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Review article

# Human growth hormone: New delivery systems, alternative routes of administration, and their pharmacological relevance

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ABSTRACT

The availability of recombinant human growth hormone (GH) has broadened its range of clinical applications. Approved indications for GH therapy include treatment of growth hormone deficiency (in children and in adults), Turner syndrome, Prader–Willi syndrome, chronic renal insufficiency and more recently, idiopathic short stature in children, AIDS-related wasting and fat accumulation associated with lipodystrophy in adults. Therapy with GH usually begins at a low dose and is gradually titrated to obtain optimal efficacy while minimizing side effects. It is usually administered on a daily basis by subcutaneous injection, since this was considered to impact upon patient compliance, extended-release GH preparations were developed and new delivery platforms – e.g., auto-injectors and needle-free devices – were introduced in order to improve not only compliance and convenience but also dosing accuracy. In addition, alternative less invasive modes of administration such as the nasal, pulmonary and transdermal routes have also been investigated. Here, we provide an overview of the different technologies and routes of GH administration and discuss the principles, limitations and pharmacological profiles for each approach.



## 1. Growth hormone: structure, secretion and functions

Human growth hormone (GH, somatotropin) is a single-chain polypeptide comprising 191 amino acids; the tertiary structure contains four helices and two disulfide bridges (Fig. 1). It is synthesized, stored, and secreted by the somatotroph cells within the lateral section of the anterior pituitary, which typically contains 3–5 mg GH and secretes between 0.5 and 0.875 mg of protein per day. Although circulating levels of GH are low (GH has an average plasma half-life of 20–30 min), pulsatile release of GH – usually in 4–8 discrete bursts – occurs throughout the day and at night with a mean peak amplitude of ~4–6 μg/l [1]. GH release is sexually dimorphic with women having more daytime GH pulses than men but a relatively modest nocturnal surge (Fig. 2) [2,3]. Basal GH levels and the frequency and amplitude of GH secretion are low in infancy, increase during childhood and reach a peak during puberty (~10 μg/l) [4,5]; secretion then gradually decreases, and a progressive decline is observed after the third decade.

The secretion profile is modulated through a complex neuroendocrine control system comprising two main hypothalamic regulators, GH-releasing hormone (GHRH, 44 amino acid peptide) and somatostatin (SS, cyclic tetradecapeptide), exerting stimulatory and inhibitory influences, respectively, on the somatotroph cell (Fig. 3). Although the interplay between these peptides is the main determinant of GH release, other physiological stimulators and inhibitors can affect GH secretion [6]. For example ghrelin, which is synthesized principally in the epithelial cells lining the fundus of the stomach, with smaller amounts produced in the placenta, kidney, pituitary and hypothalamus, can stimulate GH secretion by downregulating somatostatin release [7]. Other physiological stimulators include sleep, hypoglycemia, exercise, dietary protein, short-term fasting and arginine [8]. GH secretion can be inhibited by hyperglycemia, chronic glucocorticoid use, estradiol, and circulating concentrations of insulin-like growth factor-1 (IGF-1) through negative feedback on the hypothalamus.

The effects of GH on growth and metabolism may be direct or mediated through other hormones. The most important of these is insulin-like growth factor-1 (IGF-1) – GH acts on the liver to increase synthesis and secretion of IGF-1, which in turn stimulates division and multiplication of chondrocytes and osteoblasts, which are the primary cells in the epiphyses of long bones. GH also increases amino acid uptake and protein synthesis in muscle and other tissues [9].

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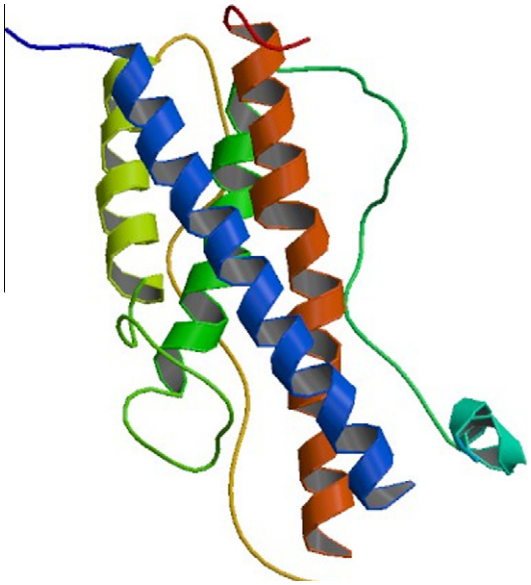

**Fig. 1.** Three-dimensional structure of human growth hormone showing the secondary structure elements (pdb file: 1 hgu; www.rcsb.org). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 2. Therapeutic uses and indications of human growth hormone

GH, extracted from human pituitary glands, was first used to treat children suffering from growth hormone deficiency (GHD) in the 1950s. Pituitary-derived GH continued to be used until 1985 when Creutzfeldt–Jacob disease was diagnosed in four patients receiving GH treatment. The availability of recombinant human GH (somatropin; from *E. coli* in 1985 and from murine cells in 1987) meant that biosynthetic GH quickly replaced pituitary-derived GH for therapeutic use. Although the initial indication was limited to the treatment of children with GHD, investigations into other therapeutic applications quickly followed (Table 1).

GH deficiency may occur as an isolated hormonal deficiency or in combination with multiple pituitary hormone deficiency as a result of hypopituitarism, tumors in the central nervous system, cranial irradiation, or other organic causes. Idiopathic growth hormone deficiency is the most common form, accounting for approximately 50–70% of cases. Growth failure is a prominent feature in children with chronic renal insufficiency and Turner syndrome; the causes being multifactorial, including reduced sensitivity to GH, rather than decreased GH levels [10]. Therefore, supraphysiological doses of GH are required for treatment in children with these conditions. In contrast, children with Prader–Willi syndrome are considered to have a hypothalamic disorder, and thus GH therapy is intended to restore physiological levels of GH [11]. The FDA approved GH therapy for GHD in adults in 1996.

Treatment with GH may be considered at three distinct dose levels [12]. Replacement therapy for GH failure in both children and adults typically consists of 0.01–0.025 mg/kg/day. Supraphysiological dosages of GH are used to treat growth deficit not due to growth hormone deficiency, such as Turner's syndrome, chronic renal insufficiency, and idiopathic short stature; GH doses for these indications are regularly around 0.05 mg/kg/day. A third pharmacological dose level is used for metabolic indications such as short-bowel syndrome or AIDS-associated cachexia; for these indications, doses range from 0.1 to 0.2 mg/kg/day. The optimal dosing schedule remains open to question; for example, it has been shown that the efficacy of a thrice weekly injection regimen was comparable to that observed in patients treated with daily GH therapy [13]. Nocturnal administration provides more physiological GH profiles than morning injections [14]. Intriguingly, comparison of the long-term effects of non-physiological continuous subcutaneous infusion versus daily subcutaneous injection (over 6 months) suggested that the effects of GH on IGF-1 and IGF-binding proteins, GH-binding protein, bone metabolism, body composition, insulin sensitivity, and lipoproteins were similar [15]. This was surprising since the constantly elevated levels of GH and IGF-1 seen in acromegaly are associated with decreased glucose tolerance, hyperinsulinemia and in some cases, diabetes mellitus [15]. These results supported the development of sustained release formulations that would reduce injection frequency.

Side effects of GH therapy are rare, but may include headache, visual problems, nausea and vomiting, fluid retention (peripheral edema), arthralgia, myalgia, paraesthesia, antibody formation, hypothyroidism and reactions at the injection site.

## 3. Conventional administration of human growth hormone via injection

GH is routinely administered by daily intramuscular (i.m.) or subcutaneous (s.c.) injection (Table 2) [16]. The pharmacokinetic/pharmacodynamic (PK/PD) parameters of approved formulations are expected to be similar; PK parameters including the median (range) maximum concentration ($C_{max}$), the area under the curve (AUC), the median (range) terminal half-life ($t_{1/2}$), and clearance (CL/F) as well as bioavailability (BA) after administration of different short-acting formulations to healthy adults are shown in Table 3. Comparison of the pharmacokinetics following i.m. and s.c. administration of GH (Humatrope®; 0.1 mg/kg in men and women) suggested that although $C_{max}$ and AUC were higher following i.m. injection, the half-life and bioavailability were greater after s.c. administration [17]. Serum GH levels achieved after s.c. injection of Nutropin AQ® at the same dose (0.1 mg/kg) in male volunteers appeared to show a higher peak and shorter half-life than those obtained with Humatrope®, perhaps pointing to the effect of formulation. In addition to the differences between absorption following i.m. and s.c. administration, studies have also tried to relate GH pharmacokinetics/pharmacodynamics to age, gender and body composition [18]. Following administration of an i.v. bolus (200 μg), younger subjects showed higher peak GH levels and AUC – the volume of distribution and clearance were higher in the older group [18].

A more recent report compared pharmacokinetics/pharmacodynamics following i.m. and s.c. administration in a group of young healthy adults performing regular physical training (Fig. 4). The bioavailability of GH was again principally influenced by the route of administration with higher $C_{max}$ and AUC of GH after i.m. injection while PD parameters were mainly determined by gender [19].

Two compartment models have been developed to describe GH pharmacokinetics/pharmacodynamics [20]. Such a model was used to analyze GH distribution kinetics after administration by intravenous bolus in human volunteers and in adults with GHD – this revealed that the major differences lay in the distribution phase – as evidenced by increases in $t_{1/2\alpha}$ (2.37 ± 0.21 and 5.21 ± 1.11 min, respectively) and the corresponding mean residence times (5.93 ± 1.11 and 12.12 ± 1.44 min, respectively). This led to a significant decrease in the mean clearance rate in GHD patients (950.9 ± 218.4 and 490.4 ± 62.6 min) [21].

Replacement GH therapy is well accepted by endocrinologists and patients; however, the need for daily injections still limits its use, and this has driven the development of long-acting formulations that provide a sustained release of GH – even though endogenous GH is released in discrete pulses. The formulations tested in experimental animals or in humans include GH microspheres, GH macrolide microparticles, crystalline GH, hyaluronate-conjugated GH and pegylated GH [22–27].

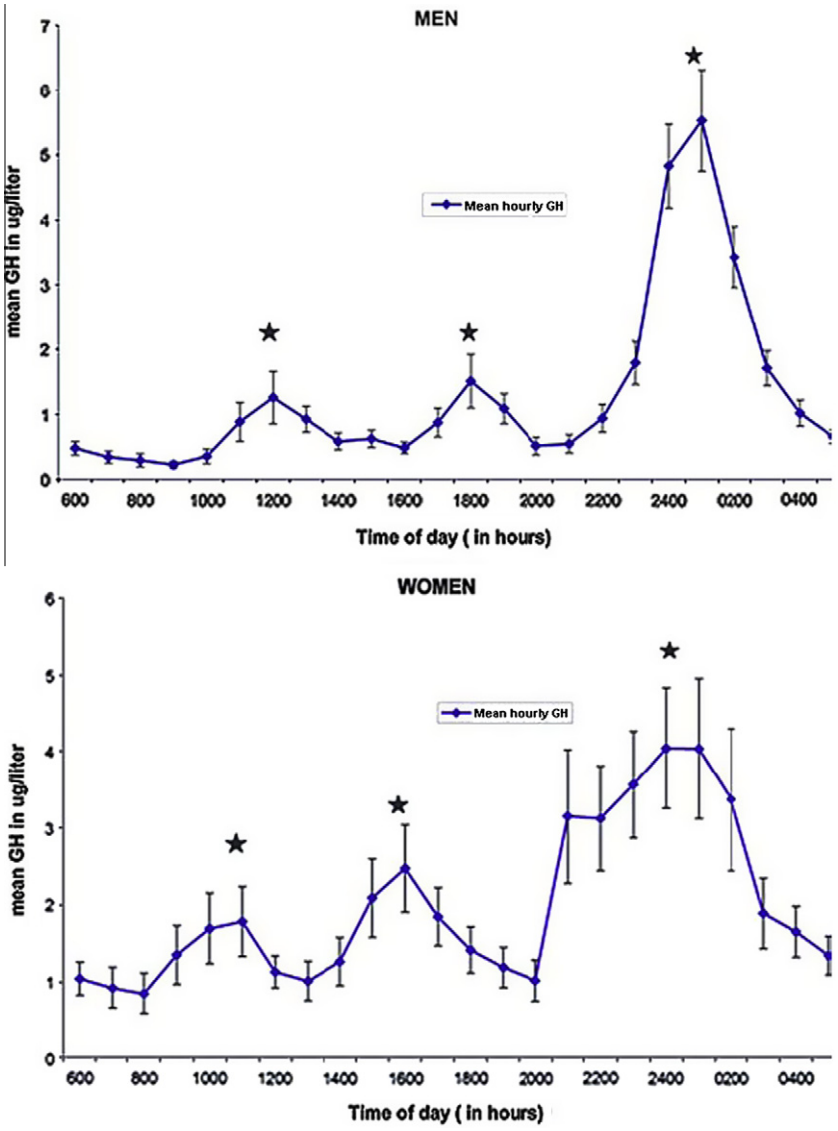


**Fig. 2.** Circardian pattern of growth hormone. The 24-h plasma profiles were determined in 93 healthy, young (18–45 years old), non-obese (BMI < 26 kg/m$^2$) and regularly fed men ($n$ = 67) and women ($n$ = 26). Data are shown as mean ± standard error (mean ± SE). Reproduced by permission from Surya et al., Complex rhythmicity of growth hormone secretion in humans, Pituitary 9 (2006) 121–125. Copyright Springer Netherlands (2006). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Studies in children using micronized zinc-stabilized GH encapsulated in poly (D,L-lactic-co-glycolic acid) biodegradable microspheres (Nutropin® depot) showed that after administration of either 0.75 mg/kg twice a month or 1.5 mg/kg once a month, GH levels were significantly increased when compared to controls [28]. The compound was clearly effective, but the principal disadvantage was that its use frequently resulted in the formation of subcutaneous nodules (60% of injections) [28]. The depot formulation was also studied in adults using a single dose of either 0.25 mg/kg or 0.5 mg/kg [29]; the results showed a substantial increase in GH concentration reaching a peak 24 h after injection. The efficacy and adverse effects were evaluated in a separate multi-dose study in which the formulation was administered every 14 days and was compared with daily somatropin injection (Nutropin AQ®) in GH-deficient subjects [30]. The results confirmed that the long-lasting GH preparation could provide effective hormone replacement therapy, maintaining normal adult serum IGF-1 levels without evidence of increased side effects.

Recent preclinical studies using a GH-containing PLGA microsphere formulation (DA-3003) demonstrated that GH concentration was sustained for 14 and 28 days in rats and monkeys, respectively, after a single administration of the new formulation [31]. The plasma concentration of IGF-1 was also increased and remained elevated for approximately 28 days in monkeys.

Although encapsulation with biodegradable polymers is very attractive, the manufacturing processes are still complicated and expensive [24]. The withdrawal of Nutropin® depot from the market was reported to be due in part to the cost of manufacturing. Moreover, since the encapsulation approaches are principally based on diffusion control and polymer degradation for slower drug release, particle sizes tend to be large (~50 μm), and as a result larger gauge needles were required for GH administration – a problem in terms of patient compliance. For example, Nutropin® depot required delivery through a specially designed 21-gauge needle. In terms of patient compliance and for market approval, administration of the drug through finer needles (29- to 31-gauge) is much preferred.

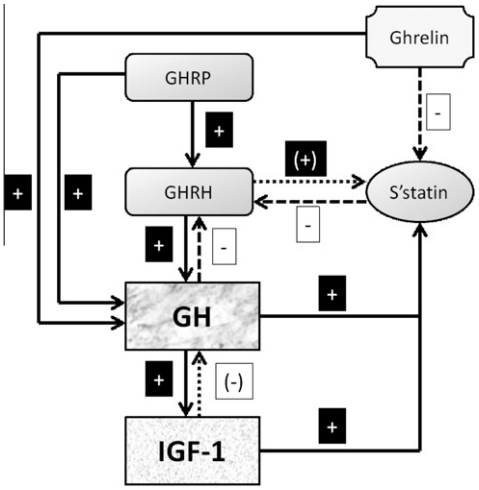


**Fig. 3.** Physiology of GH secretion. Although this is principally controlled by the interplay between GHRH and somatostatin (S' statin), other factors such as GHRP (growth hormone releasing peptide) and ghrelin can impact upon GH levels. Adapted from Veldhuis, A tripeptidyl ensemble perspective of interactive control of growth hormone secretion, Horm. Res. 60 (2003) 86–101. Copyright S. Karger A.G. 2010.

Another approach for sustained GH delivery was based on crystallization and complexation with polyelectrolytes; a monomolecular layer of positively charged poly (arginine) was applied to GH crystals to form polyelectrolyte-coated crystals, and PK/PD studies were conducted in hypophysectomized rats and monkeys [23]. The results suggested that crystals enabled *in vivo* release over several days. The efficacy of the crystalline formulation injected subcutaneously once a week appeared to be equivalent to seven daily soluble injections in the standard weight gain assay in rats and by measurement of serum IFG-I factor in monkeys. The low viscosity of the suspension also facilitated easy administration through a 30-gauge needle.

Bidlingmaier et al. analyzed the effect of hyaluronate-conjugated GH (LB03002) incorporated into microparticles versus daily recombinant GH in adults with GH deficiency [25]. Although the GH dose was 7- to 8-fold higher for the sustained release formulation, the observed maximal serum GH concentration was approximately doubled after LB03002 compared with daily GH. As expected, there was also an increase in GH half-life and in contrast to change in $C_{max}$, the AUC showed a proportionate 7- to 8-fold increase. With respect to the pharmacodynamics, the mean maximal serum IGF-1 concentration was 34–41% greater with LB03002 than with daily GH (although it was not reported whether this was statistically significant), and AUC was 7-fold greater; however, normalized to GH dose, as for GH itself, IGF-1 AUC was comparable. Another study investigated a novel formulation of recombinant GH developed as a once-a-week injection using sodium hyaluronate (HA). Microparticles were subsequently produced by spray drying technology. A single administration of the optimized formulation (1:1 GH:HA) to cynomolgus monkeys and beagle dogs for 6 days apparently induced increases in serum IGF-1 levels. The bioavailability of both formulations was equivalent to that of daily GH injection.

PEGylated GH is formulated by covalent attachment of polyethylene glycol (PEG) to GH (PEG-GH). This increases the hydrodynamic size and hence prolongs its circulatory time by reducing renal clearance. Studies have compared the pharmacokinetics of PEG-GH to those of somatropin in healthy volunteers and adults suffering from GHD [26,32,33]. In the first part of a Phase I study, healthy male volunteers (aged 25–55 years) received a single subcutaneous dose of human GH (3.6 mg, ~50 μg/kg). They were then divided into seven groups and received 3, 10, 30, 60, 100, 300 or 500 μg/kg PEGylated GH (PHA-794428) or placebo. The results showed that PHA-794428 had a prolonged elimination half-life (>20 h) and that at equivalent subcutaneous doses (50 μg/kg), PEGylation resulted in a 10- to 20-fold increase in AUC; the renal

**Table 1**
Conditions that involve treatment with human growth hormone.

| Pathology | Description |
|---|---|
| GH failure in childhood | Disorder caused principally by mutations of specific genes, e.g., GHRHR, GH1 |
| GH deficiency in adulthood | The most common cause is a pituitary adenoma or treatment of the adenoma with pituitary surgery or radiotherapy |
| Turner syndrome | Chromosomal disorder caused by a partially or completely missing X chromosome. It is a condition that only affects females. It is characterized by:<br>– short neck<br>– low hairline at the back of the neck<br>– low-set ears<br>– hands and feet that are swollen or puffy at birth<br>– soft nails that turn upward<br>Most individuals with Turner syndrome lose ovarian function in early childhood and do not start puberty at a normal age. Some have problems with specific visual-spatial coordination tasks (such as mentally rotating objects in space) and may have trouble learning mathematics (geometry and arithmetic) |
| Chronic renal insufficiency | Slowly worsening loss of the ability of the kidneys to remove wastes, concentrate urine, and conserve electrolytes<br>Principal causes:<br>– diabetes and high blood pressure<br>– Alport syndrome<br>– analgesic nephropathy<br>– glomerulonephritis of any type (one of the most common causes)<br>– kidney stones and infection<br>– obstructive uropathy<br>– polycystic kidney disease<br>– reflux nephropathy |
| Prader–Willi syndrome | Genetic disorder in which seven genes (or some subset thereof) on chromosome 15 are missing or unexpressed (chromosome 15q partial deletion) on the paternal chromosome. This condition is characterized in infancy by weak muscle tone (hypotonia), feeding difficulties, poor growth, and delayed development. In childhood affected individuals develop an insatiable appetite and chronic overeating (hyperphagia). People with this syndrome typically have intellectual impairment or learning disabilities and behavioral problems |
| Short-bowel syndrome | Malabsorption disorder caused by the surgical removal of the small intestine, or more rarely due to the complete dysfunction of a large segment of bowel. It can be classified as acquired and congenital, the last is less common<br>Caused by surgery for:<br>– Crohn's disease, an inflammatory disorder of the digestive tract<br>– Volvulus, a spontaneous twisting of the small intestine that cuts off the blood supply and leads to tissue death<br>– tumors of the small intestine<br>– injury or trauma to the small intestine<br>– necrotizing enterocolitis (premature newborn)<br>– bypass surgery to treat obesity, a now commonly performed surgical procedure<br>– surgery to remove diseases or damaged portion |
| AIDS-associated weight loss or cachexia | Involuntary loss of weight, muscle atrophy, fatigue, weakness, and significant loss of appetite |
| Idiopathic short stature | Extreme short stature – unclear etiology |

**Table 2**
Indications and usage of somatropin.

| Trade name | | Indications and usage | Administration | Dosage |
|---|---|---|---|---|
| Accretropin™ | Cangene Corporation | Growth failure in children | s.c. | 0.18–0.3 mg/kg/wk divided into 6 or 7 injections |
| | | Turner syndrome | | 0.36 mg/kg/wk divided into 6 or 7 injections |
| Genotropin®, Genotropin preservative free® | Pharmacia and Upjohn, Pfizer | Growth failure in children | s.c. | 0.24 mg/kg/wk divided into 6 or 7 injections |
| | | Replacement of endogenous GH in adults | | Start with up to 0.04 mg/kg/wk given as a daily injection, increasing the dose at 4- to 8-wk intervals according to patient requirements (max, 0.08 mg/kg/wk) |
| | | Turner syndrome | | 0.33 mg/kg/wk divided into 6 or 7 injections |
| | | Prader–Willi syndrome | | 0.24 mg/kg/wk divided into 6 or 7 injections |
| | | Idiopathic growth stature | | Up to 0.47 mg/kg/wk divided into 6 or 7 injections |
| Humatrope® | Eli Lilly and Co. | Growth failure in children | s.c., i.m. | 0.18 mg/kg/wk (divided into equal doses given on 3 alternate days, 6 times/wk, or daily) to a max of 0.3 mg/kg |
| | | Turner syndrome | s.c. | Up to 0.375 mg/kg/wk divided into equal doses given either daily or on 3 alternate days. |
| | | Replacement of endogenous GH in adults | | Start with up to 0.006 mg/kg/day, increasing the dose according to patient requirements (max, 0.0125 mg/kg/day |
| | | Idiopathic short stature | | 0.37 mg/kg divided into equal doses given 6 to 7 times/wk |
| Norditropin®, Norditropin Nordiflex® | Novo Nordisk Inc. | Growth failure in children | s.c. | 0.024 to 0.034 mg/kg 6 to 7 times/wk |
| | | Replacement of endogenous GH in adults | | Start with up to 0.004 mg/kg daily. The dose may be increased to a max of 0.016 mg/kg daily after approximately 6 wk according to patient requirements |
| | | Turner syndrome | | Up to 0.067 mg/kg daily |
| Nutropin®, Nutropin AQ® | Genentech Inc. | Growth failure in children | s.c. | Up to 0.3 mg/kg weekly divided into daily injections; pubertal patients may receive up to 0.7 mg/kg weekly in divided daily doses |
| | | Replacement of endogenous GH in adults | | Start with up to 0.006 mg/kg/day, increasing the dose according to patient requirements to a max of 0.025 mg/kg/day in patients younger than 35 yr of age and max of 0.0125 mg/kg daily in patients older than 35 yr of age |
| | | Chronic renal insufficiency | | Up to 0.35 mg/kg weekly (divided into daily injections) continued up to time of renal transplantation |
| | | Turner syndrome | | Up to 0.375 mg/kg/wk divided into equal doses 3 to 7 times/wk |
| | | Idiopathic growth stature | | Start with up to 0.3 mg/kg weekly divided into daily doses |
| Omnitrope® | Sandoz | Growth failure in children | s.c. | 0.16 to 0.24 mg/kg weekly, divided into 6 or 7 daily doses. |
| | | Replacement of endogenous GH in adults | | Start with no more than 0.04 mg/kg weekly given as a daily injection, increasing the dose at 4- to 8-wk intervals according to individual patient requirements (max, 0.08 mg/kg/wk) |
| Serostim®, Serostim LQ® | Merck Serono Inc. | AIDS-associated weight loss or cachexia | s.c., i.m. | More than 55 kg, 6 mg daily45 to 55 kg, 5 mg daily35 to 45 kg, 4 mg daily; less than 35 kg, 0.1 mg/kg |
| Saizen® | Merck Serono Inc. | Growth failure in children | s.c., i.m. | 0.06 mg/kg 3 times/wk |
| | | Replacement of endogenous GH in adults | s.c. | Start with up to 0.005 mg/kg daily. Increase the dose to no more than 0.01 mg/kg daily after 4 wk according to individual patient requirements |
| Tev-tropin™ | TEVA-Pharmaceuticals | Growth failure in children | s.c. | Up to 0.1 mg/kg 3 times weekly |
| Valtropin® | Biopartners | Growth failure in children | s.c. | 0.23 mg/kg of body weight/week (0.033 mg/kg/day) |
| | | Replacement of endogenous GH in adults | | 0.33 mg/day (equivalent to 0.005 mg/kg/day in a 66 kg adult) (6 days/week) |
| | | Turner syndrome | | 0.37 mg/kg of body weight/week (0.053 mg/kg/day) |
| Zorbtive® | Serono/Novartis | Short-bowel syndrome | s.c. | 0.1 mg/kg daily (max, 8 mg/day), rotating the injection sites. Administration for more than 4 wk has not been adequately studied. Treat moderate fluid retention and arthralgia symptomatically, or reduce the dose by 50%. Discontinue treatment for up to 5 days for severe toxicities. Upon resolution of symptoms, resume treatment at 50% of the original dose. Permanently discontinue treatment if severe toxicity persists or does not disappear in 5 days |

s.c., subcutaneous; i.m., intramuscular.

clearance of PHA-794428 was significantly lower than that of somatropin (0.1 and 9.6 l/h, respectively). It was proposed that in addition to the effect of PEGylation on glomerular filtration, it also impacted on a second receptor-mediated clearance mechanism by

**Table 3**
Pharmacokinetic parameters of short-acting GH formulations following administration to healthy adults.

| Formulation | Sex/dose/ route of administration | AUC (μg/h/l) | Maximal concentration ($C_{max;}$ μg/l) | Terminal half-life ($t_{1/2}$; $h$) | CL/F (ml/h/kg) | BA (%) |
|---|---|---|---|---|---|---|
| Nutropin® | Males 0.1 mg/kg s.c. | 643 (C.V. 12.0) | 67.2 (C.V. 29.0) | 2.1 (C.V. 20.0) | 158 (C.V. 12.0) | |
| Nutropin AQ® | Males 0.1 mg/kg s.c. | 677 (C.V. 13.0) | 71 (C.V. 17.0) | 2.3 (C.V. 18.0) | 150 (C.V. 13.0) | 81 |
| Humatrope® | Males and females mg/kg s.c. | 495 (S.D. 11.0) | 53.2 (S.D. 25.9) | 4.9 (S.D. 2.7) | 215 (S.D. 47.0) | 75 |
| | Males and females 0.1 mg/kg i.m. | 585 (S.D. 90.0) | 63.3 (S.D. 18.2) | 3.8 (S.D. 1.4) | 175 (S.D. 28.8) | 63 |
| Accretropin® | Males and females 4 mg s.c. | 255.3 (S.D. 43.0) | 29.4 (S.D. 8.3) | 3.6 (S.D. 1.3) | | 70 |
| Genotropin® | Males and females 0.03 mg/kg s.c. | | 23.0 (S.D. 9.4) | 3.0 | | 80 |

C.V.- Coefficient of variation (%); S.D.- Standard deviation.
Data.com data sources include Micromedex™ [updated 8 April 2009], Cerner Multum™ [updated 21 April 2009], Wolters Kluwer™ [updated 17 April 2009], and others. Copyright Drugs.com (2000–2009). Rxlist.com data sources include. Copyright by RxList Inc. (2009).

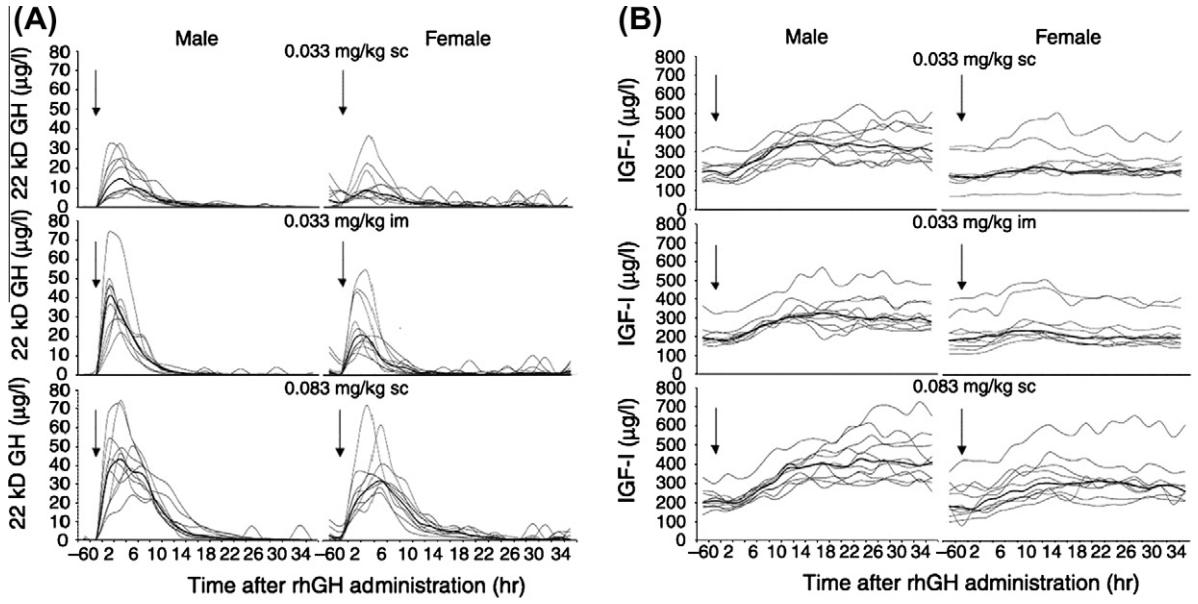


**Fig. 4.** Plasma GH concentration and IGF-1 generation following 0.033 or 0.083 mg/kg s.c. or 0.033 mg/kg i.m. administration of hGH to healthy adult males and females. Individual responses = light lines; median response = black line. Reproduced by permission from Keller et al., Pharmacokinetics and pharmacodynamics of GH: dependence on route and dosage of administration. Eur. J. Endocrinol. 156 (2007) 647–653. Copyright Society of Endocrinology (2007).

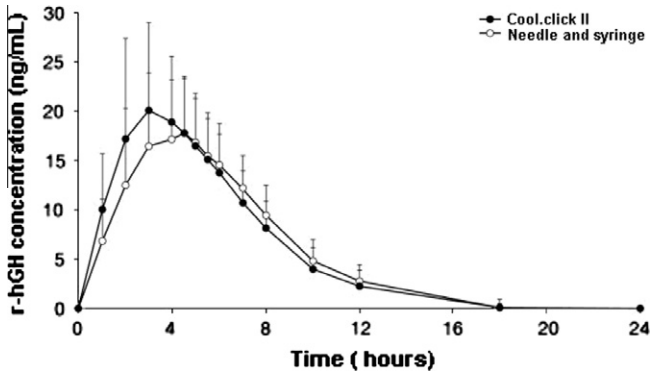


**Fig. 5.** Mean GH serum concentration vs. time profiles following subcutaneous administration of rhGH (dose 2.92 mg/subject) (mean ± SD). Reproduced by permission from Brearley et al. Pharmacokinetics of recombinant human growth hormone administered by cool.click 2, a new needle-free device, compared with subcutaneous administration using a conventional syringe and needle, BMC Clin. Pharmacol. 7 (2007) 10. Copyright BioMed Central Ltd. (2007).

reducing the affinity of the conjugate for the receptor. A sustained pharmacological response was also observed as IGF-1 levels remained elevated for up to 1 week and was confirmed by increased levels of other biomarkers. PHA-794428 was well-tolerated at doses up to 300 μg/kg – most side effects were related to fluid retention. PHA-794428 was administered to seven patients with GHD in an open-label crossover study (with washout) at doses of 20 and 60 μg/kg; the half-life was 43.3 h and IGF-1 was increased (max. conc. of 153.3 and 286.5 ng/ml at the 20 and 60 μg/kg doses, respectively. The drug was reported to be well tolerated, and there did not appear to be any production of PHA-794428 antibodies [29]. Somewhat disappointingly, a recent report from a multicentre Phase II study using PHA-794428 in GHD patients described the incidence of lipoatrophy at the injection site, which was suggested as being due to the lipolytic action of GH on subcutaneous adipose tissue [34]. Its increased prevalence in female patients might have been due to larger amounts of adipose tissue in women, although a gender-specific response was not ruled out.

Other PEGylated GH analogs have shown efficacy in animal models. Cox et al. described a PEGylated GH threonine-3 analog with a longer circulating half-life than GH following s.c. administration in rats; it was also reported to be more potent than GH at stimulating weight gain and bone growth in GH-deficient rats [35]. Pasut et al. recently described a new PEGylating agent, PEG-βAla-NHCO [36]; the pharmacokinetic profile of the GH conjugate was investigated in rats and monkeys and the potency evaluated in hypophysectomized rats. Conjugation led to an increase in

residence time in both animal models; GH half-life increased from 0.8 to 8.3 h in rats and from 3.1 to 20.8 h in monkeys. A single subcutaneous injection of GH-PEG-βAla (1 × 240 μg/rat, protein equiv) was found to be similar to the same total amount of unconjugated GH given daily over a period of six days (6 × 40 μg/rat).

## 4. New delivery devices

Compliance issues with some patients, especially children, may ultimately lead to a discontinuation of treatment because of pain and other adverse effects due to the injection procedure. In order to address this, novel administration delivery devices such as pre-filled syringes, manual injector pens, auto-injectors, injectors with hidden needles and needle-free devices have been introduced in an attempt to increase dosing accuracy and adjustability, ease of use, convenience and compliance [37,38]. Needle-based auto-injectors are devices that incorporate a pre-filled syringe or cartridge fitted with a needle. Needle-free injectors administer the drug (by definition, without a needle) by high pressure, expelling it through a fine nozzle, and can be as effective as a conventional injection [39]. Injectors are available for single and multiple doses and are suitable for a wide range of primary containers including pre-filled glass and plastic syringes and pre-filled cartridges.

A number of reports have addressed the efficacy and safety of injection devices in GH therapies, for example, the physiological responses to a needle-free injector (Medi-Jector®, Medi-Ject Corporation, MN, USA) and a multiple dose injection pen with 28G needles (Disetronic AG, Buegder, Switzerland) have been compared [40]. Use of the needle-free device (Medi-Jector®) showed fewer adverse physiological responses. Agerso et al. [39] compared the administration of a new GH formulation (Zomacton®; target dose of 1.67 mg GH) by conventional syringe and with a new needle-free device (ZomaJet® 2 Vision). The results demonstrated that administration with the ZomaJet® 2 Vision GH was bioequivalent based on AUC values; GH absorption appeared to be faster than after injection resulting in higher $C_{max}$ values. Serum concentrations of IGF-1 and free fatty acids served as pharmacodynamic markers and confirmed bioequivalence of the needle-free device. In another study, Dörr et al. determined GH pharmacokinetics after administration of Genotropin® with Genotropin® ZipTip, a needle-free device, and a fine gauge needle device (Genotropin® Pen) in children [41]. The needle-free injector was shown to be bioequivalent, and more than 20% of the patients preferred to continue using it after the study. Interestingly, statistical analysis suggested that the fine gauge needle device produced less bleeding, bruising, pain, or soreness. More recently, Brearley et al. evaluated the relative bioavailability of GH (Saizen®) administered by the cool.click™ 2 needle-free device, and a standard needle and syringe [42]. Fig. 5 shows the GH serum profiles following s.c. administration of 2.92 mg of GH to healthy volunteers by either the cool.click™ 2 device or needle injection throughout the 25-h monitoring period. Statistical assessment of the rate and extent of absorption of GH suggested that administration by cool.click™ 2 was bioequivalent to standard needle injection and demonstrated similar tolerability.

Dedicated GH delivery devices that are currently on the market include cool.click™ (Fig. 6), Serojet® and Zomajet® 2 Vision. Cool.-click™ is a modified version of Bioject's Vitajet™ 3 needle-free injection system incorporating dosage features to accurately deliver different doses of Saizen®; it received FDA market clearance in June 2000. The SeroJet® system (EMD Serono, USA) is a needle-free injection for delivering Serostim®, recombinant GH for treatment of HIV-associated wasting in adults. Zomajet® 2 Vision (Antares Pharma, Switzerland) was licensed to Ferring Pharmaceuticals for delivery of Zomacton®, GH for treatment of GH deficiency in children and Turner syndrome. This needle-free system was launched in Europe in 2003 and is a customized version of the Medi-Jector Vision® device.

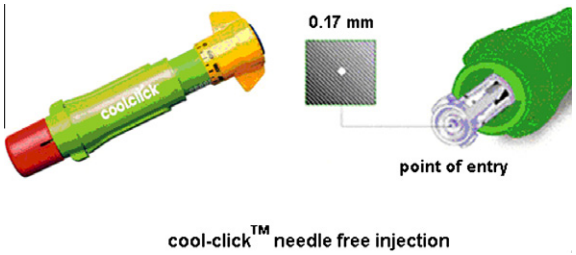


**Fig. 6.** Cool.click™ needle-free injection designed for the delivery of hGH (Merck Serono SA, Switzerland). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) Reproduced by permission from Justgrowth.com. Copyright Merck Serono S.A.–Geneva (2008).

Although needle-free injectors are recognized for their propensity to cause bruising, these devices can be superior to needle injection in terms of comfort and compliance. The advantage of this technology, compared to conventional syringe injection, is the absence of a needle which may be of benefit to children.

## 5. Alternative administration routes for the delivery of human growth hormone

The efforts to improve GH therapy and increase compliance have also led to the investigation of less invasive administration routes.

### 5.1. Intranasal delivery

The nasal route has been investigated as a non-invasive means of delivering therapeutic macromolecules due to its vascularized and permeable mucosal surfaces, no first-pass metabolism, rapid kinetics, and the ease of administration [43]. However, in the absence of a promoting agent, intranasal administration of high molecular weight molecules generally results in low bioavailability; they must overcome a diffusional barrier, which is comprised of the mucus gel layer covering mucosal membranes and an enzymatic barrier (the major obstacle for these compounds), due to secreted and membrane bound peptidases, in order to reach the systemic circulation [44]. Membrane transport is also limited by the rapid clearance of the administered formulation from the nasal cavity due to the mucociliary clearance mechanism, which decreases local residence time at the site of absorption [45].

The effectiveness of absorption promoters agents such as (i) surfactants – laureth-9, bile salts, fatty acids, phospholipids, cyclodextrins, (ii) enzymatic inhibitors, and (iii) multifunctional polymers in enhancing the nasal absorption of polar drugs has been evaluated [46,47]. These enhancers function by different mechanisms that change the permeability of the epithelial cell layer by modifying the phospholipid bilayer. The mechanisms include reduction in mucus viscosity, enzyme inhibition, and enhancement of membrane fluidity.

Sodium tauro-24,25-dihydrofusidate (STDHF) was shown to promote the nasal absorption of GH in rat, rabbit, and sheep [48]. After intranasal delivery of GH formulations containing STDHF, significant plasma GH levels were achieved (more markedly for sheep), closely mimicking the endogenous secretory pattern of GH release. Hedin et al. evaluated the efficacy of STDHF-based GH solutions administered to humans with GH deficiency [49] – the bioavailability was considerably lower (1.6–3.0%) relative to that obtained by s.c. injection. Fisher and co-workers studied the intranasal delivery of GH formulated with L-α-phosphatidylcholine

(LPC) in rats, rabbits, and sheep [50]. The bioavailability attained with the surfactant was 17.5%, 72.8%, and 16%, respectively; this corresponded to 7.6-, 52-, and 80-fold improvements in the bioavailability achieved in the absence of LPC.

The potential of using bioadhesive microspheres as a nasal delivery system for recombinant GH has also been assessed in sheep [51]. GH was administered as an aqueous solution and as lyophilized powders containing the microsphere system alone or in combination with LPC. Although GH absorption from the nasal solution was very poor, both the microsphere system alone and in combination with the biological surfactant improved uptake. Relative to s.c. injection, the estimated bioavailabilities of the nasal formulations were 2.7% and 14.4%. However, damage to cell membranes was also observed; this was most likely due to the surfactant (LPC).

Other absorption enhancers such as didecanoyl-L-α-phosphatidylcholine (DDPC) and α-cyclodextrin have also been evaluated [52]; when tested in rabbits, GH bioavailability was estimated to be 20%. It was suggested that GH was delivered via the transcellular pathway through damaged ciliated cells. The pharmacokinetics of different doses of GH in GH-deficient patients following intranasal administration using DDPC resulted in bioavailabilities of 3.8–8.9%, with respect to the applied dose [53].

More recently, a new polycarbophil-cysteine (PCP-Cys)/glutathione (GSH) gel was evaluated for the delivery of GH across nasal mucosa *in vitro* and *in vivo* [54]. The permeation of GH from the gel formulation across excised bovine nasal mucosa was improved 3-fold in the presence of PCP-Cys/GSH. The nasal administration of the PCP-Cys/GSH gel to rats resulted in a significantly increased and prolonged GH plasma concentration compared to the unmodified PC gel and physiological saline solution. Microparticulate systems containing PCP-Cys/GSH have also been evaluated for the delivery of GH *in vitro* and *in vivo* using the same animal models [55]; PCP-Cys/GSH/GH and PCP/GH microparticles exhibited similar sustained drug-release profiles. The intranasal administration of GH microparticles formulated with the thiomer resulted in a bioavailability of 8.11% which represented a 3-fold improvement compared to that of PCP/GH microparticles.

Chitosan has also been investigated as an excipient for improving intranasal absorption of GH; a powder blend (Formulation A) and granules (Formulation B) were evaluated in sheep [56]. Fig. 7 shows the serum concentration profiles after intranasal administration (Formulation A, 20 ± 1 mg; Formulation B, 19 ± 1 mg) and s.c. injection (1.7 mg); this corresponded to normalized GH doses of 0.3 and 0.03 mg/kg, respectively. The results for the two intranasal formulations were comparable. However, despite the 10-fold excess in dose, the relative bioavailabilities of Formulations A and B – when compared to s.c. injection – were only 14% and 15%, respectively. Moreover, nasal administration required the insertion of a 5-mm siliconised tracheal tube 6–7 cm into the nasal cavity and the contents emitted using a one-way bellows. Anatomical differences prevented the use of more advanced delivery systems designed for human use. Nevertheless, these seemingly aggressive conditions may have contributed to the results. Given that the daily GH dose for in adults with GH deficiency is 6–12 μg/kg (by s.c. injection), this implies that for a 70-kg adult, the total amount of GH administered daily is ~0.4–0.8 mg [56]. Although nasal delivery has the advantage that it enables pulsatile administration which better mimics endogeneous GH secretion, the dosing requirements pose a considerable challenge.

In summary, surfactant materials and bile salts have been shown to enhance GH bioavailability. However, the enhancing effect is usually correlated to the damage caused to the nasal membrane; although for other enhancers such as cyclodextrins, chitosan and selected phospholipids (e.g., LCP), the absorption enhancing effect is reported to outweigh irritation to the mucosa [49,50,52]. The possibility of delivering therapeutic amounts of GH by the intranasal route will depend on the quantities that can be dosed in a patient-friendly system and the potential nasal and systemic toxicity of the selected enhancer material.

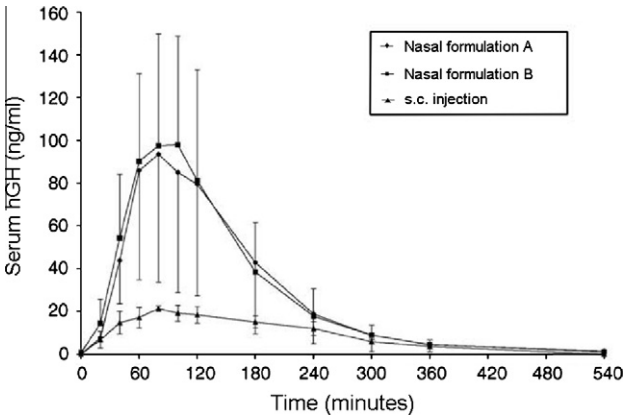


**Fig. 7.** Plasma GH concentration profile following intranasal and subcutaneous administration of GH (mean ± SD, $n = 6$). Reproduced by permission from Cheng et al., Intranasal delivery of recombinant human growth hormone (somatropin) in sheep using chitosan-based powder formulations, Eur. J. Pharm. Sci. 26 (2005) 9–15. Copyright Elsevier B.V. (2005).

### 5.2. Pulmonary delivery

Delivery into the respiratory tract is an interesting alternative for non-invasive administration of peptides and proteins for both local and systemic disorders that require fast onset and sharp pharmacokinetics; it also avoids the GI tract and the hepatic first-pass effect. The lungs contain ~300 million alveoli, each wrapped in a fine mesh of capillaries which allows passage of 5 l of blood per minute and providing a total surface area of absorption of ~70–140 $m^2$. The thickness of the alveolar epithelium (0.1–0.2 mm) permits rapid drug absorption. Pulmonary application of proteins and peptides by aerosols is principally influenced by physiological factors such as the breathing pattern, structure and function of the physiological pulmonary defense mechanisms (e.g., physiological absorption barrier, alveolar macrophages, proteases/peptidases) and specific properties of the biopharmaceuticals (e.g., molecular weight, lipophilicity, solubility in water and lipids) [57–60]. Tight junctions in alveolar type I cells (which occupy 96% of the alveolar epithelium) have a molecular size cutoff of 0.6 nm, while the value in endothelial junctions can range between 4 and 6 nm. It has also been shown that pulmonary alveolar macrophages form the first line of host defense by employing short-lived peroxidases, inflammatory, and immunomodulatory mediators [61,62]. Moreover, the protective mucus blanket which coats the airway epithelium and the surfactant that covers the alveolar epithelium also limit peptide and protein absorption from the lungs [62,63].

Another important parameter that influences pulmonary delivery of aerosol drugs, including proteins and peptides, is the particle properties of the aerosol. The aerosol must be in a size range suitable for delivery to the lungs and the aerodynamic diameter should be optimized (1–3 μm) for deposition in the alveolar region of the lung [64]. Generally, particles with diameter <1 μm are exhaled, and larger particles (>3 μm) are deposited preferentially in the tracheobronchial airways and do not reach the alveolar region.

Following intratracheal instillation of GH in rats, female rats showed higher bioavailability than males; there was also a tendency to saturation as the instilled dose was increased [65]. Colthorpe et al. compared the pulmonary deposition and pharmacokinetics of recombinant GH administered in the form of

an aerosol and instillation in rabbits [66]. The results showed that the peripheral/central deposition ratio tended to be greater for the aerosol than for the instillate. The bioavailable fraction for aerosolized GH was greater than that for instilled GH (45% vs. 16%); this was probably due to mucociliary clearance, as previously suggested for insulin [67]. A dry powder aerosol formulated with lactose and dipalmitoylphosphatidylcholine was assessed for systemic delivery of GH in rats; the powder prepared by spray drying produced particles with a diameter of 4.4 μm. The bioavailabilities following intratracheal insufflation of the dry powder and intratracheal spray-instillation of the GH solution were reported to be 23% and 8%, respectively [68]. More recently, Wolff demonstrated the delivery of GH in humans following inhalation of large porous GH powder particles; the estimated bioavailability was 5–10% [69].

Dry powder GH aerosol containing 100 μg protein and different amounts of dimethyl-β-cyclodextrin (DM-β-CD) to protect against aggregation were tested in rats [70]. The formulations were administered by insufflation through a polyethylene tube inserted into the trachea following incision between the fifth and sixth tracheal rings. Absolute bioavailabilities of 25.4%, 76.5%, and 64.0% were observed from formulations containing GH:M-β-CD at molar ratios of 10, 100, and 1000, respectively.

### 5.3. Transdermal delivery

Transdermal delivery offers several advantages over conventional routes. However, passive diffusion of biomolecules through the lipid-rich stratum corneum is restricted by their size, physicochemical properties, and susceptibility to metabolism by skin enzymes. A number of chemical and physical means to enhance transdermal delivery of peptide and protein therapeutics have been explored [71].

Radio-frequency-induced stratum corneum ablation was used to enhance GH transport in rats and guinea pigs [72]. The ViaDerm system (TransPharma Medical Ltd.) generates an electrical current at high frequency (100–500 kHz) whose passage through resistive elements in contact with the skin creates an array of small microchannels in the epidermis by microablating skin cells (Fig. 8). In the GH study, the device was applied twice on each skin area resulting in a microchannel density of 200 microchannels/cm$^2$. Dry printed patches with increasing GH content (rat: 75–450 μg; guinea pig: 50–400 μg) were then applied for up to 24 h; s.c. injection (100 μg in guinea pigs and 150 μg in rats) was used as the reference. For both animal models, plasma GH levels after stratum corneum ablation showed a dose-dependent increase up to a loading of 300 μg; for rats, at identical doses (150 μg), relative bioavailability was reported as ~75%. In the case of guinea pigs, the corresponding value was ~32% (at a dose of 50 μg). Elevated levels of IGF-1 observed after delivery in hypophysectomized rats confirmed that the GH was bioactive. The Viaderm-GH system using a 5-cm$^2$ dry patch completed Phase 1b human clinical trials but the project was stopped in Phase II.

The feasibility of using self-dissolving micropiles (SDMP) for the transdermal delivery of GH was studied in rats [73]. An aqueous solution of GH (1.4 mg in 80 μl of deionized water) was mixed with 40.2 mg dextran, and the SDMP (weight: 0.68 ± 0.5 mg; length: 3.2 ± 0.5 mm; diameter 0.6 ± 0.2 μm) were prepared by pulling the glue-like mixture with polypropylene tips. The GH-containing SDMP were inserted into the abdominal epidermis (200 μg/kg), and samples were withdrawn for up to 8 h; an i.v. injection (5 μg/kg) was used as reference. The mean maximum GH concentration and $AUC_{0-8}$ following SDMP application were 132.8 ± 11.8 ng/ml and 432.9 ± 25.3 ng h/ml, respectively (cf. 94.8 ± 20.7 ng/ml and 12.4 ± 2.1 ng h/ml after i.v. injection). Taking into account the different doses, the absolute bioavailability of GH following SDMP application was calculated to be 87.5%. Although technical feasibility was shown, the dosing requirements may impact on the practicality of using SDMP, and pharmacokinetic and safety studies in human volunteers need to be performed in order to investigate this further.

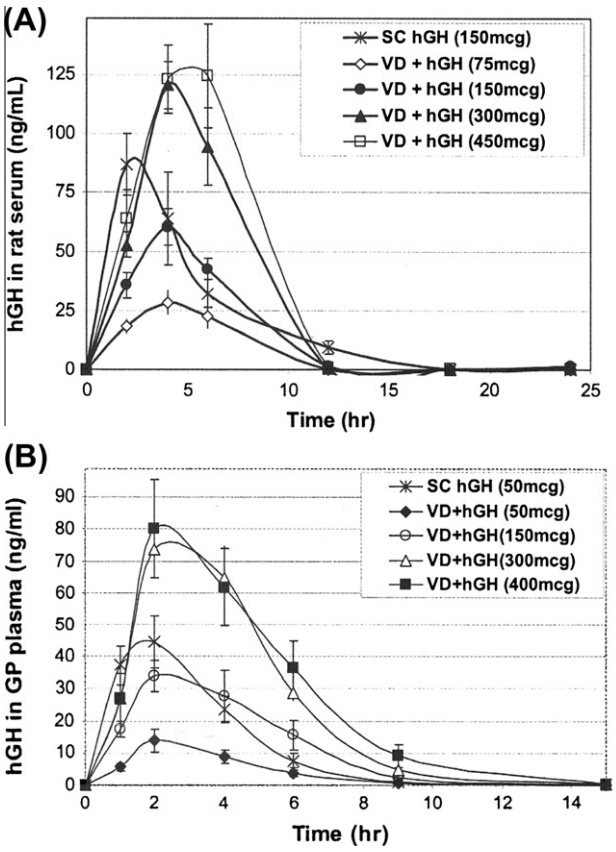


**Fig. 8.** Plasma GH levels after s.c. and transdermal administration of GH. Increasing doses of GH were applied transdermally using ViaDerm device (treated area = 1.4 cm$^2$). (A) Plasma concentration in rats; (B) plasma concentration in guinea pigs (mean ± SD, $n$ = 5–6). Reproduced by permission from Levin G. et al., Transdermal delivery of human growth hormone through RF-microchannels, Pharm. Res. 22 (2005) 550–555. Copyright Springer Science + Business Media, Inc. (2005).

## 6. Concluding remarks

The growing number of therapeutic applications of GH and the drive to provide alternatives to conventional "needle and syringe" delivery has led to the investigation of different technologies and routes of administration. Although the development of jet-injectors has provided a bioequivalent "needle-free" alternative, patients frequently prefer to continue using "needle-based" systems [41], in part due to the problems of local bruising at the application site. However, advances in the technology may reduce these effects in the future. Sustained release GH formulations have been developed, but their complex manufacturing process (aseptic processing and scaling-up) as well as the regulatory concern regarding safety mean that none are commercially available. Studies into non- or minimally invasive delivery using alternative administration routes, e.g., intranasal or pulmonary, have shown that it is technically feasible. However, relative bioavailability is poor, and the use of penetration enhancers for nasal delivery is limited by the risk of damage to sensitive mucous membranes. Pulmonary delivery systems have demonstrated efficacy in animal models; however, the large drug doses needed for achieving high

peak serum concentrations may induce local tissue reactions or systemic side effects. Finally, stratum corneum ablation technologies have proved to be as effective as s.c. administration with minor adverse effects and initial clinical results appear promising; however, the dosing requirements for GH mean that significant quantities of the protein need to be administered and this is a considerable challenge. Furthermore, the practicality of the devices needs to be evaluated on a long-term basis as they are required for treatment of chronic disorders.

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