitations in formulations such as short periods of release, initial burst, and less stable during storage.

**PLGA as biodegradable laminar implants**

As discussed before, many studies evaluated the use of PLGA in microspheres formulations. Here, another approach to control the release of formulations is the using of biodegradable laminar implants. The system based on poly (lactic/glycolic) acid (10% w/w). It was administrated in rats and compared with commercial injectable (norditropin®). Results revealed that the formulation was effective for 15 d and more potent than norditropin®. However, it is more complicated than injection cause it requires surgery to insert the implant, which may limit its application [48].

**Novel injection delivery devices**

The conventional administration of hGH via injection may lead to matters with patient compliance, avoidance of therapy and early termination of GH treatment; therefore, developing novel delivery devices is fundamental.

Devices used for hGH administration must have the following criteria: they must be practical, convenient, acceptable to patients and user-friendly. These devices for a child, therefore, it should be simple enough to operate quickly and safely. Furthermore, it can be stored at room temperature for up to several mo and are easily portable [49, 50].

The novel injection has been introduced in with an aim to enhance adjustability and dosing accuracy, ease of use, convenience and compliance. The examples of these devices are manual injector pens, pre-filled syringes, injectors with hidden needles, auto-injectors, and needle-free devices [49].

In the past years, needles with high gauge number have become available, despite that particular psychological factors which may cause noncompliance are involved in the injection steps. Several studies have investigated the safety and efficacy of the delivery devices in hGH treatment. Therefore, the jet injector’s evolution has improved over the past years.

A jet injector is a type of “needle-free injection device that can deliver hGH subcutaneously through jet injection “instead of a hypodermic needle that penetrates the epidermis. A study was done by Verrips et al compared the administration of hGH between needle-free injector (medi-jector®) and multi-dose injection pen with a 28G, it was concluded that the administration of jet injections lead to less adverse psychological reactions than a needle [51]. The same results were confirmed by Agerso et al. who compared the administration of new hGH (zomacton®) formulation which administrated both by a new needle-free device (zoma jet 2 vision) and by a conventional syringe. It was found that in jet injections, no local reactions were found. Based on AUC values, blood hGH was found to be bioequivalent. The PD markers such as free fatty acids and serum concentrations of IGF-1 were bioequivalent as well. Whereas, the hGH was found to be absorbed faster after zomacton 2 vision administration compared with a traditional syringe. These results support the use of jet injectors as an alternative to the traditional injection pens [52].

The same findings were reported when hGH (genotropin) administrated in children, it was compared to a fine gauge needle device (genotropin-pm) and a needle-free device (genotropin ZipTip™). The needle-free injector was shown to be safe and bioequivalent. Thus, after the study, more than 20% of the patients preferred to keep on using it [53].

Two free needle injector systems deliver hGH are recently available in the market. Cool. click™ (Merck Serono International SA, Geneva, Switzerland) is one of these devices, under high pressure, these devices expel hGH solution through a small nozzle in which the drug be forced through the skin and disperses it SC. It is used for patients who do not want to use a needle. Brearley et al. evaluated the relative bioavailability of hGH (Saizen®, Merck Serono) administered by a new needle-free device, cool. click™, and a syringe and standard needle. It was found that the extent and rate of absorption of hGH administrated by cool. click™ was bioequivalent and with similar tolerability to standard needle injection. The new device has the additional characteristic features of being needle-free, and assist in raising the adherence of patient and reaching real therapeutic achievement from rhGH treatment [54].

**Alternative routes for the hGH delivery**

Improving GH therapy and increasing compliance was the concern of many scientists. Therefore, search for less invasive routes such as intranasal, pulmonary, transdermal and oral delivery was established.

**Intranasal delivery**

The nasal route has been developed as a non-invasive route for delivering macromolecules since it offers many advantages as the vascularized and permeable mucosal surfaces, rapid onset of action, rapid kinetics, no first-pass metabolism, and the ease of administration [55].

Despite extensive blood supply and the large surface area of the nasal cavity, the nasal mucosa permeability is normally low for polar molecules, especially large molecular weight proteins and peptides. On the other hand, low bioavailability (1–2%) is a large obstacle of nasal delivery, which is due to the administration of high molecular weight molecules in the absence of a promoting agent. Many barriers have to be overcome in order for the drug to reach the systemic circulation like enzymatic barrier due to the presence of peptidase enzyme and, diffusional barrier, which is consisted of the mucus gel layer around the mucosal membranes [56]. In addition to that, the rapid clearance of the administered formulation from the nasal cavity hindered the membrane transport due to the mucociliary clearance mechanism, which declines the local residence time at the absorption site [57]. The peptide and proteins bioavailability is proportional inversely with their number of amino acids and molecular weight.

Several approaches have been intended to overcome these limitations and to increases nasal absorption of protein and peptide. For instance, Surfactants-laureth-9, fatty acids, bile salts, cyclodextrins, and phospholipids, multifunctional polymers and enzymatic inhibitors.

The function these enhancers work by various mechanisms including changing the epithelial cell layer permeability by modulating the phospholipid bilayer. The mechanisms include inhibition of the enzyme, reduction in mucus viscosity, and enhancement of membrane fluidity.

Various approaches have been also used in the nasal cavity for increasing the residence time of drug formulations resulting in increasing absorption of the drug. Table 6 summarizes the approaches used in the nasal cavity.
Table 6: Intranasal delivery formulations of GH

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|-------------------------|-------------------------|--------------------------|------------|
| 1  | Sodium Tauro-24, 25-  | Used STDHF on the hGH nasal absorption in three animal models (rat, rabbit, and sheep) | Rapid absorption and clearance. Bioavailability enhancement was 11-fold in rabbits and rats. Whereas, it was in sheep 21-fold. Rapid increase in plasma concentration of hGH with Cmax at 20-30 min | [58] [59] |
| 2  | L-a-phosphatidylcholine | LPC on hGH the nasal absorption in three animal species (rat, rabbit, and sheep) | The bioavailability with the LPC was 17.5% for the rats, 72.8% for rabbits, and 16% for sheep, this respectively corresponds to 7.6-, 52-, and 80-fold enhancement in the bioavailability attained in the absence of the surfactant. | [60] |
| 3  | Degradable starch microspheres (DSM)+LPC | Use of microspheres in sheep as a nasal delivery system for hGH. Microsphere system was used alone or in combination with a biological surfactant, LPC. | Increases in Cmax (9.0 ng/ml), and relative bioavailability of (2.7%) while the combination of the microsphere and LPC Cmax (55.4 ng/ml), and a relative bioavailability of (14.4%). | [61] |
| 4  | Didecanoyl-L-a-phosphatidylkholine (DDPC) | The hGH formulated with the DDPC and α-cyclodextrin the intranasally in rabbits | The absolute bioavailability was 23%. The membrane damage results from the ability of enhancers DDPC and α-cyclodextrin to interact with plasma membrane content. Bioavailability of nasal hGH was found to be low ranged between (3.8%-8.9%) whereas, following S. C. injection it was about 50%. | [62] [63] |
| 5  | Polycarbophil-cysteine (PCP-Cys)/glutathione (GSH) gel | PCP-Cys is thiomer used as multifunctional vehicle improved permeation-enhancing and mucoadhesive properties and enzymatic inhibitor for systemic, nasal polypeptide delivery. GSH is used as permeation mediator in this formulation. PCP-Cys and GSH microparticulate was dissolved in demineralized water, followed by lyophilization and micronization. | The hGH permeation from the gel formulation across “excised bovine nasal mucosa” was raised 3-fold. In rats, after intranasal administration of the PCP-Cys/GSH gel the plasma concentrations of hGH reached the blood circulation within min when compared to the unmodified PCP gel and physiological saline solution. The relative bioavailability was 8.11%. It had improvement bioavailability by 3-fold | [64] [65] |
| 6  | Chitosan nanoparticle | Two formulations were administered nasally to sheep (Formulation A) was a powder blend (20±1) mg. (Formulation B) was granules (19±1) mg. N-trimethyl chitosan chloride (TMC) as polymeric absorption enhancer and Pheroid which consists mainly of essential fatty acids | The relative bioavailability was 14% and 15% for formulations A and B, respectively. (fig. 5) Extremely high bioavailability in rats, 128.5% for Pheroid and 136.1% for TMC H-L (high molecular weight with a small degree of quaternization). | [66] [67] |
| 7  | Solutol HS15: Critical Sorb™ | Using polyglycol mono and di-esters of 12-hydroxystearic acid combined with free PEG. | Bioavailability of 10% w/v solution of the formulation was 49% in the first two h in rats. (fig. 6). | [68] |

Fig. 6: hGH serum levels in rats after hGH intranasal application with Solutol® HS15, compared to SC [68]

In conclusion; for the nasal delivery of hGH, different formulations gave various bioavailability values, and all of this is governed by the potency of the formulation. Thus the highest (49%) [68] bioavailability value found with Solutol HS15 followed by starch microspheres/IPC (14.4%) [61], LPC (16.0%) [60] and Chitosan nanoparticle (14% and 15%) [66].
Pulmonary delivery

The second non-invasive delivery route in the administration of GH is to deliver the GH directly into the respiratory tract as pulmonary drug delivery system. Improving compliance and patient satisfaction are the ultimate goals of such new delivery which leading to improved therapeutic efficacy. The lungs contain 300 million alveoli, providing a surface area 75 m², and the thickness of alveolar epithelium 0.1-0.2 mm. As a result, all of these permit rapid absorption [69]. The first concern in this route of administration is the presence of alveolar macrophages which is a natural defence mechanism [70]. Particle distribution is another fundamental factor that affects the pulmonary delivery of the aerosol drug. Suitable size range for delivery to the lungs is (1–3 µm) to be deposited in the alveolar place. Because particles more than 3 µm will not reach the alveolar region, and particles less than 1 µm will be exhaled [71].

Similarly, to intranasal, pulmonary delivery has the fast onset of action and it avoids the GI tract and the hepatic first-pass effect. On the other hand, the bioavailability of intranasal is low (<1%) in the absence of absorption enhancers. In contrast, the bioavailability resulted from a pulmonary range between 5 to 45 [72, 73].

A researcher proved that after administration intratracheal instillation of GH in rats, higher bioavailability was obtained in male more than female. This was explained that GH showed higher protein-binding in females than in males. Also, the study revealed that the Plasma levels of hGH directly proportional to dose instilled [72].

Colthorpe et al. studied the regional deposition influence on the PK of pulmonary delivered hGH in rabbits in the aerosol form and by instillation. The study discussed the effect of mucociliary clearance on the bioavailability. The result showed that bioavailability for instilled GH (16%) was lower than that aerosolized GH (45%) comparing to IV. Lower bioavailability attributed to greater mucociliary clearance on instilled form [73].

Dry powder inhaler (DPI)

DPI is considered as noteworthy for delivery of the drug [74]. Due to improved drug stability and their facility of use, dry state of the inhalers is suitable for proteins pulmonary administration like hGH. The goal of such inhalers is to enhance flow, reduce aggregation, improve drug stability, and aid in dispersion. Most DPI formulations consist of micronized drug mixed with larger carrier particle. The main approaches used in pulmonary route was summarized in table 7.

Table 7: Pulmonary delivery formulations of GH

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|-------------------------|-------------------------|-------------------------|------------|
| 1  | Dipalmitoylphosphatidylcholine (DPPC) | The dry powder aerosol formulation with lactose and DPPC was evaluated for GH systemic delivery in rats. The powder particles with a diameter of 4.4 µm were prepared by spray drying | The bioavailability was 2.3% and 8% respectively following intratracheal instillation of the dry powder and intratracheal spray-instillation of the GH solution. The hGH absorption of the dry powder from rat lung was faster and 3-fold greater after intratracheal instillation than intratracheal spray-institution of a solution. | [75] |
| 2  | Dimethyl-β-cyclodextrin (DMβCD) | Different DMβCD concentrations were prepared using a spray drying method with GH to protect against aggregation. Powder administration containing both absorption enhancer and protein stabilizing agents at optimum amounts. The stabilizing agents were the mixture of polysorbate 20, 2%, and lactose (PZL). | Absolute bioavailability values of formulations containing GH: DM-β-CD at molar ratios of 10:1, 100:1, and 1000:1 were reported as 25.4%, 76.5%, and 64.0%, respectively. 85.1% bioavailability | [76] |

The pulmonary delivery is noninvasive, safe and effective, despite that it showed limited bioavailability for rhGH.

Table 8: Transdermal delivery formulations of GH

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|-------------------------|-------------------------|-------------------------|------------|
| 1  | Radiofrequency (RF) | A dry form of hGH was applied on rats and guinea pigs base on RF thermal ablation | The bioavailability values were 75% at a dose of 300µg for rats and 33% at a dose of 50 µg for guinea in compare to SC injection. The bioavailability from SDMP was 87.5%. | [80] |
| 2  | Self-dissolving micropores (SDMP) | After mixing an aqueous solution of GH with dextran, SDMPs were prepared by pulling the mixture with polypropylene tips. | | [81] |
| 3  | Microneedle patchesa | a. Carboxymethylcellulose (CMC) and trehalose | The hGH activity was completely maintained and retained after storage at room temperature and for up to 15 mo. The rhGH has absolute bioavailability about 71% | [82] |
|    | b. Chondroitin sulfate and dextran | CMC is used to provide mechanical strength. Trehalose was used to enhance microneedle dissolution rate. The hGH was encapsulated in long dissolving microneedles made of CMC and trehalose using an aqueous. | The hGH was released after the base dissolved following the rapid dissolution of the water-soluble polymer. The absolute bioavailability of dextran was 72.8%. Meanwhile, the bioavailability of high chondroitin base was 101.3% | [83] |

---

a RF) is thermal ablation of stratum corneum to enhance GH transport across the skin. The aim of this to make an array of small microchannel in the skin through ablation on the cell.

a Microneedle patch, designed for simple, painless, self-administration of biopharmaceutical.
Transdermal delivery

Third noninvasive strategy for delivery of GH is the transdermal route. Like any other delivery route, despite its advantage, it has also limitations. Biomolecules pass through the lipid-rich stratum corneum by passive diffusion. Their passing is limited by molecular size and susceptibility to metabolism by skin enzymes [77, 78]. Numerous approaches (table 8) have been suggested to enhance the peptide and protein delivery via transdermal either by modifying the formulation of a drug or transiently enhance the permeability of the skin [79].

The absorption of rhGH in radiopharmacy techniques can be explained by the fact that dissolved rhGH diffused into tissues of the skin. On the contrary, the solid dispersion of rhGH is found in dissolving microneedle, which inserted to the skin. Consequently, the rhGH released after the base polymer dissolved within a few minutes of insertion which resulted in the high bioavailability of rhGH within 15 min T1/2.

Oral delivery system

The oral delivery system is the last noninvasive route to be discussed here. The oral delivery system is preferred due to ease of administration and high patient compliance and acceptability. However, preparation of oral dosage forms faces significant challenges that limit the applicability of drugs of large molecular size, poor lipid solubility, reduced stability and extensive enzymatic degradation such as peptidases and proteases leading to poor absorption into the gastrointestinal tract (GIT).

Many attempts have been made to solve the outstanding challenges and to improve oral bioavailability. Some were concerned with carrier effect on oral absorption of GH [84]. Others worked on structural modification and increasing lipophilicity [85]. Using penetration enhancers was also investigated [86]. Another approach was based on a formulation of solid in lipid suspension in the presence of surfactant [87]. Several researchers also worked in the formulation of fusion proteins [88, 89]. New technology was introduced to the market based on Emisphere’s eligen® Technology [90]. Liposomes also were used containing bio-enhancers and tetraether lipids [91].

The evaluation of the bioactivity of GH is crucial. It has been seen from the increase in weight gain of hypophysectomized rats after oral dosing of hGH combined with a carrier compared with the only carrier. This result proved that hGH was biologically active orally [84].

The absorption efficiency was proved to be affected by the type of dosage form. Carrier is combined with either aqueous solutions or powder-in-capsule formulations of hGH. After comparison of these two formulations; the results reported that higher efficiency was in capsule formulation than the aqueous solution. Moreover, at pH 12; there was no difference in the delivery of hGH between the two formulations. Results showed delivery of hGH at lower pH (10 and 8.4) is higher in a capsule than that of a solution [84]. Table 9 summarized the main GH formulation for oral delivery.

| No | The name of formulation | Formulation description | The results and outcomes | References |
|----|------------------------|-------------------------|-------------------------|------------|
| 1  | 4-[(2-Hydroxybenzoyl) amino] phenyl butyric Acid | Prepared by N-acetylated, non-a-aromatic amino acids. It was based on the aromatic amide substituents with hydrogen-bonding characteristics | It increased lipophilicity to promote protein absorption from the GIT in a rat’s model. The more carbon chain length, the higher absorption of rhGH due to lipophilicity enhancement in the amide portion | [85] |
| 2  | P-Glycoprotein efflux system | Novel absorption enhancer. | It makes the hGH more hydrophobic with a smaller dimension. It increases membrane fluidity due to passive diffusion through the lipid bilayer. | [86] |
| 3  | Solid-in-oil (S/O) suspension | The hGH was "complexed with an edible surfactant". Soybean oil is the continuous phase. Sucrose erucate (ER-290) is the surfactant. Sugar stabilizer such as trehalose was also used to stabilize complex form of rhGH with the surfactant, which suspending in soybean oil. | The reported bioavailability of S/O suspension was 3.3% compared to IV injection. | [87] |
| 4  | Fusion protein<sup>1</sup> Transferrin | Coding sequences of both hGH and transferrin were fused in a frame without a mutation. The bioactivity of the fused protein was enhanced by inserting helical linker, which regarded as "spacer between hGH-and transferrin". | Two fusion proteins, i.e. GT and GHT were produced. Neither hGH nor GT caused a gain in body weight. GHT promoted 2.5g of body weight gain compared to 15.2g of SC hGH. Tat peptide used as a carrier and improves rhGH absorption into cells. In the rat's intestinal cavity, the Tat rhGH levels were 1.38-fold greater than rhGH | [88] |
|    | a Tat protein transduction domain (Tat-PTD) | Tat-PTD, positively charged is a short linear peptide of (3kD), easily passed through attached intestinal cell membrane. | | |
|    | b Liposomes Containing Bio-Enhancers and Tetraether Lipids and Omeprazole | Cetylpolytrimidinium chloride as bio-enhancer Omeprazole to over come the denaturation related to low pH of encapsulation of hGH. | Relative hGH bioavailability of 3.4% compared with SC while the free hGH administered orally showed a bioavailability of only 0.01% (fig. 8). | [89] |
| 5  | PEG-PLA nanoparticles | GH was coated with enteric-coated capsules which were filled with monomethoxyl PEG-PLA nanoparticles. | The relative bioavailability was 11.06% in rats. | [90] |

<sup>1</sup> It is a physiological and natural process to improve oral delivery of protein drug. It enhances blood half-life and protein targeting.
CONCLUSION

To sum up, GH has many therapeutic indications for a long time to improve patient compliance. Several long-acting GH formulations and routes of administration have been developed. Changing from the conventional injection route to novel injection devices gave better feedback over SC injection. Many sustained release GH preparations have been developed such as GH microspheres, PEGylated GH, Prodrug formulations and GH fusion protein technology. In spite of its complexity, in the manufacturing process, they have the useful potential to be available choices. The development of noninvasive route was also studied such as intranasal, pulmonary, transdermal and oral delivery. The biggest problem in intranasal is poor and relatively low bioavailability. Even though, the penetration enhancer showed the significant increase in the bioavailability; it causes the damage to mucous membranes. In pulmonary delivery, in order to achieve high bioavailability; a high dose is required which can cause tissue reaction and systemic side effects. Transdermal drug delivery was also studied, and using stratum corneum ablation technologies was believed to be potent with fewer side effects. Up to date, although many oral formulations have been developed to deliver GH orally; yet no oral formulation is useful to overcome poor permeation, poor absorption of proteins and peptides in the GIT.

ACKNOWLEDGEMENT

The authors acknowledge Faculty of Graduate Studies at Jordan University of Science and Technology for continuous support during M. Sc. Program.

CONFLICT OF INTERESTS

Authors would like to dedicate this article to German Academic Exchange Service (DAAD) for the support and encouragement of postgraduate students in the M. Sc. program in Pharmaceutical Technology.

REFERENCES

1. J Cázares-Degadillo, A Ganem-Rondero, YN Kalla. Human growth hormone: new delivery systems, alternative routes of administration, and their pharmacological relevance. Eur J Pharm Biopharm 2011;78:278–88.
2. JD Veldhuis. A tripeptidyl ensemble perspective of interactive control of growth hormone secretion. Horm Res 2003;60:86–101.
3. P Zizzari, MT Bluett-Pajot. Ghrelin, from GH secretagogue to orexigenic peptide. Corresp Metab Horm Diabetes Nutr 2004;8:58–65.
4. H Balaji. Review article ghrelin—a review. Asian J Pharm Clin Res 2013;6:9.
5. J Alba-Roth, OA Muller, J Schoplh, K von Werder. Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. J Clin Endocrinol Metab 1998;87:1186–9.
6. LM Carlsson, S Rosberg, RV Vitango, WL Wong, K A Bertsson-Wikland. Analysis of 24 h plasma profiles of growth hormone (GH)-binding protein, GH/GH-binding protein complex, and GH in healthy children. J Clin Endocrinol Metab 1993;77:356–61.
7. SYL Bernat, G Pal, A Koskiakkoff, W Li. Functional promiscuity of squirrel monkey growth hormone receptor toward both primate and nonprimate growth hormones. Mol Biol Evol 2002;19:1083–92.
8. S Surya, K Symons, E Rothman, AL Barkan. Complex rhythm of growth hormone secretion in humans. Pituitary 2006;9:121–5.
9. MA Preece. Diagnosis and treatment of children with growth hormone deficiency. Clin Endocrinol Metab 1982;1:1–24.
10. G Mc vault. The psychological consequences and quality of life in adults with growth hormone deficiency. Growth Horm IGF Res 2000;10:563–8.
11. JS Christiansen, KW Kastrup, H Ørskov, C Binder. Absorption of human growth hormone injected subcutaneously and intramuscularly in growth deficient dwarfs. PR 1981;15:1540.
12. EL Company. Humatrope, somatropin (rDNA origin) for injection; 2009.
13. N Vahl, N Müller, T Lauritsen, JD Christiansen, JØ Jørgensen. Metabolic effects and pharmacokinetics of a growth hormone pulse in healthy adults: relation to age, sex, and body composition. J Clin Endocrinol Metab 1997;82:3612–8.
14. A Keller, Z Wu, J Kratzsch, E Keller, WF Blum, A Kniss, et al. Pharmacokinetics and pharmacodynamics of GH: dependence on route and dosage of administration. Eur J Endocrinol 2007;156:47–53.
15. S Sakhare, A Yadav, P Jadhav. Design, development and characterization of mucoadhesive gastro spheres of carvedilol. Int J Appl Pharm 2016;8:37–42.
16. E Reiter, K Atte, T Moshang Jr, B Silverman, S Kemp. A multicenter study of the efficacy and safety of sustained-release GH in the treatment of naïve pediatric patients with GH deficiency. J Clin Endocrinol Metab 2001;86:4700–6.
17. Y Wei, Y Wang, A Kang, W Wang, S Ho, J Gao, et al. A novel sustained-release formulation of recombinant human growth hormone and its pharmacokinetic, pharmacodynamic and safety profiles. Mol Pharmacol 2012;9:2039–48.
18. M Bidlingmaier, J Kim, C Savoy, M Kim, N Ebrecht, S de la Motte, et al. Comparative pharmacokinetics and pharmacodynamics of a new sustained-release growth hormone (GH), LB03002, versus daily GH in adults with GH deficiency. J Clin Endocrinol Metab 2006;91:2926–30.
19. D Cook, B Biller, M Vance, A Hoffman, L Phillips, K Ford, et al. The pharmacokinetic and pharmacodynamic characteristics of a long-acting growth hormone (GH) preparation (Nutropin Depot) in GH-deficient adults. J Clin Endocrinol Metab 2002;87:4508–14.

Fig. 8: hGH Plasma after oral administration of liposomal encapsulated hGH. (+Omep) or (−Omep) indicates whether animals were pre-treated with omeprazole or not [91]
20. H Kwak, W Shim, M Choi, M Son, Y Kim, H Yang, et al. Development of a sustained-release recombinant human growth hormone formulation. J Controlled Release 2009;137:160–5.

21. C Govardhan, N Khalaf, C Jung, B Simone, A Higbie, S Qu, et al. Novel long-acting, crystal formulation of human growth hormone. Pharm Res 2005;22:1461–70.

22. S Kim, Hahn, M Kim, D Kim, Y Lee. Development of a novel sustained release formulation of recombinant human growth hormone using sodium hyaluronate microparticles. J Controlled Release 2005;104:323–35.

23. F Jordan, A Naylor, C Kelly, S Howdle, A Lewis, L Illum. Sustained release hGH microsphere formulation produced by a novel supercritical fluid technology: in vivo studies. J Controlled Release 2010;141:153–60.

24. K Brodbeck, S Pushpala, J McHugh. Sustained release of human growth hormone from PLGA solution depots. Pharm Res 1999;16:1825–9.

25. A Hoffman, B Biller, D Cook, J Baptista, B Silverman, L Dao, et al. Efficacy of a long-acting growth hormone (GH) preparation in patients with adult GH deficiency. J Clin Endocrinol Metab 2005;90:6431–40.

26. R Webster, E Didier, P Harris, N Siegel, J Jadler, L Tlbury, et al. PEGylated proteins: evaluation of their safety in the absence of definitive metabolism studies. Drug Metab Dispos 2007;35:9–16.

27. P Touraine, G D'Souza, Ione Kourides, Roger Abs, Paul Barclay, Ruja Xie, et al. Lipatrophy in GH-deficient patients treated with a long-acting pegylated GH. Eur J Endocrinol 2009;161:533–40.

28. R Webster, R Xie, E Didier, R Finn, J Finnessy, A Edgington, et al. PEGylation of somatropin (recombinant human growth hormone) in young adults with growth hormone deficiency: impact on its clearance in humans. Xenotransplantation 2008;13:340–51.

29. M Rasmussen, L Jensen, T Anderson, T Klimgaard, J Madsen, A Harris, E Chlipala, D Smith, S Carlson, D Doherty. Multiple doses of pegylated long-acting growth hormone are well tolerated in healthy male volunteers and possess a potential once-weekly treatment profile. Clin Endocrinol (Oxf) 2010;73:769–76.

30. J de Schutter, M Rasmussen, Z Gucev, A Eliakim, T Battelino. Long-acting pegylated human GH in children with GH deficiency: a single dose, a dose-escalation trial investigating safety, tolerability, pharmacokinetics and pharmacodynamics. Eur J Endocrinol 2012;166:401–9.

31. K Racz, M Cocalescu, V Olynik, M Popa, V Popovici, K Radiuk, et al. OR7-6: safety and efficacy of ARX201: a six-month doseringaging study of a novel long-acting pegylated growth hormone in young adults with growth hormone deficiency. Growth Horm IGF Res 2014;24:S20.

32. G Cox, M Rosendahl, E Chlipala, D Smith, S Carlson, D Doherty. A long-acting, mono-PEGylated GH. Eur J Endocrinol 2012;166:533–40.

33. G Cox, M Rosendahl, E Chlipala, D Smith, S Carlson, D Doherty. Long-acting pegylated somatropin (recombinant human growth hormone) is a potent stimulator of weight gain and bone growth in hypophysectomized rats. Endocrinology 2007;148:1590–7.

34. M Rasmussen, M Albrecht, T Anderson, M Olsou, P Christiansen. A phase 2, multicenter, multiple-dose, open-label, parallel group, active controlled, safety, tolerability, the pharmacokinetic and pharmacodynamic study of ACP-001 in adult patients with Growth Hormone Deficiency (AGHD). Growth Horm IGF Res 2010;20:259–67.

35. M Rasmussen, M Olsou, M Albrecht, T Anderson, M Olsou, P Christiansen. A phase 2, a new needle-free device, compared with a fine gauge needle. J Pediatr Endocrinol Metab 2003;16:383–92.

36. G Cox, M Rosendahl, E Chlipala, D Smith, S Carlson, D Doherty. Ghrelin is a potent stimulator of weight gain and bone growth in hypophysectomized rats. Endocrinology 2007;148:1590–7.

37. G Cox, M Rosendahl, E Chlipala, D Smith, S Carlson, D Doherty. A phase 1, 2 doseescalation study of a novel long-acting pegylated growth hormone in young adults with growth hormone deficiency. Growth Horm IGF Res 2014;24:S20.

38. M Roth, V Popovici, P Vanuga, J Payer, M Pfeifer, M Billingermaier, et al. Once-weekly, CTP-modified hGH (MOD-4023) is effective in growth hormone deficient adults: a phase II, dose and frequency finding study. Growth Horm IGF Res 2012;22:884–5.

39. E Kim, D Jang, S Yang, M Lee, K Jin, H Cha, et al. Controlled release of human growth hormone fused with a human hybrid Fc fragment through a nanoporous polymer membrane. Nanoscale 2013;5:4262–9.

40. S Nagam, A Jyothi, J Poojitha, S Aruna, R Nandendla. A comprehensive review on hydrogels. Int J Pharm Res 2016;8:19–23.

41. K Manohar, W Ravis, D-Golden, A Banga. Controlled release of human growth hormone following subcutaneous administration in dogs. Int J Pharm 1997;152:1993–8.

42. G Tae, J Kornfield, J Hubbell. Sustained release of human growth hormone from in situ forming hydrogels using selfassembly of fluoropolymer-ended poly(ethylene glycol). Biomaterials 2005;26:5529–65.

43. H Yoo, Photo-cross-linkable and thermo-responsive hydrogels containing chitosan and Pluronic for sustained release of growth hormone (hGH). J Biomat Sci Polym Ed 2007;18:1429–41.

44. U Shinde, M Joo, H Moon, B Jeong, Sol-gel transition of PEG-PAF aqeous solution and its application for hGH sustained release. J Mater Chem 2012;22:6072–9.

45. M Park, C Chun, S Ahn, M Ki, C Cho, S Song. Sustained delivery of human growth hormone using a polyelectrolyte complexloaded thermosensitive polyphosphazene hydrogel. J Controlled Release 2010;147:529–39.

46. N Singh, Q Nguyen, B Kim, D Lee. Nanostructure controlled sustained delivery of human growth hormone using injectable, biodegradable, pH-temperature responsive nanohybrid hydrogel. Nanoscale 2015;7:3904–53.

47. V Phan, T Thambi, H Duong, D Lee. Poly(amino carbonate urethane)-based biodegradable, temperature and pH-sensitive injectable hydrogels for sustained human growth hormone delivery. Sci Rep 2016;6:29978.

48. J García, M Dorta, O Munguia, M Llabrés, J Farínja. Biodegradable laminar implants for sustained release of recombinant human growth hormone. Biomaterials 2002;23:4759–64.

49. E Fidotti. A history of growth hormone injection devices. J Pediatr Endocrinol Metab 2001;14:947–501.

50. H Dumas, P Panayiotopoulos, D Parker, V Pongpairochana. Understanding and meeting the needs of those using growth hormone injection devices. BMC Endocr Disord 2006;6:5.

51. GH Verrips, R Hirasing, M Fekkes, T Vogels, S Vanhorick, H De Waal. Psychological responses to the needle-free Medi-Jector or the multidose disetronic injection pen in human growth hormone therapy. Acta Paediatr 1998;87:154–8.

52. H Agero, J Pedersen, S Gappi, P Thomann, B Jesusek, T Senderovitz. Pharmacokinetics and pharmacodynamics of a new formulation of recombinant human growth hormone administered by Zomataf 2 Vision, a new needle-free device, compared to subcutaneous administration using a conventional syringe. J Clin Pharmacol 2002;42:1262–8.

53. H Dörr, S Zabransky, E Keller, B Otten, C Parshk, L Nyman, et al. Are needle-free injections a useful alternative for growth hormone therapy in children? safety and pharmacokinetics of growth hormone delivered by a new needle-free injection device compared to a fine gauge needle. J Pediatr Endocrinol Metab 2003;16:383–92.

54. E Brealey, A Priestley, J Scott, M Christen. Pharmacokinetics of recombinant human growth hormone administered by cool.click™ 2, a new needle-free device, compared with subcutaneous administration using a conventional syringe and needle. BMC Pharmacol Toxicol 2007;10:1.

55. L Ilium. Nasal drug delivery-possibilities, problems and solutions. J Controlled Release 2003;97:187–98.

56. L Jorgensen, E Bechgaard. Intranasal permeation of thyrotropin-releasing hormone: in vitro study of permeation and enzymic degradation. Sct Title Pharm 1994;107:231–7.

57. A Harris, I Nilsson, Z Wagner, U Alkner. Intranasal administration of peptides: nasal deposition, biological
response, and absorption of desmopressin. J Pharm Sci 1986;75:1085–8.

58. P Baldwin, C Klingbeil, C Grimm, J Longenecker. The effect of sodium tauro-24,25-dihydrofusidate on the nasal absorption of human growth hormone in three animal models. Pharm Res 1990;7:547–52.

59. L Hedin, B Olsson, M Dicalalusy, C Flagg, AS Petersson, S Rosberg. Intranasal administration of human growth hormone (hGH) in combination with a membrane in patients with GH deficiency: a pharmokinetic study. J Clin Endocrinol Metab 1993;76:962–7.

60. A Fishr, N Farraj, D O’Hagan, I Gill, B Johansen, S Davis, et al. Effect of L-alpha-lysophosphatidylcholine on the nasal absorption of human growth hormone in three animal species. Int J Pharm 1991;74:47–56.

61. L Ilhun, N Farraj, S Davis, B Johansen, D O’Hagan. Investigation of the nasal absorption of biosynthetic human growth hormone in sheep-use of a bioadhesive microsphere delivery system. Int J Pharm 1990;63:207–11.

62. C Agerholm, Lonebøth, P Johansen, M Nielsen, F Ellin. Epithelial transport and bioavailability of Intranasally administered human growth hormone formulated with the absorption enhancers didecanoyl-L-a-phosphatidylcholine and a-cyclodextrin in rabbits. J Pharm Sci 1994;83:5–8.

63. T Laursen, B Grandjean, J Jørgensen, J Christiansen. Bioavailability and bioactivity of three different doses of nasal growth hormone (GH) administered to GH-deficient patients: comparison with the intravenous and subcutaneous administration. Eur J Endocrinol 1996;135:309–15.

64. V Leitner, D Gaggi, A Schnürch. Thiomers in noninvasive polypeptide delivery: in vitro and in vivo characterization of a polycarboxyl-systeine/glutathione gel formulation for human growth hormone. J Pharm Sci 2004;93:1682–91.

65. V Leitner, D Gaggi, A Krauland, A Schnürch. Nasal delivery of human growth hormone: in vitro and in vivo evaluation of a thiomers/glutathione microparticulate delivery system. J Controlled Release 2004;100:87–95.

66. Y Cheng, A Dyer, I Gill, M Hinchcliffe, R Nankervis. Intranasal delivery of recombinant human growth hormone (somatropin) in sheep using chitosan-based powder formulations. Eur J Pharm Sci 2005;26:69–15.

67. D Steyn, L Plessis, A Kotzé. Nasal delivery of recombinant human growth hormone: in vivo evaluation with pheroild™ technology and n-trimethyl chitosan chloride. J Pharm Sci 2010;13:263–73.

68. L Illum, F Jordan, A Lewis. CriticalSorb: a novel efficient nasal delivery system for human growth hormone based on solutol HS15. J Controlled Release 2012;162:194–200.

69. R. Aga, M. Iqbal, M. Arif, R. Kinget, N. Verheke. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir Res 2001;2:198–209.

70. C Lombry, D Edwards, V Prêt, R Vanbever. Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules. Am J Physiol Lung Cell Mol Physiol 2004;286:L1002–8.

71. H Chrystyn. Is total particle dose more important than particle distribution? Respir Med 1997;91:7–9.

72. H Folkesson, I Hedin, B Westrom. Lung to blood passage of human growth hormone after intratracheal instillation: stimulation of growth in hypophysectomized rats. J Endocrinol 1992;134:197–203.

73. P Colthorpe, S Farr, I Smith, D Wyatt, G Taylor. The influence of regional deposition on the pharmacokinetics of pulmonary delivered human growth hormone in rabbits. Pharm Res 1995;12:356–9.