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Report

The Effect of Sodium Tauro-24,25-Dihydrofusidate on the Nasal Absorption of Human Growth Hormone in Three Animal Models

Patricia A. Baldwin,[1,2] Corine K. Klingbeil,[1] Carla J. Grimm,[1] and John P. Longenecker[1]

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The ability of a novel permeation enhancer, sodium tauro-24,25-dihydrofusidate (STDHF), to increase the systemic delivery of human growth hormone (hGH) after intranasal administration was investigated in rat, rabbit, and sheep. Formulations of hGH with STDHF exhibited greatly enhanced nasal absorption at concentrations of STDHF above its critical micelle concentration. The increase in bioavailability was 11-fold in rats and in rabbits and 21-fold in sheep for formulations containing 0.5% STDHF as compared to those without STDHF. Glycocholate or taurocholate at 0.5% was three to five times less effective than STDHF at enhancing hGH absorption in rats. Additionally, the pulsatile absorption kinetics observed after intranasal delivery more closely resemble the endogenous secretory pattern of hGH than those obtained following subcutaneous administration.

KEY WORDS: nasal delivery; sodium taurodihydrofusidate (STDHF); human growth hormone; bioavailability; absorption enhancer.

INTRODUCTION

The widespread therapeutic use of proteins and peptides is dependent on the availability of a safe, convenient, and noninvasive method of delivery. Administration via the nasal cavity is an attractive alternative to parenteral delivery due to the ease of administration, the highly vascularized mucosal surfaces of the nasal passages, the potential advantages (relative to oral delivery) of no first-pass metabolism, and the rapid absorption kinetics (relative to subcutaneous or intramuscular injection). However, in the absence of an absorption enhancer, intranasal administration of proteins and peptides larger than approximately 10 amino acids generally results in extremely low bioavailabilities, typically in the range of 0 to 3% (1–3).

Recent studies have demonstrated the efficacy of a novel compound, sodium tauro-24,25-dihydrofusidate (STDHF; Fig. 1), in enhancing the intranasal absorption of insulin in both animal studies (4,5) and human clinical trials (6). After intranasal delivery of insulin formulations containing STDHF, high transient serum insulin levels were achieved, closely mimicking the normal postprandial kinetic pattern of pancreatic insulin release. This "pulsatile" kinetic profile observed after intranasal administration may represent a significant therapeutic advantage in the treatment of diseases by hormonal proteins and peptides which are naturally secreted in a pulsatile manner.

Another protein for which nasal delivery may be advantageous is human growth hormone (hGH). The current therapy for children with human growth hormone deficiency is intramuscular (im) or subcutaneous (sc) injection of hGH, two or three times per week. Both methods of delivery result in sustained high levels of the hormone lasting for 12 to 24 hr following injection (7,8). Studies have shown that growth rates improve when the total dose per week is divided into a larger number of injections, resulting in more frequent hGH pulses of shorter duration (8–11). However, increased dosing frequency is unacceptable to most patients. A formulation of hGH which could be administered intranasally might allow more frequent dosing to gain patient acceptance.

Daugherty and co-workers (12) have recently studied intranasal delivery of hGH in an anesthetized rat model using several different surfactants as permeation enhancers. They found that while both polyoxyethylene-9-lauryl ether (Laureth-9) and deoxycholate functioned effectively as permeation enhancers (absolute bioavailabilities of 60–80 and 13–23%, respectively), their use resulted in severe damage to the nasal mucosa. Glycocholate was also studied as an enhancer, and although the toxicity of this compound to the nasal mucosa was much less than that observed for the other surfactants, the bioavailability was much lower (7–8%).

In previous studies with insulin (5), STDHF provided a good combination of low toxicity and high bioavailability. In the work presented here, the ability of STDHF to enhance the intranasal absorption of hGH was determined. Formulations of STDHF with hGH were studied in rat, rabbit, and sheep in order to address the questions of pharmacokinetics following intranasal dosing, bioavailability, dose response, dependence on STDHF concentration, and effect of surfactant structure on delivery.

[1] California Biotechnology Inc., 2450 Bayshore Parkway, Mountain View, California 94043.

[2] To whom correspondence should be addressed.

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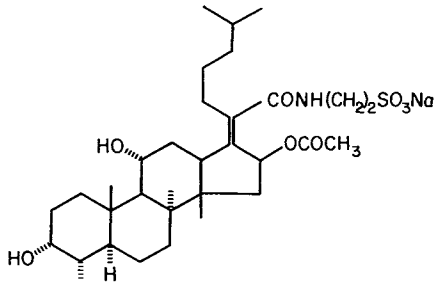

Fig. 1. Structure of sodium tauro-24,25-dihydrofusidate.

MATERIALS AND METHODS

Materials

Human growth hormone was produced using the methods of Friedman and co-workers (13) under contract to California Biotechnology Inc. (Mountain View, CA) by Invitron Corp. (St. Louis, MO). It was purified at the Garvan Institute (Sydney, Australia) and supplied as a lyophilized powder. Sodium tauro-24,25-dihydrofusidate dihydrate (STDHF) and sodium glyco-24,25-dihydrofusidate dihydrate (SGDHF) were obtained from Leo Pharmaceuticals (Ballerup, Denmark). Sodium glycocholate was from Sigma (St. Louis, MO) and sodium taurocholate was from CalBiochem/Behring (La Jolla, CA). All surfactants were of the highest purity available.

Preparation of hGH Solutions

Surfactant solutions at the concentrations (w/v) specified were prepared in 20 m*M* sodium phosphate buffer, pH 7.4. Sufficient hGH was then added to bring the solution to the specified concentration (1 to 5 mg/ml). The solution was gently rocked for approximately 10 min at room temperature until the hGH had dissolved and was then allowed to equilibrate at 4°C overnight prior to use. For experiments where hGH was administered without a permeation enhancer, a different method of solution preparation was employed since the hGH dissolved slowly in the absence of STDHF. The hGH was dissolved at twice the final concentration in 3 m*M* NaOH by gently rocking for 10 min. The sample was then diluted by half to the final concentration with 40 m*M* sodium phosphate buffer, pH 7.4. These samples were also stored at 4°C overnight prior to use. No differences in the chromatographic behavior of hGH prepared in the presence or absence of STDHF could be detected by size-exclusion or reverse-phase HPLC (data not shown). Osmolality of these solutions ranged from 55 mOsm/kg in the absence of STDHF to 69 mOsm/kg for solutions containing 1% STDHF as measured by vapor pressure depression (Wescor, Inc., Logan, Utah).

Administration of hGH to Rats

Male Sprague–Dawley rats (Simonsen, Gilroy, CA, 240–290 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg), with additional doses given intravenously as necessary. The surgical procedure was based on those of Salem (14) and Hirai and co-workers (15,16). A trachea tube was inserted to assist breathing. For delivery to the nasal cavity, a 5-cm cannula (PE 90) was inserted through the esophagus and the nasopharyngeal opening and tied in place, leaving approximately 2.5 cm of the cannula exposed. Intranasal delivery in rats was accomplished using 80 μl of the test solution (hGH concentration ranged from 1 to 5 mg/ml for doses of 0.3 to 1.5 mg/kg) drawn up in a 100-μl Hamilton syringe. After attachment of a 5-cm piece of PE 10 tubing to the syringe, the tubing was inserted completely into the esophageal cannula so that the end was even with the end of the guide cannula and the test solution was injected. Blood samples (0.5 ml) were drawn from a catheter (PE 90) in the right jugular vein and diluted with heparinized saline. Blood volume was replaced with an equivalent volume of phosphate buffered saline.

For comparison, hGH was delivered to some rats directly via the nares. No esophageal cannula was inserted for these experiments; however, a trachea tube was inserted to assist breathing. The test solution (20 μl, 4 mg/ml hGH, 0.3 mg/kg dose) was delivered 1 cm into the nasal cavity using PE 10 tubing attached to a Hamilton syringe. For studies involving parenteral administration of hGH, a jugular vein catheter was inserted as described above for collection of blood samples. Growth hormone (100 μl of 0.8 mg/ml in buffer, 0.3 mg/kg dose) was administered subcutaneously (sc) into the loose skin between the scapulae of the rat, intramuscularly (im) in the thigh, intraperitoneally (ip), or intravenously (iv; 150 μl of 0.533 mg/ml in buffer, 0.03 mg/kg dose) through a catheter inserted into the femoral vein.

Plasma was separated and stored at 4°C for less than 48 hr prior to analysis of growth hormone using an immunoradiometric assay (Tandem-R, Hybritech, Inc., San Diego, CA). Plasma hGH concentrations were corrected for the dilution with heparinized saline. Endogenous rat growth hormone does not cross-react in this assay and the assay does not detect fragments of hGH (12). In addition, the presence of STDHF in the samples does not interfere in this assay.

Administration of hGH to Rabbits

New Zealand male rabbits (R & R Rabbitry, Stanwood, WA, 3.0 to 4.0 kg) were fasted overnight and weighed on the morning of the experiment. Animals were anesthetized by im administration of xylazine (4 mg/kg) followed by ketamine (25 mg/kg). A 22 g × 1.25-in. Teflon IV catheter (Abbocath, Chicago, IL) was inserted into the medial artery of the ear and taped into place for collection of blood samples. Intranasal administration of a solution of 5 mg/ml hGH in 0.5% STDHF was accomplished using a Hamilton syringe with 3 cm of PE 10 tubing attached. The hGH formulation was delivered 1.5 to 2 cm into the nares while the rabbit was lying on its sternum with its head elevated 5.5 cm. The dose was 0.1 mg/kg; one-half of the dose (~40 μl) was delivered to each nostril.

Blood samples were allowed to clot at 4°C overnight in borosilicate glass tubes and the serum was removed and analyzed for hGH within 48 hr as described above. We have not observed any cross-reactivity of endogenous rabbit growth hormone with this assay.

Administration of hGH to Sheep

Delivery of hGH to sheep and collection of blood sam-

ples was accomplished using a previously described method (5). Formulations containing 10 mg/ml hGH with or without 0.5% STDHF were delivered 7 cm into the ventral nasal meatus using a total volume of 10 μl per kg divided equally between the nostrils (0.1 mg/kg dose). Blood samples were allowed to clot in borosilicate glass tubes and the serum was separated and stored at −20°C for later analysis of hGH by immunoradiometric assay as described above. No cross-reactivity with endogenous sheep growth hormone has been observed with this assay.

Analysis of Data

Differences between experimental groups were assessed by a two-tailed *t* test. Estimations of area under the curve (AUC) of plots of plasma hGH concentration versus time were calculated using the trapezoidal rule. AUCs were not extrapolated beyond the time course of the experiment.

RESULTS AND DISCUSSION

Nasal Absorption of hGH in Rats

In order to assess the efficacy of STDHF as an enhancer of hGH absorption after intranasal administration, anesthetized rats were used as a model system. The plasma hGH concentrations observed as a function of time after iv bolus injection, sc injection, or intranasal delivery through the nasopharyngeal opening using 0.5% STDHF as an absorption enhancer are shown in Fig. 2. As expected, the plasma levels decline rapidly after a bolus iv injection but remain elevated at a nearly constant level for more than 2 hr after subcutaneous injection. Nasal delivery of hGH with 0.5% STDHF results in peak hGH levels between 20 and 30 min after administration, followed by a decline in plasma levels over the next 2 hr.

The bioavailability relative to the iv dose for several different routes of administration in the rat model is shown in Table I. For hGH injected im, ip, or sc, the bioavailability is in the range of 22–26%. For hGH in 0.5% STDHF administered in an 80-μl dose volume via the nasopharyngeal route, the bioavailability is 32%. However, for all delivery routes other than iv, hGH levels have not fallen to zero at 150 min (the last time point taken; see Fig. 2). Since the hGH plasma levels decline much more rapidly after intranasal administration than after im, ip, or sc dosing, the total AUC is likely to be greater for the parenteral routes than for the nasal route.

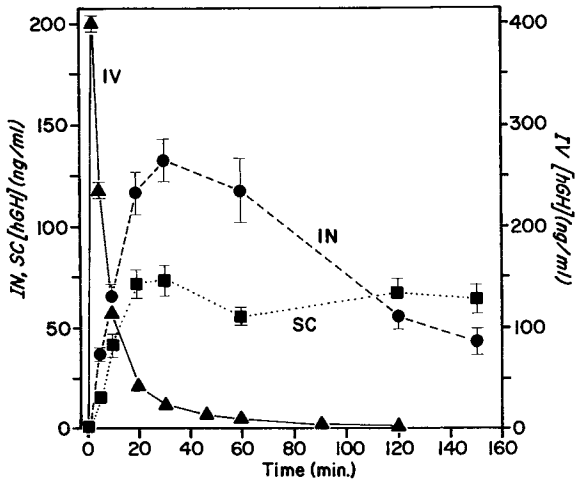

Fig. 2. Kinetics of hGH absorption in rats following iv (▲), sc (■), or intranasal (●) dosing. The iv (0.03 mg/kg; $N = 8$) and sc (0.3 mg/kg; $N = 6$) doses were administered as solutions of hGH in buffer. For the intranasal dose (0.3 mg/kg; $N = 20$), 80 μl of a formulation of 0.1% hGH in 0.5% STDHF was administered through the nasopharyngeal opening.

Administration of hGH in 0.5% STDHF directly into the nasal cavity through the nares in a volume of 20 μl results in a bioavailability that is approximately one-half that observed for administration via the nasopharyngeal opening (Table I). This may be due to the smaller volume of administration or to more efficient ciliary clearance of the protein from the nasal cavity following administration via the nares in the absence of an esophageal cannula.

A dose/response curve in the rat model obtained by increasing the hGH concentration in a constant administration volume is shown in Fig. 3. For all doses, the STDHF concentration was 0.5% and the dose was administered via the nasopharyngeal opening in a volume of 80 μl. Since the dose/response curve is linear between 0 and 1.5 mg/kg, the bioavailability does not change as a function of dose within this range. A similar result was observed by Daugherty and coworkers (12), who found that nasal delivery of hGH resulted in a bioavailability that was independent of dose when glycocholate was used as a permeation enhancer. However, delivery of hGH across other mucosal surfaces [e.g., rectal (17)] and delivery of other polypeptides via the nasal route [e.g., nafarelin acetate (18)] have resulted in nonlinear dose–response curves with increasing bioavailability at higher drug concentrations. The nonlinearity has been ascribed to the increased activity of the drug at the higher concentration or to saturable binding and/or metabolism by proteolytic enzymes (18). Since the dose/response curve for nasally administered hGH is linear, these do not appear to be important factors affecting the nasal delivery of hGH using STDHF within this dose range.

The effect of changes in STDHF concentration on the bioavailability of hGH delivered to rats is demonstrated in Fig. 4. Delivery is negligible in the absence of the permeation enhancer and is also poor at 0.1% which is below the critical micelle concentration (CMC) of STDHF [0.16% (19,20)]. Above the CMC at 0.3% STDHF, the AUC is increased approximately fivefold. Apparently, the optimal STDHF concentration is at or slightly above the CMC. Although the

Table I. Bioavailability of hGH in Rats

Formulation & delivery route	Dose (mg/kg)	% bioavailability (±SE)	*N*
hGH in buffer, iv injection	0.03	100	8
hGH in buffer, im injection	0.3	23.4 (±1.1)	6
hGH in buffer, ip injection	0.3	25.4 (±3.8)	3
hGH in buffer, sc injection	0.3	22.0 (±2.2)	6
hGH in 0.5% STDHF, nasopharyngeal opening	0.3	31.7 (±3.5)	20
hGH in 0.5% STDHF, nares	0.3	17.2 (±4.3)	6

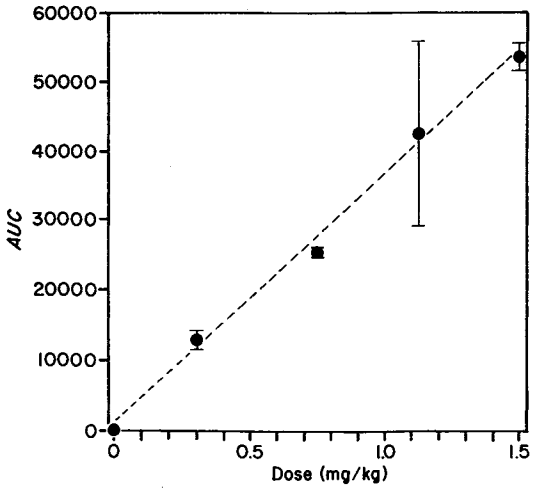

Fig. 3. Area under the curve (AUC; ng * min/ml) as a function of hGH dose in rats. Dose was altered by varying the concentration of hGH in a 0.5% solution of STDHF. Data are displayed as mean ± SE of results in 3 to 20 rats. $R^2 = 0.995$ for the mean values.

mechanism of STDHF-enhanced hGH absorption is not fully understood, the results presented here suggest two alternative hypotheses. One possibility is that the presence of at least a small number of STDHF micelles is important for delivery across the mucous membrane. Alternatively, monomeric STDHF may be the form responsible for permeation enhancement and the greatest concentration of monomeric STDHF is achieved at or slightly above the CMC.

The observation that intranasal absorption of hGH *decreases* as the concentration of STDHF is increased from 0.3 to 1.0% ($P < 0.05$ for difference) suggests that gross alterations in the mucosal membrane are not responsible for the enhancement of hGH absorption by STDHF, since greater changes in membrane morphology are observed at higher STDHF concentrations (21,22). In addition, although it is important to be at or slightly above the CMC for optimal delivery, an increase in the number of STDHF micelles beyond a certain level (in this case 0.3%) does not aid delivery.

The effect of surfactant structure on the intranasal delivery of hGH is shown in Fig. 5, where the AUC values of the plasma hGH versus time curves for the taurine and glycine derivatives of dihydrofusidic acid and cholic acid are compared. Both glycocholate and taurocholate have been used clinically for the delivery of insulin (23) and glycocholate has recently been shown to enhance absorption of recombinant methionyl-hGH (12). These data show that the dihydrofusidate derivatives are three to five times more effective than the analogous trihydroxy bile salts as permeation enhancers for nasally delivered hGH ($P < 0.001$). Interestingly, there is no difference between the glycine and the taurine conjugates for either steroid nucleus ($P < 0.05$). This suggests that the structure of the steroid nucleus and not the conjugated side chain is the critical factor in the enhancement mechanism.

Comparison of Nasal hGH Absorption Between Species

Due to the small body weight and limited possible volume of delivery in the rat model, it is not possible to formulate hGH at the concentrations and the hGH:STDHF ratios that will be used in clinical trials. In order to study formulations which more closely approximate those which might be used in humans, we studied the intranasal absorption of hGH with 0.5% STDHF in rabbits and sheep. Figure 6 compares the kinetic profiles observed after intranasal administration of hGH to sheep, rabbits, and rats (via both routes of administration). The kinetic profiles observed in sheep, rabbit, and rat (via the nares) are quite similar and the AUCs for these three dose-corrected curves are not statistically different ($P < 0.05$). The curve for the administration to the rat via the nasopharyngeal route has a significantly higher C_{max} and AUC as discussed above. In all three species, intranasal administration using STDHF as a permeation enhancer results in a "pulsatile" pharmacokinetic profile, i.e., rapid absorption followed by rapid clearance of hGH.

The AUCs obtained for hGH delivered in the presence and absence of 0.5% STDHF for all three species are compared in Table II. For all species, the delivery in the absence

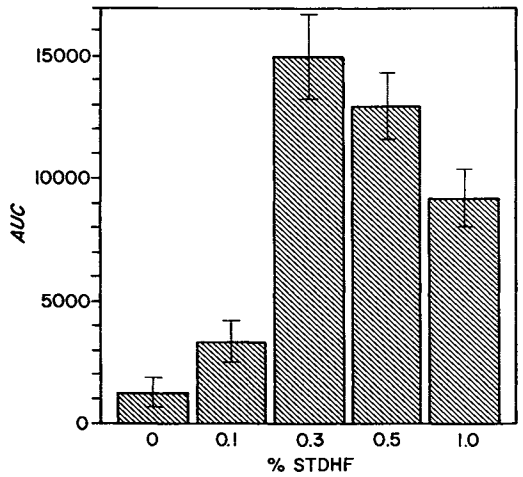

Fig. 4. AUC as a function of STDHF concentration (w/v) in hGH/STDHF formulations. The in dose was 0.3 mg/kg. Data are displayed as mean ± SE for a minimum of five rats.

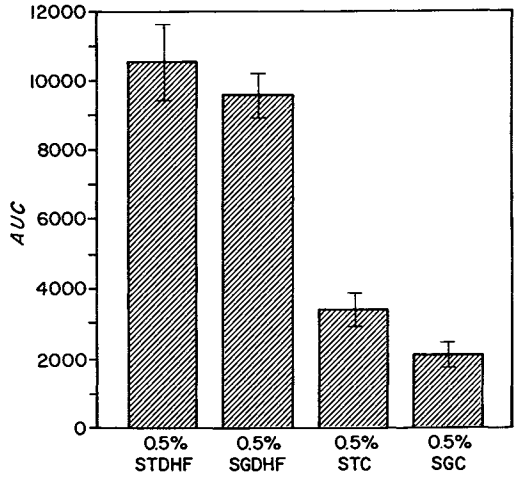

Fig. 5. AUC as a function of surfactant in formulations containing 0.1% hGH and 0.5% surfactant. Data are displayed as mean ± SE for six rats per experiment.

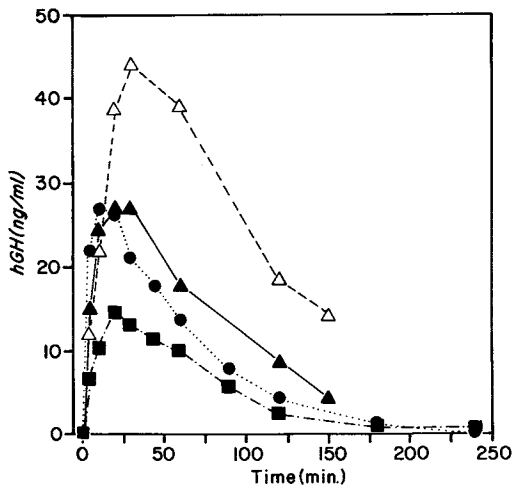

Fig. 6. Kinetics of hGH absorption following intranasal delivery in 0.5% STDHF to sheep (■), rabbit (●), and rat. For the rat, kinetics are shown for delivery via the nares (▲) and for delivery via the nasopharyngeal opening (△). Data points for the rat data were adjusted to a dose of 0.1 mg/kg based on the linear dose–response curve.

of a permeation enhancer is quite low. STDHF significantly improves the delivery of hGH with an 11-fold enhancement in rat and rabbit and a 21-fold enhancement in sheep.

The transitory elevation of serum hGH levels observed following intranasal dosing more closely resembles the pharmacokinetics observed for endogenous hGH secretion than does the profile observed after sc dosing. Normal secretion of hGH occurs in pulses resulting in elevated plasma levels lasting for about 2 hr (24,25). Although the importance of the episodic secretory pattern of hGH is not well understood, mimicking this pattern may be more effective in stimulating growth than the sustained hGH blood levels which result from the normal subcutaneous therapy. Recently, Clark and co-workers (26) have shown that iv pulsatile dosing resulting in transient GH levels is more effective at producing growth in hypophysectomized rats than the same dosing pattern given subcutaneously or than a continuous infusion of GH. In addition, the response to a continuous GH infusion declines over time, whereas pulsatile GH remains effective. One possible explanation of these data is that GH receptors are eventually down-regulated by continuous exposure to GH, but down-regulation does not occur with intermittent exposure. Many studies of growth rates in humans during hGH therapy for pituitary dwarfism also demonstrate that growth rates decline after several years of treatment with hGH administered two or three times weekly by the sc or im routes (9,10). The pulsatile administration of hGH achieved by the intranasal route may prevent this decline in efficacy of the treatment. Since it is not clear whether the important parameters for maximal response in patients are the total amount of bioavailable hGH, the peak serum level, the frequency and timing of administration, or a combination of these factors, an analysis of the optimal therapeutic dose level and dosing schedule by the intranasal route must await the results of clinical studies in growth hormone-deficient children.

Table II. Effect of 0.5% STDHF on hGH Delivery in Different Species

Animal	N	Dose (mg/kg)	AUC[a] with STDHF	AUC without STDHF	Increase in delivery
Rat[b]	14	0.3	13227 (±1260)	1257 (±507)	11-fold
Rabbit	8	0.1	1948 (±405)	182 (±29)	11-fold
Sheep	6	0.1	1240 (±142)	58 (±15)	21-fold

[a] AUC = area under the serum level vs time curve (ng * min/ml) ± SE.
[b] Nasopharyngeal model.

CONCLUSIONS

These data show that the fusidate derivative, STDHF, can enhance absorption of hGH across the nasal mucosa. The pulsatile kinetics observed following intranasal administration resemble the kinetic profile of hGH levels observed following endogenous secretion and, as a result, may offer an improved therapy. Although the mechanism of STDHF-enhanced absorption is not understood, an STDHF concentration at or slightly above the CMC is apparently required for optimal delivery of hGH. The bioavailability of hGH is three- to fivefold greater when the permeation enhancer is either the taurine or the glycine derivative of fusidate (STDHF or SGDHF) as compared to the similar cholate derivatives (STC or SGC). The work presented here suggests that intranasal hGH administration with STDHF may be a viable alternative to existing parenteral therapies.

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