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Pulmonary delivery of TH9507, a growth hormone releasing factor analogue, in the dog

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Abstract

A modified growth hormone releasing factor (GRF; TH9507), a 44 amino acid peptide analogue of natural human growth hormone releasing factor, is being developed for the treatment of age-associated conditions resulting from diminished growth hormone (GH) secretion. The inhalation route of administration is being considered as an alternative to subcutaneous injection. A study was undertaken in dogs to investigate the absorption of TH9507 following pulmonary delivery. Male beagle dogs were administered TH9507 by intratracheal dry powder insufflation and subcutaneous injection at doses of approximately 375 and 38 μg/kg, respectively. In a separate study, male and female dogs received 100 μg/kg intravenously. Blood samples were collected at selected sampling times after dosing and plasma levels of TH9507 were measured by radioimmunoassay. The bioavailability by the inhaled route was 41% relative to subcutaneous dosing, with an absolute bioavailability estimated at 13%. No significant difference was observed for the terminal half-life of TH9507 after intratracheal (39 min) and subcutaneous (26 min) administrations. The mean residence time (MRT) was greater following intratracheal administration (74 min versus 52 min; $P < 0.01$). These data indicate that the delivery of the TH9507 by the inhalation route may provide a suitable alternative to subcutaneous injection.

Keywords: Powder inhalation; Bioavailability; Peptide; hGRF

1. Introduction

Growth hormone releasing factor (GRF) binds to cell membrane receptors in the pituitary gland to stimulate secretion of growth hormone (GH) and subsequently, insulin-like growth factor-1 (IGF-1) which is released by the action of GH in the liver or locally in peripheral target tissues (Casanueva, 1992). Ageing is associated with diminished GH and IGF-1 secretion (Rudman et al., 1990). This may result in

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conditions such as sleep disturbance (Van Cauter et al., 2000), reduced immune response (Clark, 1997; Kelley, 1989; Khorram et al., 1997), muscle wasting in chronic obstructive pulmonary disease (COPD) (Pape et al., 1991) and slow recovery following hip fracture (Hedström et al., 1999).

Human GRF (hGRF) is a 44 amino acid peptide, which acts in conjunction with the inhibitory hormone somatostatin to regulate GH secretion in a pulsatile manner (Casanueva, 1992; Marshall et al., 1996; Van Cauter and Plat, 1996). The elimination half-life of hGRF following intravenous injection of the peptide in healthy subjects is relatively short, about 7 min, due to rapid inactivation by plasma dipeptidylaminopeptidase (Frohman et al., 1986). The modification of hGRF by the addition of a transhexenoyl moiety on the N-terminal end of the peptide to form the analogue TH9507, under development by Theratechnologies, resulted in the stabilisation of the molecule (Abribat et al., 2001a). In vitro studies showed that TH9507 was more resistant than hGRF to degradation in human plasma (Abribat et al., 2001a). Furthermore, in vivo studies demonstrated an increase in plasma elimination half-life in animals and humans while retaining the stimulatory pharmacological activity of GRF on the GH–IGF-1 axis (Abribat et al., 2001a,b). In addition, multiple subcutaneous injections of TH9507 administered to pigs resulted in increased circulating levels of IGF-1 and GH, including pulsatile GH release, thereby mimicking the normal physiological pattern of GH secretion (Abribat et al., 2001a). The safety and efficacy of TH9507 is currently under investigation in various phase II clinical studies targeting age-related sleep disorders, immune response and hip fracture recovery in the elderly, and muscle wasting in COPD patients.

Treatment with TH9507 may involve daily subcutaneous injections over prolonged periods. As an alternative to dosing by injection, efficient absorption and GH secretion following pulmonary delivery has been reported for other GRF analogues (Pinski et al., 1993; Smith et al., 1994). TH9507 has a molecular weight of 5136 Da, similar to that of insulin (5808 Da), which is under development as an inhaled product (Patton et al., 1999; Skyler et al., 2001). The present study is a preliminary investigation, undertaken in dogs, to assess the bioavailability of a dry powder formulation of TH9507 following intratracheal dosing to the lungs.

2. Materials and methods

2.1. Preparation of TH9507 formulations

TH9507 (*N*-[*trans*-3-hexenoyl]-hGRF(1–44)NH_2 acetate) was prepared at Theratechnologies by solid phase peptide synthesis according to a 9-fluorenylmethoxycarbonyl (Fmoc) strategy. Ramage amide resin was used as a solid support and Fmoc-amino acids with appropriate protection on the side chains were used for peptide synthesis along with a suitable coupling reagent.

A dry powder formulation of TH9507 suitable for administration to the lungs from a PennCentury dry powder insufflator (Penn-Century, Philadelphia, PA, USA) was prepared by spray-drying TH9507 (Lot No. FF-01-003; >95% purity) from aqueous solution without the addition of any other excipients. The formulation was stored in closed containers at ambient temperature. The powder particles had a mean geometric particle diameter of 0.9 μm (range: 0.6–2.2 μm) as measured over 20 particles by scanning electron microscopy.

TH9507 for subcutaneous dosing was prepared under aseptic conditions and reconstituted in water (0.5 mg/ml). Sterility was assured by filtration through a low-protein binding 0.2 μm filter and testing for endotoxin using the limulus amoebocyte lysate test. Solution for intravenous injection comprised 100 μg/ml TH9507 in water containing 5% (w/v) mannitol. This was also passed through a 0.2 μm filter prior to injection. Analysis of the filtered solutions confirmed the nominal TH9507 concentrations in the formulations.

2.2. Characterisation of powders for intratracheal administration

Quantitative analysis of solutions of the TH9507 powder was performed by gradient reverse-phase HPLC and UV detection. Chemical purity was measured against related substance peaks following spray-drying and upon storage for the duration of the animal studies.

Emission and dispersion efficiency from the PennCentury device were assessed using an Andersen cascade impactor (USP apparatus 1). The device was loaded with approximately 5 mg and operated using an air line as detailed in Section 2.3. A sampling

airflow rate of 28.3 l/min through the impactor was established and one or two doses were emitted from the device into the sampling port. Residual drug in the device was determined by flushing the powder chamber and delivery needle with water and analysing the sample by HPLC. The emitted dose (ED) was determined by subtraction of the amount of residual drug recovered from the device from the pre-determined device load. The emitted fraction (EF) equates to the ED as a percentage of device load. The fine particle fraction (FPF) was obtained from the amount of drug recovered from stages below the 5.8 μm impactor cut-off, expressed as a percentage of the emitted dose.

2.3. Animal studies

The procedures for the care and treatment of the animals were approved by the UK Home Office for the intratracheal and subcutaneous dosing at Inveresk Research (Tranent, Scotland) and the Institutional Animal Care and Use Committee of ITR Laboratories (Montreal, Canada) for the intravenous dosing.

2.3.1. Intratracheal and subcutaneous dosing

An investigation into the systemic bioavailability of TH9507 following intratracheal delivery to the lungs was conducted in male beagle dogs ($n = 5$, 3 years old and weighing 12–15 kg). Each animal had a surgically prepared permanent tracheostome to facilitate repeat intrapulmonary dosing. In the current study, each dog was dosed with TH9507 on two occasions at least 7 days apart. On the first occasion a dose of 0.5 mg TH9507 dissolved in 1 ml water was administered by subcutaneous injection into the right flank.

For intratracheal administration, the second dosing occasion, a flexible endotracheal tube was attached to the PennCentury device and the tip of the tube was inserted to about 1 cm proximal to the carina. The device was connected to an air line controlled by an automated solenoid air pressure valve to deliver approximately 5 mg powder with 60 ml of air over 0.5 s, at the beginning of inspiration. After dosing, residual powder was washed from the delivery device, but the washings were not analysed. The pharmacokinetic analysis was thus based on the nominal emitted dose (5 mg). On both occasions, venous blood samples (2 ml) were taken from each dog immediately before dosing and following dosing at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180 and 240 min.

2.3.2. Intravenous dosing

As part of a toxicity study, single intravenous bolus injections of TH9507 formulation were administered daily for 28 days to male and female beagle dogs (three animals per sex; approximately 7 months old and weighing 7.1–9.0 kg) at a dose of 100 μg/kg. After the first and last doses, venous blood samples (4 ml) were taken from each animal immediately before dosing and following dosing at 5, 15, 30, 60, 90, 120, 240 and 1440 min.

2.4. Analytical procedures

Each blood sample was collected into a heparinised tube and the plasma separated by centrifugation at 4 °C and 3000 rpm for 10 min. The plasma was stored at below −20 °C in plain plastic tubes prior to shipment on dry ice for analysis by a radioimmunoassay (RIA) having a lower limit of quantification of 0.6 ng/ml.

2.4.1. Radioimmunoassay

TH9507 was measured in unextracted plasma with a double antibody radioimmunoassay procedure, using ^{125}I-Tyr^{10}-hGRF(1–44)NH_2 as tracer (Amersham Biosciences, Piscataway, NJ, USA), TH9507 (Lot No. FHEXGRF0002A) as standard and a polyclonal rabbit anti-human GRF (Peninsula Laboratories, San Carlos, CA, USA). This antiserum (RIN-8061 Lot No. 024633-1) has an IC_{50} of 10 pg/tube, a cross reactivity of 100% with hGRF(1–40)NH_2 and hGRF(1–44)NH_2 and against porcine GRF (67%), hGRF(1–40)COOH (60%), hGRF(1–37)COOH (24%), hGRF(1–29)NH_2 (12%), hGRF(30–44)NH_2 (1%) and rat GRF (1%). This assay was conducted in a 20 mM phosphate buffer, pH 7.2, containing 0.1% BSA (RIA grade), 0.15 M NaCl, 10 mM EDTA, 0.1% sodium azide and 0.1% Tween 20 (all reagents from Sigma, St. Louis, MO, USA). The 500 μl incubate consisted of a standard (or unknown), first antibody at a final dilution of 1/400,000 and tracer (ca. 11,000 cpm) in polypropylene tubes. Seven standards ranging from 3 to 3000 pg/tube were used to generate a standard curve. The incubation was over 20–24 h at 4 °C. The second antibody (goat anti-rabbit; Cederlane Laboratories, Ontario, Canada) was added at a dilution of

1/10. After centrifugation, the tubes were incubated for 16–18 h then centrifuged and the supernatant discarded. The resulting pellets were counted for radioactivity.

2.4.2. Pharmacokinetic analysis

Plasma concentration–time profiles are presented as geometric means with the corresponding asymmetrical standard errors (S.E.) obtained through log-transformation and back-transformation.

Pharmacokinetic parameters were calculated for each animal by non-compartmental analysis (Gibaldi and Perrier, 1982) using WinNonlin® (version 3.1, Pharsight Corporation, Mountain View, CA, USA). For the intratracheal and subcutaneous dosing, corrections for baseline-interference were made by assuming a linear increase in background concentrations from mean pre-dose levels of 0.8–1.4 ng/ml at 240 min following subcutaneous injection and subtracting the interpolated background levels from the measured values at each sampling time. Subtraction of pre-dose levels alone did not appear to produce meaningful estimates of pharmacokinetics. The increase in baseline interference may be due to dosing with TH9507, and although the increase may not be truly linear with time, this assumption has little effect on the overall findings.

The maximum plasma concentrations (C_{max}) following the intravenous administrations were calculated by log-linear back-extrapolation to time of dosing, whereas for the intratracheal and subcutaneous administrations C_{max} (and t_{max}) are the observed values. Terminal half-lives ($t_{1/2,z}$) were calculated according to $t_{1/2,z} = \ln(2)/\lambda_z$. The areas under the plasma concentration–time curves (AUC) were calculated by the linear-trapezoidal method and extrapolated to infinity (AUC_{inf}) according to $AUC_{inf} = AUC_{last} + C_{last}/\lambda_z$, where AUC_{last} is the calculated AUC up to the last quantifiable observation, C_{last} the plasma drug concentration at the last quantifiable observation and λ_z the rate constant determined from the log-linear terminal phase by least-squares regression. The mean residence time (MRT) was extrapolated to infinity (MRT_{inf}) according to $MRT_{inf} = AUMC_{inf}/AUC_{inf}$, where $AUMC_{inf} = AUMC_{last} + ((t_{last} \times C_{last})/\lambda_z) + (C_{last}/\lambda_z^2)$ and $AUMC_{last}$ is the calculated area under moment curve up to the last quantifiable observation. The relative bioavailability (F_{rel}) was calculated from $F_{rel} = (AUC_{i.t.}/AUC_{s.c.})(D_{s.c.}/D_{i.t.})$, where subscripts denote the routes of administration and D is the dose. Total body clearance (CL) was calculated from $CL = D/AUC_{inf}$.

Summary statistics are presented as the arithmetic mean with corresponding standard deviation (S.D.), unless otherwise stated. Confidence limits were calculated using Microsoft Excel® (Microsoft Corp., USA) from mean$\pm$(S.D.$/t\sqrt{n}$), where the t-value is from the Student's t-distribution. The harmonic mean $t_{1/2,z}$ and confidence limits correspond to the arithmetic mean (and S.D.) λ_z values calculated for each dog. The ratio estimate for F_{rel} from geometric mean AUC_{inf}/D values and the corresponding 95% confidence interval was calculated from the log-transformed values for AUC_{inf}/D by normal approximation ($S.D._{ratio} = (S.D._{\log(AUC_{inf}/D_{i.t.})}^2 + S.D._{\log(AUC_{inf}/D_{s.c.})}^2)^{1/2}$).

3. Results

3.1. Characterisation of powders for intratracheal dosing

Spray-drying of TH9507 produced microparticles suitable for dosing to the lungs. Chemical purity was unaffected by the formulation process. Emitted fractions from the PennCentury device were 93 ± 2.4% (w/w) (mean ± CV, $n = 8$) with fine particle fractions in the range of 28.8–31.0% ($n = 3$). No changes in particle size, powder dispersion or chemical purity occurred during storage at ambient temperature over the 6-week period from the preparation of the spray dried powder to dosing the animals.

3.2. Animal studies

3.2.1. Intratracheal and subcutaneous dosing

The dogs exhibited no adverse effects as a result of dosing with TH9507 by either route of administration. The measured mean plasma TH9507 concentrations are shown in Fig. 1. The mean C_{max} values for the intratracheal (5 mg/dog) and subcutaneous (0.5 mg/dog) doses were 34 and 17 ng/ml, respectively, both observed at 30 min (median t_{max}). The subcutaneous doses appeared to produce two major peak concentrations in each animal at approximately 15 and 30 min.

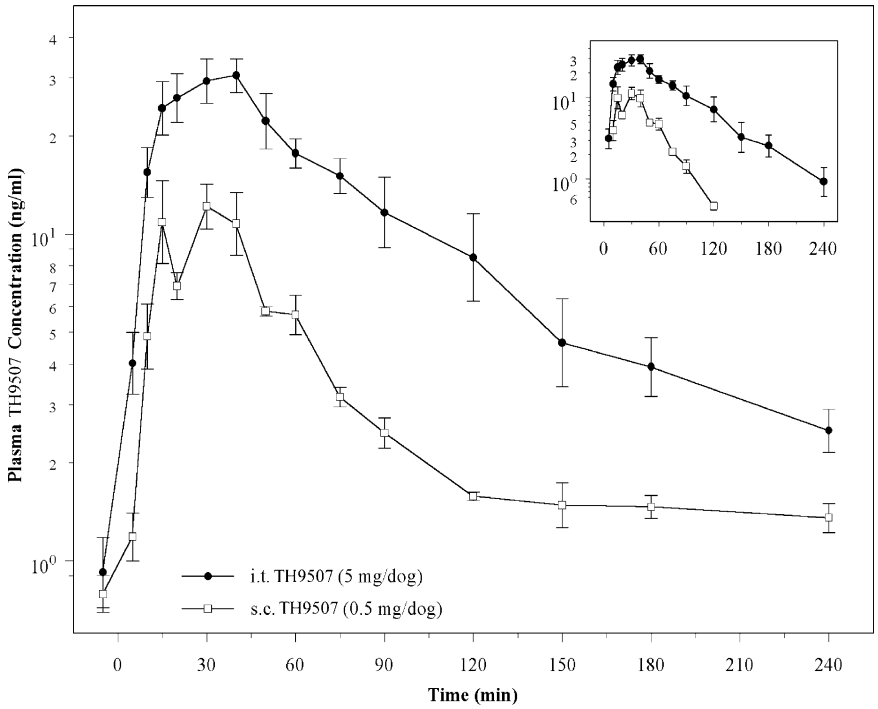

Fig. 1. Plasma TH9507 concentration following intratracheal and subcutaneous administration to beagle dogs. *Main:* geometric mean ± S.E. ($n = 5$) measured plasma TH9507 concentration and *inset:* geometric mean ± S.E. ($n = 5$) plasma TH9507 after correction for baseline interference.

This double peak is probably an artefact resulting from proportionally greater assay variability at lower concentrations. By the last observation (240 min), the blood concentrations were approaching the baseline levels for both routes of administration (Fig. 1).

Measurable pre-dose plasma concentrations indicated assay interference by canine GRF or other blood compounds. The apparent asymptotical characteristic of the TH9507 plasma level curve (150–240 min) after subcutaneous administration, suggests that this interference may have increased during the study. Accordingly, the pharmacokinetic analysis has been performed with a correction for baseline under this assumption (Section 2.4.2).

Pharmacokinetic parameters calculated from corrected plasma TH9507 concentrations (AUC, C_{max}, t_{max}, $t_{1/2}$, λ_z, F_{rel} and MRT) are listed in Table 1. The bioavailability following intratracheal delivery relative to subcutaneous dosing was approximately 41% indicating high efficiency in pulmonary absorption. The AUC added by extrapolation was within 0.8–5.1% of total AUC_{inf} for both routes. The mean residence time

Table 1
Selected pharmacokinetic parameters (mean ± S.D.) for TH9507 after single s.c. and i.t. dosing to beagle dogs ($n = 5$)

	Dose (mg)	C_{max} (ng/ml)	t_{max}[a] (min)	$t_{1/2,\lambda_z}$[b] (min)	AUC_{inf}[c] (ng min/ml)	F_{rel}[d] (%)	MRT_{inf} (min)
s.c. dosing	0.5	17 (6)	30 (15–30)	26 (17–56)	604 (138)	–	52 (6)
i.t. dosing	5	35 (10)	30 (15–40)	39 (32–50)	2617 (1036)	41 (23–74)	74 (12)

[a] Median and range.
[b] Harmonic mean with 95% confidence limits.
[c] Geometric mean values were 592 and 2445 (ng min/ml) after s.c. and i.t. dosing, respectively.
[d] Geometric mean with 95% confidence limits.

Table 2
Selected pharmacokinetic parameters (mean ± S.D.) for TH9507 after a single i.v. dose to beagle dogs ($n = 5$)

Dose (μg/kg)	100
$t_{1/2,\lambda_z}$ [a] (min)	25 (18–39)
C_{max} (ng/ml)	427 (118)
AUC_{inf} [b] (ng·min/ml)	5301 (2145)
CL (ml/min/kg)	21 (7)
MRT_{inf} (min)	18 (3)

[a] Harmonic mean with 95% confidence limits.
[b] Geometric mean value was 4981 ng min/ml.

of 74 min following pulmonary delivery was greater than the 52 min following subcutaneous injection ($P < 0.01$, paired Student's *t*-test) indicating an extended duration of systemic absorption from the lung compared to subcutaneous dosing.

3.2.2. Intravenous dosing

Pharmacokinetic parameters calculated for intravenous dosing are listed in Table 2. For one female animal, the terminal half-life could not be obtained accurately from the available plasma concentration data. Values, therefore, are summarised only for the remaining five animals. Plasma profiles showed a bi-exponential decline with a mean terminal half-life of 25 min. The total AUC for a 100 μg/kg i.v. dose was estimated to be 4981 ng min/ml (geometric mean), with less than 4% from extrapolation to infinity, but on average 33% from back extrapolation to time of dosing. Clearance was calculated at 21 ml/(min kg) and MRT_{inf} at 18 min.

With reference to the mean AUC calculated from the intravenous study, the absolute bioavailability of TH9507 following intratracheal dosing was 13% and after subcutaneous administration 32%. Possible inaccuracies in these estimates may arise from the high degree of back-extrapolation in the AUC calculated for the intravenous dose and additionally because doses were administered to different groups of animals.

4. Discussion

The present study demonstrates that TH9507 can be formulated by spray-drying to produce dry powder microparticles suitable for inhalation to the deep lung (Heyder, 1982).

In beagle dogs, the extent of absorption from the lungs was about 41% relative to a subcutaneous dose, with absolute bioavailability estimated at 13%. The absorption of only 32% following subcutaneous injection indicates a susceptibility of TH9507 for inactivation at the injection site as reported for hGRF(1–29)NH_2 (Rafferty et al., 1985; Pinski et al., 1993).

Absolute bioavailability for insulin absorption from the lungs in the same animal model, using a dry powder formulation of similar dispersion characteristics as the TH9507 formulation, is approximately 3%. In a clinical study, when administered to healthy adult males from a dry powder inhaler, the bioavailability relative to subcutaneously administered Humulin S (Eli Lilly, Basingstoke, UK) was 18% (Hardy et al., 2002). This equates to an absolute bioavailability of approximately 15% based on efficient insulin absorption following subcutaneous injection (Jacobs et al., 1993; Heinemann et al., 1997). Presumably, the improved targeting of the alveolar absorption site with clinical inhaler use is the primary contributor to the five-fold increase in bioavailability. Assuming that these observations are predictive for TH9507, a peptide of similar molecular weight to insulin, the absolute bioavailability of TH9507 in man is expected to be considerably higher than the 13% found in dogs.

In conclusion, these preliminary findings indicate that the delivery of a TH9507 dry powder formulation by the inhalation route may provide a suitable alternative to subcutaneous administration. A clinical study is required in order to establish the efficiency of inhalation delivery in man and to evaluate the pharmacodynamics of TH9507 on GH and IGF-1 production.

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