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Albutropin: a growth hormone–albumin fusion with improved pharmacokinetics and pharmacodynamics in rats and monkeys

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Abstract

Growth hormone (GH) replacement therapy is used to treat GH deficiency. Treatment requires daily administration because of the short plasma $t_{1/2}$ of GH. Albutropin, human GH fused at its N-terminus with human serum albumin, should be cleared from the circulation more slowly than GH. Pharmacokinetic and pharmacodynamic studies of albutropin were conducted in rats and monkeys. After subcutaneous (s.c.) dosing in rats, a twofold decrease in clearance and a fourfold increase in plasma half-life were seen with albutropin compared to GH. In monkeys, s.c. administered albutropin (0.3 mg/kg) had a sixfold longer terminal half-life and an eightfold slower clearance than GH (0.3 mg/kg). A single subcutaneous administration of albutropin (0.3, 1.5 and 4 mg/kg) increased plasma insulin-like growth factor 1 (IGF-1) levels for up to 7 days. Seven consecutive daily s.c. injections of GH at 0.3 mg/kg resulted in an increase in IGF-1 equivalent to that induced by a single administration of albutropin at 4 mg/kg. Albutropin (1–20 μg/kg) dosed daily, every other day or every 4 days significantly increased cumulative body weight gain and tibial epiphyseal growth plate width in hypophysectomized rats compared to equimolar doses of GH. These results suggest that albutropin could be given less frequently than GH and achieve therapeutic effects in patients.

Keywords: Growth hormone; Pharmacokinetic; (Monkey); IGF-1 (insulin-like-growth-factor)

1. Introduction

Human growth hormone (GH) is a 191-amino-acid pituitary protein that stimulates the production and release of insulin-like growth factor-1 (IGF-1) into the systemic circulation. IGF-1 is instrumental in the promotion of linear growth in children, and in the control of metabolism in adults. It is regulated through complex feedback mechanisms involving GH, insulin-like growth factor-1 binding protein 3 (IGF-BP3) and their complexes. A deficiency of GH results in inadequate circulating IGF-1 levels and is manifested as abnormal linear growth in children. In adults, this deficiency results in decreased lean body mass, reductions in exercise tolerance, muscle mass/strength, cardiac performance, bone density and neuropsychological disturbances (Baskin et al., 1998).

Currently, GH supplementation is approved for the treatment of adult and pediatric GH deficiency, adult acquired immune deficiency syndrome (AIDS)-associated wasting, Turner syndrome and chronic renal insufficiency. Clinical investigations exploring GH as a treatment for idiopathic short stature, constitutional delay of growth and development, children who are small for gestational age, skeletal dysplasias and other diseases are ongoing (Baskin et al., 1998; Vance and Mauras, 1999).

The majority of the GH preparations currently available require daily administration; hence, compliance can be a problem, especially in adolescents. In adult GH deficiency, daily administration and concomitant side effects (e.g. injection site discomfort, transient edema and arthralgia) limit the therapeutic utility of existing formulations. A long-acting form of GH has the potential to reduce discomfort by requiring fewer injections and possibly by minimizing the adverse events associated with peaks and troughs in plasma concentration that occur with daily injection.

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Two strategies to create a long-acting form have been explored. The first approach is to modify the formulation of the GH to allow the product to be slowly released from the injection site over a period of time. This sustained release form requires fewer injections; however, the active agent is not changed, so the elimination of GH remains unchanged and the dose of GH must be increased to cover the longer dosing interval. One sustained release form of GH has been approved for use in GH-deficient children. Marketed as Nutropin Depot®, it is GH encapsulated in poly(lactide-coglycolide) microspheres. Twice monthly administration is recommended and the drug needs to be administered in multiple injection sites if the patient exceeds 30 kg (Physicians Desk Reference, 2002). A 2-year trial in GH-deficient children has shown that growth rates are significantly ($p < 0.001$) lower in children given Nutropin Depot® than in children given daily GH (Silverman et al., 2002). Nutropin Depot® is not currently approved for use in adults.

The second approach is to modify the GH molecule itself, so that the active drug is cleared more slowly from the systemic circulation. This would improve utility and compliance by requiring less frequent administration, but would not require injection of the large mass of drug required for a sustained release formulation. By avoiding the peaks and troughs of daily GH administration, it is also possible that a long-acting GH would have an improved side effect profile compared to the current formulations requiring daily subcutaneous (s.c.) injections.

A fusion protein therapeutic, albutropin, derived from the joining of human serum albumin and human GH genes has been developed. The recombinant fusion product is produced in *Saccharomyces cerevisiae*. By fusing GH to human serum albumin, which is cleared slowly from the plasma (elimination $t_{1/2}$ of 19 days; Peters, 1996), albutropin may achieve a comparable or improved pharmacologic profile of GH with decreased dosing frequency. Although such a long-acting GH product whose plasma levels are sustained may not mimic physiologic GH release, there is functional evidence from both GH-deficient rats (Gargosky et al., 1994) and in human studies (Laursen et al., 1994, 2000) that intermittent and continuous administration of GH result in equivalent formation of the IGF-1/IGF-1BP3/acid labile subunit ternary complex, which regulates the concentration of free IGF-1. In adults, continuous administration of GH for 6 months resulted in changes in the IGF–IGFBP3 axis that were comparable to daily administration. Insulin sensitivity and lipoprotein analysis indicated that continuous and intermittent GH exposure had similar safety profiles (Laursen et al., 2001). Additionally, the approved sustained release formulation of GH does promote growth in GH-deficient children (Reiter et al., 2001; Silverman et al., 2002).

This paper describes preclinical pharmacokinetic and pharmacodynamic profiles of albutropin in rat and monkey studies. Pharmacokinetic measurements were taken to evaluate the pharmacokinetic profile of albutropin and GH following single i.v. or s.c. doses in cynomolgus monkeys and rats. The pharmacodynamic studies were conducted to compare the effects of a single administration of albutropin with seven consecutive daily injections of GH on IGF-1 induction in cynomolgus monkeys. In rats, the growth-promoting effects of intermittent injections of albutropin were compared to daily GH injections in young, hypophysectomized rats. The results from these studies provide a foundation for the use of intermittent dosing of albutropin for the treatment of GH-deficient children and adults.

2. Methods and materials

2.1. Preparation and purification of albutropin

Albutropin fusion protein (molecular weight approximately 89 kDa) was produced as a recombinant protein composed of recombinant human serum albumin genetically fused at its C-terminus to the N-terminus of recombinant GH. The yeast strain used for the production of albutropin (strain BXP10) was a genetically modified form of the yeast *S. cerevisiae* laboratory strain AH22 that has been optimized for the production of recombinant human serum albumin with minimal post-transcriptional modification. Albutropin was produced using the modified yeast strain in a fermenter. The supernatants from multiple fermentations were pooled and purified. The composition of the final purified albutropin product was verified by N-terminal sequencing and ELISA. Levels of bioburden, endotoxin and residual yeast DNA were within limits considered acceptable for administration to humans.

For pharmacokinetic studies in rats, recombinant human GH was obtained from National Institute of Biological Standards and Controls (NIBSC) at an initial concentration of 1.95 mg/ml. It was diluted with phosphate-buffered saline to 200 μg/ml for the s.c. and to 40 μg/ml for the i.v. studies. For the pharmacodynamic studies, lyophilized recombinant GH (2.6 IU/mg) was obtained from Accurate Chemical and Scientific Corp. Albutropin was initially prepared as a 1.0 mg/ml solution in phosphate-buffered saline and used undiluted for s.c. dosing. It was diluted to a final stock concentration of 0.2 mg/ml for i.v. dosing. Recombinant human serum albumin (Sigma) was prepared at a final concentration of 0.2 mg/ml. For the rat pharmacodynamic studies, all s.c. test solutions were given at a volume of 0.1 ml/rat.

For monkey pharmacokinetic/IGF-1 studies, recombinant human growth hormone (GH, Genotropin®) was purchased from Pharmacia (Lot No. 36028A51, 3 IU/mg). Stock solutions of GH were prepared using the supplied diluent and stored refrigerated for the 7-day duration of the dosing period. Albutropin was supplied as a 3.8 mg/ml solution and was administered at concen-

Table 1
Experimental design of rat pharmacokinetic study

Drug	Dose (μg/kg)	Route, *N*	PK sampling schedule
Group 1 albutropin	800	s.c., 4	2, 5, 8, 12, 24 and 30 h
Group 2 albutropin	100	i.v., 8	15, 60, 90 min, 2, 3, 5, 12 and 24 h
Group 3 GH	100	s.c., 3	20 min, 1, 2, 3, 4, 6 and 8 h
Group 4 GH	20	i.v., 3	20 min, 1, 2, 3, 4, 6 and 8 h

trations between 0.6 and 3.8 mg/ml to achieve the doses of 0.3, 1.5 and 4.0 mg/kg.

2.2. Pharmacokinetic and pharmacodynamic studies in Sprague–Dawley rats and cynomolgus monkeys

2.2.1. Pharmacokinetic studies

Single-dose pharmacokinetic studies were conducted in Sprague–Dawley rats and cynomolgus monkeys. Pharmacodynamic studies were conducted using multiple-dose schedules in rats and a single-dose schedule in monkeys. All animal experimentation was conducted in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and under the supervision and approval of the Institutional Animal Care and Use Committees of Human Genome Sciences, Covance Laboratories, and Hilltop Lab Animals. Rats were housed either individually or two per cage in rooms with a 12-h light/dark cycle. Access to water (municipal supply) and noncertified rodent chow was provided ad libitum. Monkey studies were conducted in accordance with Covance Standard Operating Procedures and with generally recognized good scientific practices. Monkeys were individually housed with environmental controls for the animal room set to maintain a temperature of 18–29 °C and a 12-h light/dark cycle. Noncertified primate diet was provided ad libitum. Certified primate treats, fruit or other appropriate treats were provided. Tap water was available to the monkeys from a well supply ad libitum.

To compare the pharmacokinetics of albutropin and GH in rats, four groups of Sprague–Dawley rats (270–290 g), three to eight female rats per group, were obtained with surgically implanted indwelling jugular vein and carotid artery catheters from Hilltop Lab Animals. The rats were randomly assigned to four treatment groups (see Table 1). Rats were administered a single s.c. or i.v. injection of GH (20 μg/kg i.v. or 100 μg/kg s.c.) or albutropin (100 μg/kg i.v. or 800 μg/kg s.c.). With the exception of the predose sample, which was collected under isoflurane anesthesia, blood collection was performed in unanesthetized animals. Blood samples (approximately 0.25 ml) were collected in EDTA-coated microtainers for ELISA analyses of albutropin plasma concentration at the times outlined in Table 1. After each sampling, the blood volume was replaced with an equal volume of sterile 0.9% saline. Samples were stored on wet ice for up to 1 h prior to centrifugation and plasma

Table 2
Treatment schedule for monkey pharmacokinetic/pharmacodynamic study

Group	Treatment	Dose (mg/kg)	Route, number of monkeys (male/female)	PK sampling schedule	Plasma IGF-1 sampling schedule
1	Vehicle	0	s.c., 2/2	Prior to dosing and at 0.5, 6, 12, 24, 48, 72, 96 and 120 h after dosing	Prior to dosing and at 6, 12, 24, 48, 72, 96, 120, 144 and 216 h after dosing
2	albutropin	4	i.v., 2/2	Prior to dosing and at 5 min, 1, 6, 12, 18, 24, 30, 36 and 48 h after dosing	Prior to dosing and at 6, 12, 24, 48, 72, 96, 120, 144 and 216 h after dosing
3	albutropin	4	s.c., 2/2	Prior to dosing and at 0.5, 6, 12, 24, 48, 72, 96 and 120 h after dosing	Prior to dosing and at 6, 12, 24, 48, 72, 96, 120, 144 and 216 h after dosing
4	albutropin	1.5	s.c., 2/2	Prior to dosing and at 0.5, 6, 12, 24, 48, 72, 96 and 120 h after dosing	Prior to dosing and at 6, 12, 24, 48, 72, 96, 120, 144 and 216 h after dosing
5	albutropin	0.3	s.c., 2/2	Prior to dosing and at 0.5, 6, 12, 24, 48, 72, 96 and 120 h after dosing	Prior to dosing and at 6, 12, 24, 48, 72, 96, 120, 144 and 216 h after dosing
6	GH	0.3 (for 7 days)	s.c., 2/2	Prior to dosing and at 0.5, 1, 2, 4, 8, 12, 18 and 24 h after the first dose; 0.5, 1, 2, 4, 8, 12, 18, 24, 36, 48, 60 and 72 h after the last dose	Prior to dosing and at 6, 12, 24, 48, 72, 96, 120, 144, 168, 192 and 216 h after dosing

Table 3
Treatment schedule for hypophysectomized rat study

Group	Treatment	Number of rats	Treatment schedule (days)	Route	Dose level (μg/rat)	Dose volume (ml)
1	vehicle	10	daily, days 1–13	s.c.	NA	0.1
2	human serum albumin	10	daily, days 1–13	s.c.	20	0.1
3	albutropin	10	daily, days 1–13	s.c.	20	0.1
4	albutropin	10	QOD, 1, 3, 5, 7, 9, 11, 13	s.c.	20	0.1
5	albutropin	10	Q4D, 1, 5, 9, 13	s.c.	20	0.1
6	GH	10	daily, days 1–13	s.c.	5	0.1
7	GH	10	QOD, 1, 3, 5, 7, 9, 11, 13	s.c.	5	0.1
8	GH	10	Q4D, 1, 5, 9, 13	s.c.	5	0.1
9	albutropin (boiled)	10	daily, days 1–13	s.c.	5	0.1
10	sham hypophysectomy/vehicle	10	daily, days 1–13	s.c.	NA	0.1

harvest. Plasma samples were stored at approximately −20 °C prior to analysis.

Twenty-four cynomolgus monkeys (Covance Research Products, Alice, TX) were assigned to one of six treatment groups (two males/two females per group, Table 2). These groups received a single s.c. injection of either vehicle or albutropin (dose levels from 0.3 to 4.0 mg/kg) or albutropin administered i.v. at a dose of 4.0 mg/kg. A separate group of monkeys received daily s.c. injections of GH (0.3 mg/kg) for 7 days.

Venous blood samples (approximately 1 ml) were collected into EDTA-containing microtubes for assay of plasma albutropin, GH and monkey IGF-1 (i.e. monkey pharmacodynamic arm of the study) by ELISA as described in Table 2. Samples were stored on ice for up to 1 h prior to centrifugation and plasma harvest. Plasma samples were then stored at approximately −80 °C.

A commercial sandwich ELISA kit specific for detection of human growth hormone (BioClin) was used for evaluation of the rat plasma samples. This kit detects human growth hormone in plasma by means of an antibody sandwich ELISA format. This kit was initially used to measure the concentration of albutropin in rat plasma. For these plasma samples, an albutropin standard curve (1.2–100 ng/ml) was used and the concentrations of albutropin in rat plasma were interpolated from this curve.

A more specific ELISA format was used for the detection of albutropin in monkey plasma. Instead of using the anti-GH detector antibody supplied in the BioClin kit, an anti-human serum albumin detector antibody was substituted as the detector antibody. By using the BioClin microplate, and the anti-human serum albumin detector antibody, and an albutropin standard curve, the concentrations specifically reflect the concentration of albutropin in plasma samples from the monkeys. The concentration of albutropin in the plasma (ng albutropin per ml of plasma) was interpolated from an albutropin standard curve ranging from 0.625 to 40 ng/ml albutropin.

Standard pharmacokinetic parameters, including clearance (CL or CL/F), volume of distribution (Vd or Vd/F), half-life ($t_{1/2}$), area under the plasma concentration versus time curve (AUC), maximal observed plasma concentration (C_{max}) and time to maximal observed plasma concentration (T_{max}), were obtained from plasma albutropin or GH concentration/time curves by noncompartmental analysis using the modeling program WinNonlin (Pharsight, version 3.1). Plasma albutropin or GH concentration data were uniformly weighted for this analysis. The AUC was calculated using the log-linear trapezoidal analysis for the i.v. data and the linear-up/log-down trapezoidal method for the s.c. data. Plasma concentration profiles for each rat (with the exception of the s.c. albutropin data) or monkey were analyzed individually, and mean ± standard error of the mean (S.E.M.) values for the pharmacokinetic parameters are reported in Tables 4–6. A compartmental analysis was conducted on the data from monkeys given s.c. albutropin or GH and the data from the rat given s.c. albutropin data in order to determine the absorption half-life following s.c. dosing. The albutropin and GH plasma data following s.c. dosing were fit to a one-compartment model using first-order input and output. Data were weighted as the reciprocal of the square of the predicted plasma concentration ($1/C_{pred}^2$) for this analysis. There were insufficient data available for the determination of absorption half-life from the rats dosed s.c. with GH.

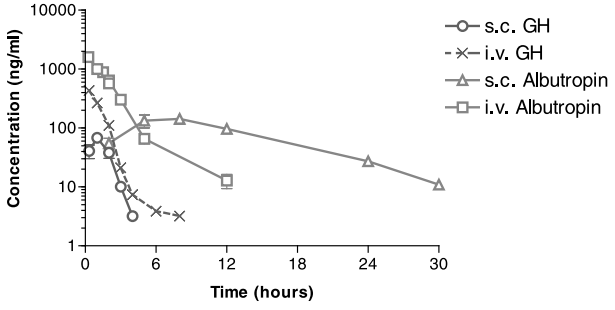

Fig. 1. Mean (± S.E.M.) plasma albutropin or GH concentrations (ng/ml) following a single i.v. or s.c. dose of albutropin or s.c. GH in rats ($n = 3–8$ per dose/route).

Table 4
Mean (± S.E.M.) pharmacokinetic parameters following single-dose i.v. or s.c. administration of albutropin and GH in Sprague–Dawley rats

Parameter		Estimate[a]	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
		Drug						
		Albutropin			GH			
Dose		100 μg/kg i.v.	800 μg/kg s.c.	800 μg/kg s.c.	20 μg/kg i.v.	20 μg/kg i.v.	100 μg/kg s.c.	100 μg/kg s.c.
AUC	h μg/ml	3.27	2.07	0.19	0.67	0.03	0.12	0.01
AUC/dose	(h μg/ml)/μg/kg)	3.3×10^{-2}	2.6×10^{-3}	2.4×10^{-4}	3.4×10^{-2}	1.5×10^{-3}	1.2×10^{-3}	1.0×10^{-4}
CL or CL/F	ml/h/kg	30.61	397.48	41.13	30.14	1.46	820.91	94.15
C_{max}	μg/ml	1.88	0.18	0.02	0.56	0.06	0.07	0.01
C_{max}/dose	(μg/ml)/μg/kg)	1.9×10^{-2}	2.3×10^{-4}	2.5×10^{-5}	2.8×10^{-2}	3.0×10^{-3}	7.0×10^{-4}	1.0×10^{-4}
MRT	h	2.05	12.55	0.39	1.3	0.02	1.53	0.08
Absorption $t_{1/2}$	h	NA	4.21	0.16				
Terminal $t_{1/2}$	h	2.96	5.93	0.36	3.09	0.05	0.66	0.08
T_{max}	h	0	6.5	0.87	0	0	1	0
Vz or Vz/F	ml/kg	130.88	3396.53	363.27	134.4	8.5	776.58	100.73
Vss	ml/kg	62.82						
Bioavailability	%	NA	7.9		NA		3.6	

Parameters were generated for individual rats, and the mean (± S.E.M.) data are presented here.
[a] For the 100 μg/kg i.v. dose only, complete curves were not obtained from individual rats; therefore, mean data were analyzed for this dose group.

2.2.2. Pharmacodynamic studies

Hypophysectomized (interaural method) or sham-operated Sprague–Dawley male rats, 3–4 weeks of age, were obtained from Taconic Laboratories. During a post-surgical acclimation period of 3 weeks, rats were examined and weighed twice weekly to eliminate animals deemed to have incomplete hypophysectomy evidenced by weight gain similar to that of sham-operated rats. Those rats with incomplete hypophysectomized were eliminated from the study. The average body weights of the hypophysectomized and sham rats were 70 and 150 g, respectively, at the time of the experiment.

Rats were injected s.c. with albutropin, vehicle, human serum albumin or inactivated albutropin (boiled) once daily for 13 consecutive days (days 1–13; QD), every other day (days 1, 3, 5, 7, 9, 11 and 13; QOD) or every fourth day (days 1, 5, 9 and 13; Q4D). Additional groups of rats received GH by s.c. injection once daily for 13 days, every other day or every fourth day. Albutropin or human serum albumin (20 μg/rat) was administered s.c. in an injection volume of 0.1 ml/rat. The dose of GH was 5 μg/rat, a dose of growth hormone that was equimolar with the amount of GH in a corresponding 20 μg/rat dose of albutropin. The treatment groups are summarized in Table 3. Following injection, rats were weighed daily for up to 14 days. Rats were euthanized on day 14 (24 h after the final dose) by overexposure to CO_2. The right tibia was harvested, fixed, decalcified and processed for histological examination of the epiphyseal growth plate (toluidine blue staining of sections taken midway through the thickness of the tibia). The average tibial epiphyseal growth plate area (± S.E.M., reported in mm^2) was determined from the epiphyseal growth plate area of five tibial sections using computerized morphometry (IPLab Spectrum).

Plasma samples for IGF-1 analyses were obtained at the times described in Table 2. Samples were analyzed for IGF-1 concentration using an ELISA developed by Nichols Research Laboratories and implemented by Quest Diagnostics (Nichols Institute). Plasma IGF-1 values were normalized to percent change from each animal's pre-study value.

Body weight gain was evaluated using repeated measures analysis of variance (ANOVA) with heteroscedastic compound symmetric covariance structure. Epiphyseal plate area data were subjected to a one-way ANOVA followed by a Dunnett's post hoc test. The alpha values for the repeated measures ANOVA as well as the one-way ANOVA/Dunnett's were assigned to be 0.05. In the monkey pharmacodynamic study, the mean IGF-1 response for each dose group was statistically compared against the mean response of the vehicle control group. These comparisons were performed in the context of a one-way analysis of variance

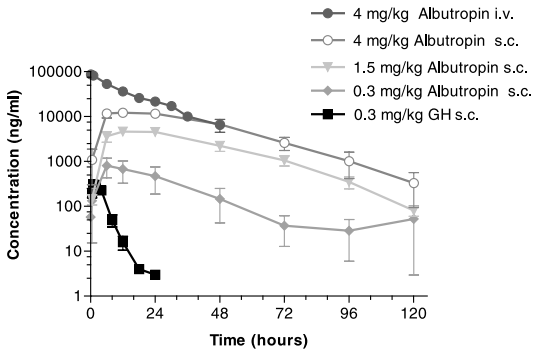

Fig. 2. Mean (± S.E.M.) plasma albutropin or GH concentrations (ng/ml) following single-dose i.v. or s.c. administration of albutropin or s.c. GH in cynomolgus monkeys ($n = 4$ per dose/route).

Table 5
Mean (± S.E.M.) pharmacokinetic parameters following single-dose i.v. or s.c. administration of albutropin in cynomolgus monkeys

Parameter		N	0.3 mg/kg s.c.		1.5 mg/kg s.c.		4.0 mg/kg s.c.		4.0 mg/kg i.v.	
			Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
AUC	h ng/ml	4	24310	14548	226325	36640	629608	99570	1474442	138534
AUC/Dose	(h ng/ml)/(ng kg)	4	0.08	0.05	0.15	0.02	0.16	0.02	0.370	0.030
CL or CL/F	ml/h/kg	4	24.6	7.1	7.1	1.0	6.8	1.0	2.8	0.3
C_{max}	ng/ml	4	808.5	376.3	5089.0	509.5	13690.8	1646.9	87486.9	1313.8
C_{max}/dose	(ng/ml)/(ng/kg)	4	3.0×10^{-3}	1.3×10^{-3}	3.4×10^{-3}	3.4×10^{-4}	3.4×10^{-3}	4.1×10^{-4}	0.022	0.000
$t_{1/2\ elim}$	h	4	14.3	1.4	12.8	1.2	14.7	2.9	14.8	3.5
$t_{1/2\ absorb}$	h	4	2.8	0.9	7.7	2.4	7.6	2.3		
MRT	h	4	25.3	2.4	35.0	2.1	35.5	3.5	20.8	4.4
T_{max}	h	4	6.0	0.0	13.5	3.8	16.5	4.5	0.0	0.0
Vz or Vz/F	ml/kg	4	520	169	133	28	133	6	56	8
Bioavailability	%	4					43			

(ANOVA) at each time point followed by a multiple comparisons test (alpha value of 0.05).

3. Results

3.1. Pharmacokinetic and pharmacodynamic studies in Sprague–Dawley rats and cynomolgus monkeys

3.1.1. Pharmacokinetic studies

Plasma albutropin and GH concentrations (mean ± S.E.M.) from the rat pharmacokinetic study are presented in Fig. 1 and the results of the pharmacokinetic analyses are presented in Table 4. The terminal half-life of albutropin in the rat was about 3 h after i.v. administration and 6 h after s.c. administration. The terminal half-life of GH in rats was similar to albutropin after i.v. administration (3 h), but considerably shorter after s.c. administration (0.7 h). Apparent clearance of i.v. albutropin (30.6 ml/h/kg) was the same as the clearance of i.v. GH (30.1 ml/h/kg). Albutropin was absorbed slowly in the rat compared to GH, reaching a C_{max} of 200 ng/ml about 6 h after s.c. injection of 800 μg/kg. Recombinant human GH reached its C_{max} of 70 ng/ml more quickly at 1 h after s.c. dosing with 100 μg/kg. The s.c. bioavailability of both albutropin (7.9%) and GH (3.6%) were very low relative to i.v. administration of the respective proteins in the rat.

Visual inspection of the plasma concentration data revealed no obvious differences in plasma concentration profiles from the albutropin-treated monkeys, therefore the male and female monkeys were analyzed together. There appeared to be differences between males and females in the plasma concentration curves from the GH-injected monkeys, so these profiles were analyzed separately.

After single i.v. or s.c. doses of 0.3, 1.5 and 4.0 mg/kg, albutropin was detectable in the plasma of most monkeys through the duration of the sampling period (48 h after i.v. dosing and 120 h after s.c. dosing; Fig. 2). Two monkeys in the 0.3 mg/kg albutropin dose group had detectable plasma concentrations for 96 h after dosing. In contrast, plasma concentrations of GH were below the limit of quantitation by 24 h after dosing. Results of the non-compartmental pharmacokinetic analyses are provided in Tables 5 and 6. The terminal half-life of albutropin was 13–15 h in the cynomolgus monkey following i.v. or s.c. injection and 2–3 h following s.c. injection of GH. The mean residence time of s.c. albutropin was at least sixfold greater than that of s.c. GH. Clearance was 2.8 ml/h/kg after the i.v. dose of 4 mg/kg and approximately apparent clearance was 7 ml/h/kg after s.c. dose levels of 1.5 and 4

Table 6
Mean (± S.E.M.) pharmacokinetic parameters following single-dose i.v. or s.c. administration of GH in male and female cynomolgus monkeys

Parameter		Day 0				Day 6			
		Females		Males		Females		Males	
		Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
AUC	h ng/ml	1314	72	1951	56	1260	22	1723	131
AUC/Dose	(h ng/ml)/(ng kg)	4.4×10^{-3}	2.0×10^{-4}	6.5×10^{-3}	2.0×10^{-4}	4.2×10^{-3}	1.0×10^{-4}	5.7×10^{-3}	4.0×10^{-4}
CL/F	ml/h/kg	229.0	12.5	153.9	4.4	238.1	4.2	175.2	13.3
C_{max}	ng/ml	263.8	77.5	410.1	73.9	248.8	31.7	346.3	16.5
MRT	hr	4.2	0.8	4.0	0.3	3.6	0.6	4.1	0.4
$t_{1/2\ absorb}$	h	0.61	0.22	0.45	0.03	0.68	0.14	0.56	0.13
$t_{1/2\ elim}$	h	1.7	0.4	2.8	1.1	1.6	0.4	2.4	0.0
T_{max}	h	3.0	1.0	1.5	0.5	2.0	0.0	2.0	0.0
Vz/F	ml/kg	563	149	618	253	541	143	599	43

mg/kg. Apparent clearance was 24.6 ml/h/kg after the lowest s.c. dose of 0.3 mg/kg. In contrast, the apparent plasma clearance rate of s.c. injected GH was 229 ml/h/kg in the female monkeys and 154 ml/h/kg in the male monkeys (Table 6). The absorption of albutropin after s.c. dosing was slower than that of s.c. administered GH, with an absorption half-life of 2.8 h for the 0.3 mg/kg of albutropin and a mean of 0.5 h for the 0.3 mg/kg GH dose. The s.c. bioavailability after a dose of 4 mg/kg was approximately 43% relative to the i.v. dose of 4 mg/kg as determined from the ratio of the s.c. to i.v. area under the plasma concentration curve (AUC).

3.1.2. Pharmacodynamic studies

Albutropin dosed daily, every other day or every 4 days significantly increased body weight gain compared to vehicle (phosphate-buffered saline; PBS) controls (Fig. 3). Heat-inactivated (boiled) albutropin had no significant effect on body weight gain. Similarly, GH administered at a dose level equimolar to those of albutropin induced a significant increase in body weight gain with every day and every other day dosing. However, no significant growth was achieved with every fourth day dosing of GH compared to PBS controls. In every other day and every 4-day dosing regimens, the weight gain in rats treated with albutropin was greater than that of regimen-matched GH treatment groups. Although the mean weight gain induced by GH administration every 4 days was not statistically different from either daily human serum albumin (a protein control) or inactivated albutropin control administration, the effect of every fourth day administration of human serum albumin or boiled albutropin was not assessed, thus a more appropriate comparison with regimen-matched controls was not possible in this study. However, it is clear that intermittent dosing of albutropin increases in body weight gain in hypophysectomized rats.

Albutropin dosed daily, every other day or every 4 days significantly increased tibial epiphyseal plate area compared to vehicle controls (Fig. 4A and B). Heat-inactivated albutropin had no effect on epiphyseal plate growth. Recombinant GH also increased epiphyseal plate growth when given daily and every other day; however, when administered every 4 days, GH failed to increase significantly tibial epiphyseal plate area (Fig. 4B). When dosed QD or QOD, there were no significant differences between albutropin and GH. Albutropin on a Q4D schedule significantly increased tibial epiphyseal plate area compared to GH on a Q4D schedule.

Plasma IGF-1 concentrations were increased in a dose-dependent fashion in monkeys given a single administration of albutropin by the s.c. and i.v. routes (Fig. 5). The IGF-1 concentrations began to increase significantly within 12 h after dosing and peaked 72–120 h after the injection of albutropin (4 mg/kg i.v. or s.c.). In the 4 mg/kg s.c. and i.v. treatment groups, plasma IGF-1 concentrations appeared to be increased above predose values for at least 7 days. At the 1.5 mg/kg s.c. dose, plasma IGF-1 concentrations were significantly increased above vehicle controls at 120 h only, although there was a trend towards increased plasma IGF-1 between days 4 and 7. A single injection of 0.3 mg/kg albutropin did not significantly increase IGF-1 concentrations compared to the vehicle control group. Seven consecutive s.c. injections of GH at 0.3 mg/kg (2.1 mg/kg total cumulative dose over 7 days of administration) increased plasma IGF-1 concentrations to the same extent as a single i.v. or s.c. administration of albutropin (4.0 mg/kg).

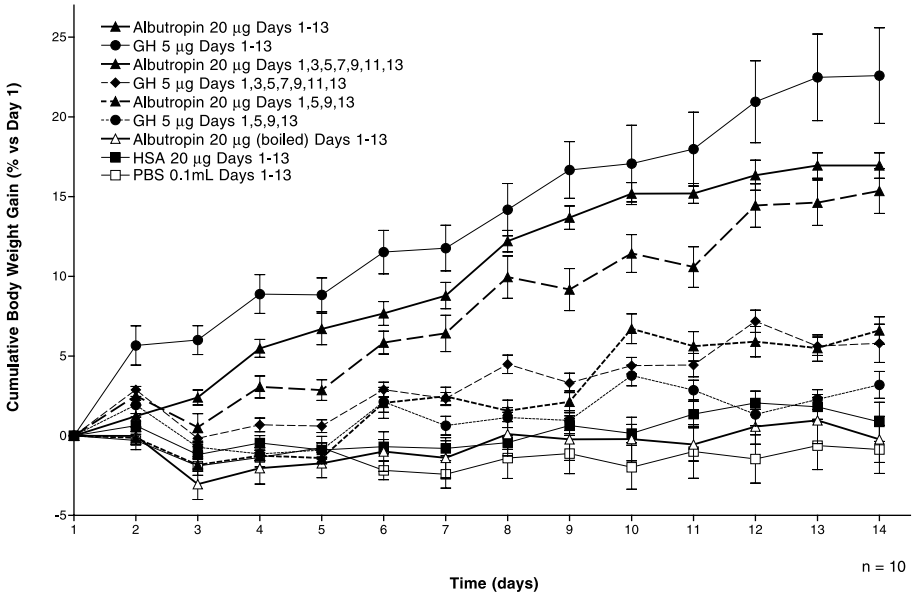

Fig. 3. Mean (± S.E.M.) cumulative body weight gain (% of day 1) for hypophysectomized rats treated with either albutropin or GH administered s.c. using various dosing regimens ($n = 10$ per treatment group).

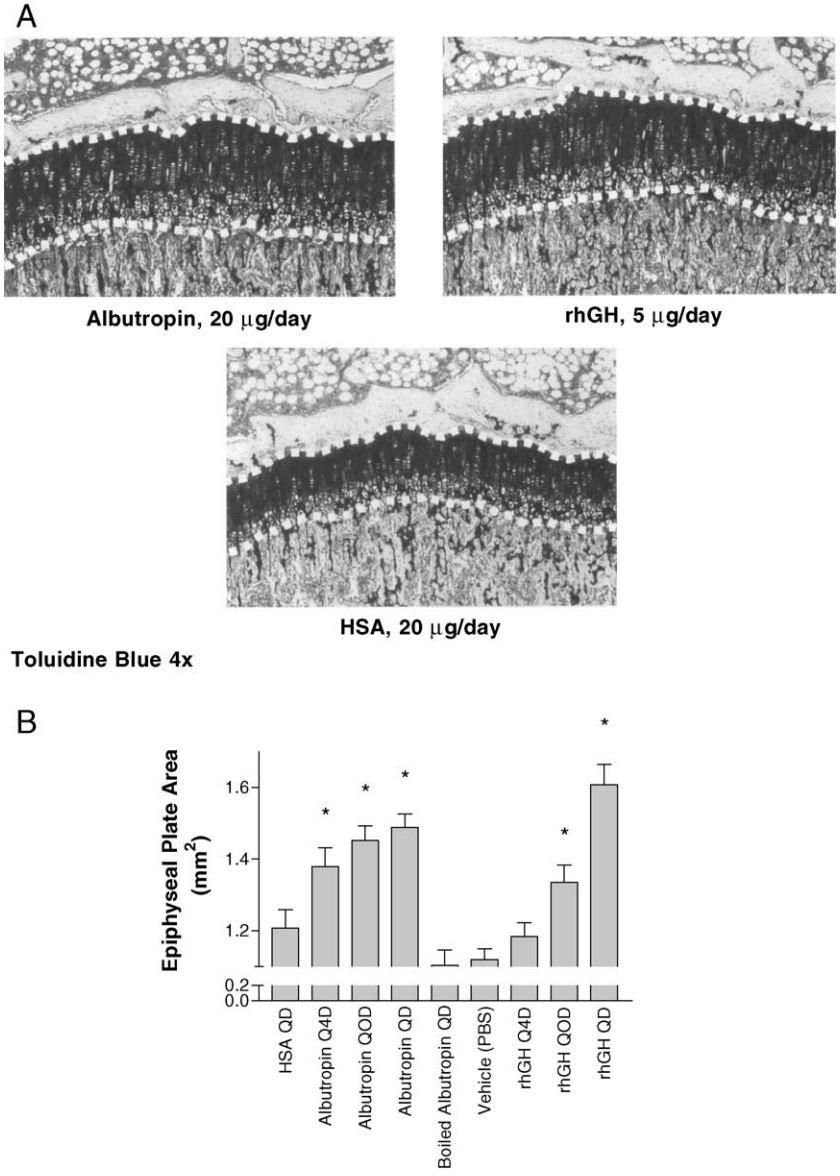

Fig. 4. Effect of albutropin on rat tibial epiphyseal plate growth (A) histologically and (B) as measured by computerized morphometry (mean tibial epiphyseal growth plate area ± S.E.M.) on histologic sections.

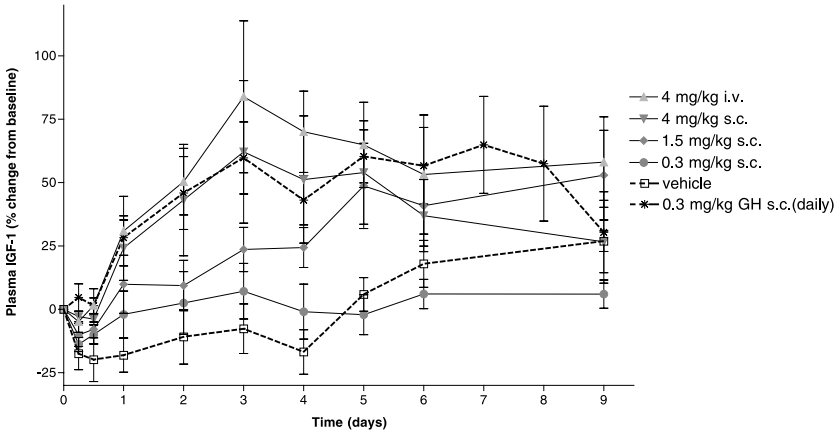

Fig. 5. Mean (± S.E.M.) percent change in IGF-1 concentrations in monkey plasma following single i.v. or s.c. administration of albutropin at 0.3–4.0 mg/kg or 7 daily s.c. administration of GH ($n = 4$ per dose/route). All IGF-1 concentrations were normalized to their baseline values prior to study initiation.

4. Discussion

Although GH supplementation is effective in the treatment of GH deficiency in children and adults, the disadvantages of daily injections over extended periods of time limit its use by physicians in certain patient populations as well as increase the risk of dosing error, the number of care givers, the cost of treatment and/noncompliance rates (Davies et al., 2000; Reiter et al., 2001). Especially important in certain populations, such as children of short stature who may not understand the implications of not following the prescribed GH dosing regimen, is the necessity of compliance to achieve the optimal benefit from GH therapy (Reiter et al., 2001). The issue of finding a more suitable alternative to daily GH injections and subsequent compliance gains further importance as GH-deficient children transition into adults with a continuing need for GH treatment (Vahl et al., 2000). The requirement of daily therapy is largely due to recombinant GH's short plasma half-life and has led to the development of a sustained release form of GH (Reiter et al., 2001). We report here that albutropin, a recombinant human serum albumin/human growth hormone fusion protein, has a pharmacokinetic profile in the rat and, especially, monkey that is longer in duration than that of GH. This unique pharmacokinetic profile allows for intermittent administration of albutropin to achieve pharmacodynamic effects in both growth-hormone-deficient rat and normal monkey as evidenced by growth and elevations in plasma IGF-1 levels, respectively.

In general, albutropin offers a superior pharmacokinetic profile compared with that of GH when administered s.c. in the rat. Although albutropin and GH are poorly absorbed s.c. in the rat and have similar terminal half-lives following i.v. administration, there are substantial differences in plasma clearance of albutropin compared to GH. Specifically, plasma is cleared of albutropin at less than half the rate of GH following s.c. dosing. The terminal half-life and mean residence time of albutropin were approximately eight times longer than that of GH in rats following s.c. administration. In addition, the volume of distribution following s.c. dosing is much larger for albutropin than for GH.

In an effort to examine whether the pharmacokinetic advantages in the rat translated to a pharmacodynamic benefit, the possibility that albutropin might stimulate growth in GH-deficient hypophysectomized rats with dosing regimens less frequent than daily was tested at equimolar albutropin and GH dose levels. Albutropin administered daily, every other day and even every fourth day significantly increased both cumulative body weight gain and tibial epiphyseal plate parameters. These growth-promoting effects were generally greater than those induced with equimolar dose levels of GH administered at the same intermittent dosing schedules. Of note, the every fourth day administration schedule for albutropin shows a clear benefit over GH since albutropin enhanced both body weight gain and bone growth, while GH showed no significant effects on either parameter.

In monkeys, albutropin administered at the same dose of GH by mass (0.3 mg/kg) as GH showed an improved pharmacokinetic profile relative to GH: The half-life was six times longer for albutropin, the mean residence time was about 5- to 10-fold greater and clearance was 8-fold slower than GH. In addition, the absorption of albutropin was slower than that of GH as evidenced by the greater half-life of absorption of albutropin compared to GH. Pharmacodynamically, the long circulation time of albutropin relative to GH in the monkey resulted in a prolonged IGF-1 response measured in blood plasma following a single s.c. injection. Subcutaneous administration of a single dose of albutropin increased circulating IGF-1 concentrations in a dose-dependent manner in the normal cynomolgus monkey. At the highest albutropin dose, IGF-1 concentrations were elevated above baseline for as long as 7 days after a single administration. This IGF-1 response was equivalent to the response achieved by seven consecutive daily administrations of GH at 0.3 mg/kg (2.1 mg/kg total dose). Thus, the enhanced circulation time of a single dose of albutropin resulted in substantial pharmacodynamic improvement over a single dose of GH, raising the possibility that albutropin could offer similar growth enhancement with reduced dosing frequency compared with standard GH treatment regimens.

The concept of reduced dosing frequency with a GH product received some attention with the development of Nutropin Depot®. This sustained release formulation of GH is effective and approved for use in pediatric populations; however, comparisons to historical controls have revealed that 1- and 2-year growth rates are significantly ($p < 0.001$) lower in children given Nutropin Depot® (1-year growth rate 8.2 ± 1.8 cm/year) than in children treated with GH (one-year growth rate 10.1 ± 2.8 cm/year) (Silverman et al., 2002). The local effects of subcutaneously administered Nutropin Depot® include nodules, erythema, pain at the injection site, headache and vomiting (Silverman et al., 2002). Preclinical toxicology studies in both rat and monkey have shown that s.c. administration of albutropin produces no local reactions compared to vehicle. Given the medical need for a less frequently administered form of GH, the pharmacologic properties of albutropin in this study in monkeys and rats suggest that this product warrants clinical testing. The sustained activity of albutropin in the rat and monkey support its potential utility as an agent that requires only intermittent administration to attain a therapeutic benefit that is currently achieved with daily dosing.

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