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Recombinant human parathyroid hormone 1–34: Pharmacokinetics, tissue distribution and excretion in rats

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Abstract

The objective of this work was to characterize the preclinical pharmacokinetics, tissue distribution, and excretion profiles of recombinant human parathyroid hormone (1–34) [rhPTH (1–34)] in healthy rats. Pharmacokinetic properties of ^{125}I-rhPTH (1–34) were examined after a single subcutaneous (s.c.) and intravenous (i.v.) bolus injection, respectively. Tissue distribution and urinary, fecal, and biliary excretion patterns of ^{125}I-rhPTH (1–34) were also investigated following a single s.c. injection. Our results suggested that rhPTH (1–34) was rapidly distributed and cleared in a bi-exponential manner after a single i.v. bolus injection. Following a single s.c. administration, rhPTH (1–34) exhibited rapid and considerable absorption and declined in a mono-exponential manner, with the absolute bioavailability and elimination half-life of 65% and 3.4–4.1 h, respectively. The TCA-precipitated radioactivity was widely distributed and rapidly diminished in most tissues/organs. Approximately 91% and 2% of the total radioactivity was recovered in urine and feces by 72 h postdosing, respectively; whereas 6% excreted into bile up to 24 h postdosing. These findings indicated high absolute bioavailability, rapid absorption and disposition of rhPTH (1–34) following a single s.c. administration in healthy rats. The accumulation of rhPTH (1–34) in tissues/organs examined appeared to be low. The major elimination route was urinary excretion.

Keywords: Recombinant human parathyroid hormone (1–34); Pharmacokinetics; Distribution; Excretion; Bioavailability

1. Introduction

Recombinant human parathyroid hormone (1–34) [rhPTH (1–34)], the N-terminal (1–34) fragment of full-length human parathyroid hormone [hPTH (1–84)], is the first anabolic agent approved for the treatment of osteoporosis (Quattrocchi and Kourlas, 2004). hPTH (1–84) is an endogenous polypeptide of 84 amino acids (9.5 kDa) that is the most important peptide regulator for calcium and phosphate ion homeostasis in blood and bones (Habener et al., 1984; Mannstadt et al., 1999; Gardella and Juppner, 2000). hPTH has been recognized as an anabolic agent on bones since 1930s (Reeve, 2002). The earliest studies in humans revealed that low doses of synthetic hPTH (1–34) had anabolic effect on bone and was effective for the treatment of postmenopausal osteoporosis in aged women (Reeve et al., 1976). Structure–activity studies have shown that the classic biological actions of hPTH reside in the 1–34 region of the mature protein (Potts et al., 1982), which is responsible for the binding affinity to its receptor (Bergeron et al., 1981). Pharmacodynamic studies also indicated that hPTH (1–34) exhibited the full spectrum biological effects on bone tissues as that of hPTH (1–84) (Kimmel et al., 1993).

The cloning, expression and large-scale purification (Suzuki et al., 1998) of rhPTH (1–34) allowed numerous in vitro and in

Abbreviations: rhPTH (1–34), recombinant human parathyroid hormone (1–34); TCA, trichloroacetic acid; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; C_{max}, the maximum plasma concentrations; T_{max}, the time to reach C_{max}; AUC_{0-T}, area under the plasma concentration versus time curve from 0 h to the last measured time point; $AUC_{0-\infty}$, area under the plasma concentration versus time curve from 0 h to extrapolation to infinity; $t_{1/2\alpha}$, half-life at α phase; $t_{1/2\beta}$, half-life at β phase; Cl, total body clearance; Cl/*F*, total body clearance corrected for bioavailability; *V*/*F*, volume of distribution corrected for bioavailability; V_{ss}, volume of distribution at the steady-state; %ID/g tissue/organ, percent of the total injected dose per gram of tissues or organs; %ID/ml liquid, percent of the total injected dose per milliliters of liquid; ANOVA, analysis of variance

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vivo studies to examine the structure, function and potential clinical utility of this peptide. Recent experimental investigations, including animal and human studies, showed that rhPTH (1–34) was significantly effective in the treatment of osteoporosis and prevention of bone fracture coupled with remarkably low toxicity. Daily injections of low dose rhPTH (1–34) can prevent bone loss and stimulate new bone formation in patients (Finkelstein et al., 1994, 1998). It was shown to preserve or increase bone mineral density (BMD), bone mass and strength, maintain bone quality and reduce the fracture risk in humans and various animal species, including osteoporotic women (Lindsay et al., 1997; Neer et al., 2001), men (Kurland et al., 2000; Orwoll et al., 2003), children (Koch, 2001), monkeys (Jerome et al., 2001; Turner et al., 2001), greyhounds (Podbesek et al., 1983), rabbits (Hirano et al., 1999; Mashiba et al., 2001), rats (Ejersted et al., 1995; Sato et al., 1997) and mice (Alexander et al., 2001; Zhou et al., 2003). Based on the results of preclinical and clinical trials, Forteo™ (Teriparatide), a recombinant human PTH (1–34) product, was approved by the U.S. Food and Drug Administration in December 2002 as the first anabolic agent that actually stimulates new bone formation (Quattrocchi and Kourlas, 2004). It is approved for the treatment of postmenopausal osteoporosis in women as well as primary or hypogonadal osteoporosis in men who are at high risk for fracture.

A good understanding of the pharmacokinetic properties in proper animal models is crucial to any novel drugs including biotech products such as rhPTH (1–34). Moreover, proper knowledge on the distribution and excretion is vital to investigate the major target sites and interpret the in vivo disposition of this peptide. Considering its diverse biological actions (Qin et al., 2004) and safety concerns (Crandall, 2002; Quattrocchi and Kourlas, 2004), detailed in vivo pharmacokinetic and disposition studies of rhPTH (1–34) by the proposed administration route (s.c.) are required. In addition, novel combination therapies of rhPTH (1–34) with conventional therapeutic agents (Binkley and Krueger, 2005; Garces and Garcia, 2006), such as anti-resorptive agents (Hunziker et al., 2000), basic fibroblast growth factor (Iwaniec et al., 2002), growth hormone (Mosekilde et al., 2000) or raloxifene (Deal et al., 2005), also require preclinical pharmacokinetic information, which can assist in the design and optimization of the combination regimens in animal models. Such knowledge is useful in the understanding of the complicated dose–response relationships in animals and lays the foundation for the establishment of starting dosing regimen design in the clinical combination trials (Tang et al., 2004). Furthermore, the in vivo pharmacokinetic data obtained in the preclinical species is one of the key tools used in predicting the human parameters, using various extrapolation approaches such as allometric scaling (Obach et al., 1997; Jolivette and Ward, 2005). It would be more important if the peptide is used in combination with other drugs in patients.

Previous studies made some investigation on the pharmacokinetics of hPTH (1–34) following different administration routes in humans (Kent et al., 1985; Lindsay et al., 1993; Fraher et al., 1995; Kohno et al., 1998) and rats (Codrons et al., 2003, 2004). Compared to numerous pharmacodynamic studies that have been assessed in humans and various animal models including rats, however, no peer-reviewed study has been designed and carried out specifically for acquiring the pharmacokinetic information of the large-scale produced peptide given in escalating doses by single s.c. injection in rats. More importantly, no data are available to elaborate the tissue distribution and excretion patterns of the genetically engineered N-terminal fragment 1–34 of hPTH. In the present study, we simultaneously investigated the pharmacokinetic profiles of an rhPTH (1–34) in healthy Wistar rats following a single s.c. and i.v. bolus administration, respectively. Furthermore, the tissue distribution and excretion patterns of ^{125}I-radiolabeled peptide after a single s.c. administration in rats were also investigated.

2. Materials and methods

2.1. Materials

The tested recombinant 1–34 N-terminal fragment of hPTH (4118 Da; p*I* 8.3), was produced in *Escherichia coli* and supplied by southwestern China Biotech Pilot Base Co. Ltd. (Chongqing, China). The purity was proved to be greater than 98.0% by high performance liquid chromatography and SDS-PAGE, and the specific activity was 1.08×10^4 IU/mg peptide. All rhPTH (1–34) solutions were formulated in 50 mM phosphate-buffered saline (PBS; pH 7.4) and stored at −80 °C until use. The peptide samples were frozen once only to avoid the degradation caused by freeze-thaw cycles. High specific activity (^{126}I content normally <0.005%), carrier-free protein iodination Na^{125}I (100 mCi/ml in 0.01 M NaOH solution, pH 7–11) was purchased from Amersham Biosciences Limited (Amersham, UK). Iodogen (1,3,4,6-tetrachloro-3alpha,6alpha-diphenylglucoluril) iodination reagent was purchased from Pierce (Rockford, IL, USA). The other common chemicals were provided by standard commercial sources and were of the highest quality available.

2.1.1. Radioiodination and purification of ^{125}I-rhPTH (1–34)

The tested rhPTH (1–34) was radiolabeled with Na^{125}I according to the mild Iodo-Gen method as previously described (Fraker and Speck, 1978) with some modification. Briefly, 54 μg peptide (18 μg/ml) was incubated with 2 mCi (1 Ci = 3.7×10^7 Bq) of carrier-free Na^{125}I in a calibrated reaction vial with 375 μg of Iodo-gen reagent for 30 min at room temperature with gentle shaking. After incubation, the iodinated peptide was removed and purified on a Sephadex G-75 gel column (Sigma, St. Louis, MO, USA) equilibrated with sterile and pyrogen-free 50 mM PBS (pH 7.4), to separate free ^{125}I from peptide-bound ^{125}I with the same buffer. The fraction containing ^{125}I-labeled rhPTH (1–34) was collected and the remaining fractions were discarded. The harvested ^{125}I-rhPTH (1–34) was more than 98.5% precipitable with trichloroacetic acid (TCA) (10%, v/v) in blank rat plasma, and its specific radioactivity was 2.17×10^5 Bq/μg (5.86 μCi/μg) peptide. Analysis of ^{125}I-rhPTH (1–34) preparations by SDS-PAGE indicated no major contamination by free ^{125}I or degradation products of the peptide.

2.1.2. Final formulation preparation

The ^{125}I labeled rhPTH (1–34) fraction was mixed with unlabeled rhPTH (1–34) in 50 mM PBS (pH 7.4) to achieve the final mixture peptide solutions for dosing used in rat experiments. For each of the three dosage groups, a stock mixture solution of ^{125}I-labeled and unlabeled rhPTH (1–34) was prepared based on the concentration of the peptide to be administered. In this way, each animal in the three different groups would receive a dose of 5, 10, or 20 μg/kg peptide, respectively, while with the same ^{125}I activity of 29 μCi/kg.

2.2. Animals

Healthy Wistar rats (male and female) weighing 220–250 g were purchased from Laboratory Animal Center, Tianjin Institute of Pharmaceutical Research (Tianjin, China). Prior to any experimental procedure, animals were acclimated for at least 1 week in Institute of Radiation Medicine of Chinese Academy of Medical Sciences & Peking Union Medical College (Tianjin, China), where all the studies were carried out. Four rats were housed in each cage in a room with controlled temperature (25 ± 1 °C) and humidity (55 ± 5%) and a 12-h light/dark cycle. Animals had free access to standard laboratory diet and water before the experiments. The animal experimental protocols were approved by the Institutional Ethical Committee for Care and Use of Laboratory Animal of National Institute for the Control of Pharmaceutical & Biological Products in accordance with the governmental guidelines on animal experimentation, National Institutes of Health "Principles of Laboratory Animal Care" and the United States Department of Agriculture "Animal Welfare Act".

2.3. Validation for radioactivity determination in plasma, tissues and excreta by TCA precipitation assay

Precipitation of the iodinated peptide by ice-cold 10% TCA was used to determine the peptide concentrations. The blank rat plasma, tissue homogenate and excreta samples were prepared and used in the preliminary experiments according to the protocol described below to examine the reliability of the TCA precipitation assay in the determination of radioactivity in rats. When ^{125}I-rhPTH (1–34) was incubated in vitro in blank rat plasma and tissue homogenate samples at 37 °C for up to 2 h, greater than 96% of the added radioactivity was TCA precipitable and recovered in the TCA precipitated pellet. However, after i.v. administration in rats, the ratio of TCA precipitable radioactivity to total radioactivity in the samples decreased time-dependently. Conversely, greater than 98% of the total radioactivity recovered from the excreta was not TCA precipitable and recovered in the supernatant. In addition, SDS-PAGE of plasma, tissue and excreta samples showed that only intact peptide was present in the TCA precipitation, while small-molecule fragments in the supernatant. Hence, TCA precipitable radioactivity rather than total radioactivity was used to calculate the ^{125}I-rhPTH (1–34) concentration in rat plasma and tissue homogenate samples. To obtain the mass balance information, however, total radioactivity instead of TCA precipitated radioactivity recovered from the excreta was used in the excretion studies. A series of calibration standards were prepared by adding seven concentrations (0–50 ng/ml) of radiolabeled rhPTH (1–34) into the blank plasma, tissue and excreta samples that were examined. The relationship between the added concentrations and counted radioactivity of the standards was evaluated. The results showed good relationships ($r^2 > 0.98$) and recovery (>95%) for the entire matrix and the linear range of the assays was found to be 0–50 ng/ml (data not shown). Plasma, tissue, urine, feces, and bile samples from blank rats were also counted to determine the background counts for each matrix.

2.4. Pharmacokinetic studies

The ^{125}I-rhPTH (1–34) was administered as a single s.c. or i.v. bolus injection in 50 mM PBS (pH 7.4) to rats. Dose volumes (3 ml/kg) were calculated based on the pretreatment body weights of animals, rounded to the nearest 0.01 ml and measured carefully in calibrated syringe to minimize the variability of the administered amount of rhPTH (1–34) to rats.

The rats were grouped randomly (four groups, $n = 6$ per group) based on their genders and body weights. Each rat in the three s.c. dosing groups was administrated a single s.c. injection at the nape of the neck with the mixture solution of unlabeled and ^{125}I-labeled rhPTH (1–34) at dosage of 5, 10 or 20 μg/kg, respectively. Another group of rats received a single i.v. bolus injection, via the tail vein, at the dosage of 10 μg/kg. Blood samples (∼100 μl) were collected from tail vein into heparinized tubes prior to dosing and 5, 10, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7 h after administration. In order to prevent the rhPTH (1–34) degradation, blood samples were immediately processed and plasma was then harvested by centrifugation at 2000 × *g* and 4 °C for 10 min. The ^{125}I-rhPTH (1–34)-associated radioactivity in the plasma samples was determined after precipitation with TCA (10%, v/v). Briefly, each plasma aliquot (50 μl) was added with 0.45 ml of ice-cold TCA (10%, v/v), vortex-mixed and incubated on ice for 30 min. The mixture were then centrifuged at 2000 × *g* and 4 °C for 10 min, and the supernatant, containing free ^{125}I or ^{125}I associated with fragmented peptide, was aspirated from each sample. The resultant TCA precipitate was then counted in a LKB Wallac Gamma Counter (Pharmacia LKB, Uppsala, Sweden) to determine the amount of ^{125}I radioactivity that remained associated with precipitable peptide. The result was expressed as nanograms of TCA-precipitable peptide per milliliter (ng/ml).

2.5. Tissue distribution studies

Four groups of rats ($n = 6$ per group) were administered a single s.c. injection at 10 μg/kg dose by the same way as described above. In an attempt to minimize the organ-specific uptake of ^{125}I by the thyroid gland, all animals also received a dose of 50 mg sodium iodide intraperitoneally, 12 h before the ^{125}I-rhPTH (1–34) dosing. The rats in the four groups were sacrificed by decapitation at 0.5, 1, 4, or 24 h postdosing, respectively, and blood samples (300–400 μl) were collected from the trunk into heparinized tubes immediately. Blood aliquots

were immediately processed, and the resultant plasma samples were analyzed for TCA-precipitable radioactivity in the gamma-counter as described above. The tissues or organs, including brain, thyroid, heart, lungs, liver, spleen, stomach, intestine, kidneys, testicle/uterus, muscle, adipose, femur and vertebra were excised, trimmed of extraneous fat, residual muscle and connective tissue, thoroughly rinsed of residual blood or contents with ice-cold 50 mM PBS (pH 7.4), and blotted dry. For radioactivity assay, small slices of tissues/organs were individually weighed, recorded, and homogenized twice at 8000 rpm for 30 s each time in 1 ml of ice-cold TCA (10%, v/v) immediately with samples in an ice bucket. Homogenization was performed using a Heidolph Diax 900 homogenizer (Heidolph Instruments GmbH & Co. KG, Germany). The homogenates were then centrifuged at $2000 \times g$ for 15 min at 4 °C and the pellet was used for counting in the gamma-counter.

The radioactivity in various rats tissues was expressed as the percent of the total injected dose recovered in each tissue/organ divided by the weight (in grams) of that tissue/organ, and further compared with that in plasma at all time points. The values therefore have units of percent per gram (%ID/g tissue/organ) and percent (%), respectively. For plasma, the volume (in ml) was used (i.e. %ID/ml) instead of the weight.

2.6. Excretion studies

To study the mass balance of the injected radioactivity, the urine, feces, and bile samples were measured for the total instead of TCA-precipitated radioactivity to reflect the total excretion of radioactivity during the period examined.

2.6.1. Urinary and fecal excretion

Urine and feces were collected the day before drug dosing for the background count in these matrices, and throughout the experiment for the measurement of total excreted radioactivity. Each of the rats ($n = 6$) received a single s.c. injection at 10 μg/kg dose by the same way as described above, and was then individually placed in a stainless-steel metabolic cage that allowed separate collection of urine and feces. They were provided with standard food and water ad libitum throughout the experiment. Total voided urine and total excreted feces samples, if present, were collected from each rat at intervals of 0–1, 1–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–36, 36–48, 48–60 and 60–72 h postdosing. The cages were rinsed with 20% EDTA to wash the urine residue, and the resulted fluid was collected together with the urine samples. The volume of each collected urine sample was recorded separately and 1 ml aliquots of each sample were directly counted for the total ^{125}I radioactivity in the gamma-counter. Fecal samples were individually weighed and homogenized with sufficient water to permit reasonably accurate pipetting and counted as that of urine.

2.6.2. Biliary excretion

Healthy Wistar rats were anesthetized by intraperitoneal injection with 0.5 ml of a cocktail containing ketamine (75 mg/kg) and xylazine (5 mg/kg). Following a midline abdominal incision, the common bile duct was exposed and the distal end ligated with a silk suture. The bile-duct was cannulated with a small length of PE-10 tubing (Clay Adams™, Becton Dickinson, Sparks, MD, USA) for collection of bile samples, and closed with surgical clips. After the rats woke up, ^{125}I-rhPTH (1–34) was then administrated as a single s.c. injection at dose of 10 μg/kg. Bile samples of each rat were collected at 0–1, 1–2, 2–4, 4–6, 6–8, 8–12, 12–18 and 18–24 h postdosing, and the volume of each collected samples was recorded separately. Then, the samples were processed by the same way as that of plasma, except that the total instead of TCA-precipitated radioactivity was counted.

2.7. Pharmacokinetic modeling and analyses

The concentration versus time profiles were obtained for each individual animal, and compartmental pharmacokinetic modeling and pharmacokinetic parameter calculation were performed using WinNonlin Program version 1.0 (Scientific Consulting Inc., Cary, NC).

After s.c. dosing and i.v. administration in rats, the plasma concentration versus time profiles were well fitted to a one-compartment and two-compartment open model, respectively, with a first order elimination rate constant. Model selection was guided by visual inspection of the plasma concentration versus time profiles, lowest Akaike's Information Criterion value (Akaike, 1976) and examination of residual variances. The basic parameters that were calculated include the maximum plasma concentrations (C_{max}), the time to reach C_{max} (T_{max}), area under the plasma concentration versus time curve from 0 h to extrapolation to infinity ($AUC_{0-\infty}$), half-life at α phase ($t_{1/2\alpha}$), half-life at β phase ($t_{1/2\beta}$), total body clearance (Cl), total body clearance corrected for bioavailability (Cl/*F*), volume of distribution corrected for bioavailability (*V*/*F*) and volume of distribution at the steady-state (V_{ss}). Dose-normalized C_{max} and $AUC_{0-\infty}$ were determined by dividing C_{max} and $AUC_{0-\infty}$ by the corresponding dosage, respectively.

2.8. Statistical analysis

All the data are expressed as mean ± standard deviation (S.D.), except where otherwise noted. Dose proportionality after a single s.c. administration of different dosages was determined by comparison of the dose-normalized C_{max} and $AUC_{0-\infty}$ across dosage levels using an ANOVA and linear regression analysis. Statistic analysis was performed using GraphPad Prism® program Version 3.0 (GraphPad Software Inc., San Diego, CA). Statistical significance was set as $P < 0.05$.

3. Results and discussion

rhPTH (1–34) is the first approved anabolic agent for osteoporosis treatment. To our knowledge, however, there are only limited data available on both preclinical and clinical pharmacokinetics and disposition of this peptide (Quattrocchi and Kourlas, 2004). The present studies report the pharmacokinetic properties, distribution and excretion patterns of rhPTH (1–34) in rats.

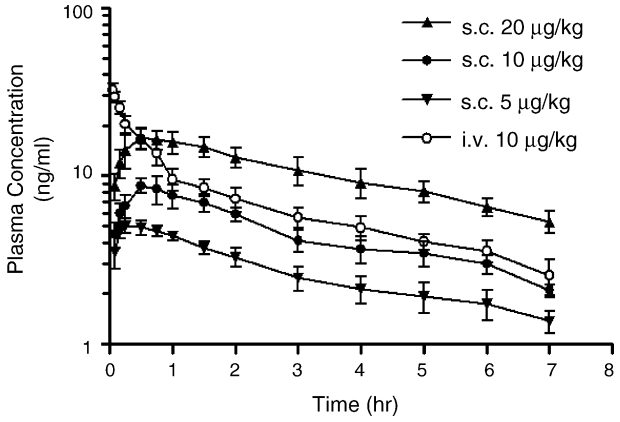

Fig. 1. The plasma concentration-time profiles of ^{125}I-rhPTH (1–34) following a single s.c. (5, 10 and 20 μg/kg, respectively) and i.v. bolus (10 μg/kg) administration in rats ($n = 6$ per group). Symbols are observed data.

3.1. Pharmacokinetics of rhPTH (1–34)

The estimated plasma TCA-precipitable peptide concentration versus time profiles followed mono-exponential and bi-exponential decline in rats after a single s.c. and i.v. bolus injection (Fig. 1), respectively. The corresponding compartmental pharmacokinetic parameters generated by fitting plasma concentration profile to a one-compartmental (s.c.) or two-compartmental (i.v.) open model are listed in Table 1.

Following a single i.v. injection at 10 μg/kg dose, TCA-precipitable peptide was rapidly distributed and then eliminated from rat plasma with $t_{1/2\beta}$ of 3.7 ± 0.9 h. After a single s.c. dosing, plasma TCA-precipitable peptide concentration profiles showed a rapid initial absorptive phase followed by a relatively slower $t_{1/2\beta}$ of 3.4–4.1 h in a mono-exponential fashion, which suggested a first order process. The short elimination half-life might be predominantly due to the degradation of the peptide in kidneys, liver and/or other peripheral sites including the skeleton as that of hPTH (1–84) (Martin et al., 1979). The absolute bioavailability after s.c. administration were estimated to be 79 ± 16%, 65 ± 7% and 82 ± 10% for 5, 10 and 20 μg/kg dose, respectively.

Dose linearity of the pharmacokinetics over the s.c. dosage range examined (5–20 μg/kg) was demonstrated. The C_{max} and $AUC_{0-\infty}$ values of the three doses indicated an apparent dose-proportionality (Fig. 2). The four-fold increase in dosage led to an approximately four-fold increase in C_{max} (4.8 ng/ml versus 16.6 ng/ml) and $AUC_{0-\infty}$ (25.2 ng h/ml versus 105.3 ng h/ml) (Table 1). Although T_{max} and $t_{1/2\alpha}$ seemed to increase with the dosages ($P < 0.05$), there were no significant differences for other parameters including Cl/F and $t_{1/2\beta}$ among the three dosages analyzed by ANOVA ($P > 0.05$). Therefore, our results support linear rather than non-linear plasma pharmacokinetics of rhPTH (1–34) across the investigated dosage range in rats (5–20 μg/kg).

The previously reported pharmacokinetic studies showed differing results in humans (Lindsay et al., 1993; Fraher et al., 1995) and rats (Codrons et al., 2003, 2004; Frolik et al., 2003), depending on the administration routes, assay methods, and pharmacokinetic models used. Lindsay et al. (1993) found that after s.c. injection of hPTH (1–34) to osteoporotic patients, the serum concentration increased rapidly with T_{max} of 15–45 min, and eliminated with $t_{1/2\beta}$ of 75 ± 5 min. The V and Cl of hPTH (1–34) were 100 l and 790 ml/min, respectively. However, Fraher et al. (1995) concluded from their investigation that $t_{1/2\beta}$ of hPTH (1–34) was 10.2 ± 0.5 min and Cl was 2.0 ± 0.4 l/min after bolus i.v. administration in healthy subjects. The T_{max}, $t_{1/2\beta}$ and absolute bioavailability in patients were also reported to be approximately 30 min, 3 h and 95%, respectively, after s.c. injection (Brixen et al., 2004; Quattrocchi and Kourlas, 2004) by other researchers. Similarly, previous studies on the pharmacokinetics of rhPTH (1–34) in rats also gave differing findings (Leone-Bay et al., 2001; Codrons et al., 2003, 2004; Frolik et al., 2003). In the present studies, similar T_{max} and $t_{1/2\beta}$, while a little lower

Table 1
Pharmacokinetic parameters estimated by one- or two-compartmental model analysis following a single i.v. or s.c. injection of ^{125}I-rhPTH (1–34) to Wistar rats ($n = 6$ per group)

Parameter	Dose and route of administration				
	i.v. 10 μg/kg	s.c. 5 μg/kg	s.c. 10 μg/kg	s.c. 20 μg/kg	P value
T_{max}[a] (h)	NA	0.29 ± 0.08	0.48 ± 0.06	0.56 ± 0.14	<0.05
$t_{1/2\alpha}$[b] (min)	15.6 ± 3.6	3.0 ± 1.2	2.7 ± 1.2	6.0 ± 1.8	<0.05
$t_{1/2\beta}$ (h)	3.7 ± 0.9	3.5 ± 0.8	3.4 ± 0.3	4.1 ± 0.9	>0.05
$AUC_{0-\infty}$ (ng h/ml)	63.9 ± 10.9	25.2 ± 5.5	42.6 ± 4.7	105.3 ± 13.3	>0.05
C_0 or C_{max}[c] (ng/ml)	34.2 ± 2.0	4.8 ± 0.2	7.8 ± 1.0	16.6 ± 2.8	>0.05
Cl or Cl/F[d] (ml/h/kg)	161 ± 27	182 ± 24	202 ± 21	200 ± 14	>0.05
V_{ss} or V/F[e] (l/kg)	0.7 ± 0.1	0.99 ± 0.04	1.18 ± 0.17	1.12 ± 0.23	>0.05
F_{abs}[a,f] (%)	NA	79 ± 16	65 ± 7	82 ± 10	>0.05

P values are obtained by evaluating the pharmacokinetic parameters across the three s.c. dosage groups using one-way ANOVA followed by Newman–Keuls t test. C_{max} and $AUC_{0-\infty}$ were normalized by the corresponding dosages when conducting comparison between the three s.c. administration groups.

[a] NA, not applicable for this administration route.

[b] $t_{1/2\alpha}$, represents distribution half-life and absorption half-life for i.v. and s.c. administration route, respectively.

[c] C_0 or C_{max}, for i.v. and s.c. administration route, respectively.

[d] Cl or Cl/F, for i.v. and s.c. administration route, respectively.

[e] V_{ss} or V/F, for i.v. and s.c. administration route, respectively.

[f] Calculated as (mean $AUC_{0-\infty\,s.c.}$/mean $AUC_{0-\infty\,i.v.}$) × (dose i.v./dose s.c.) × 100.

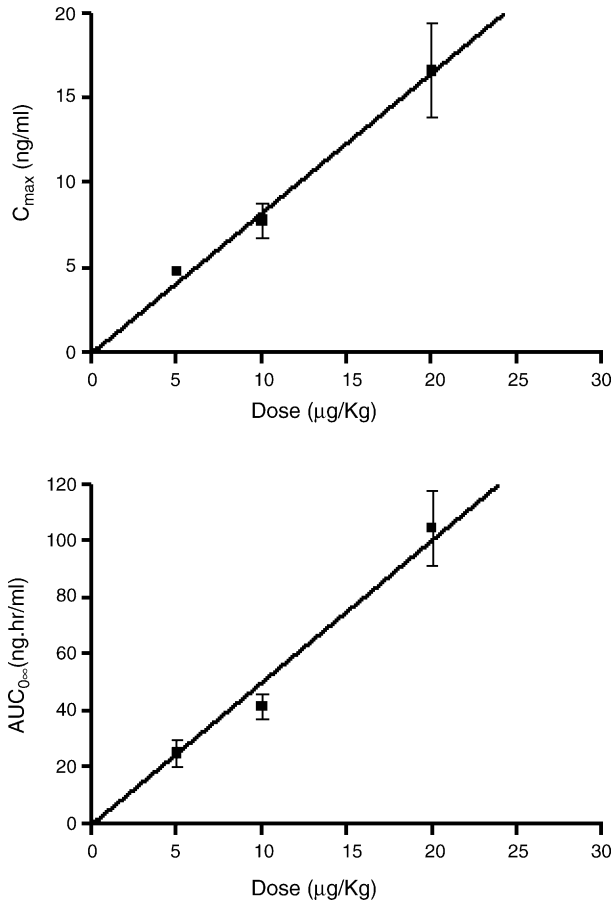

Fig. 2. The profiles of the mean plasma rhPTH (1-34) C_{max} and $AUC_{0-\infty}$ after a single s.c. administration versus the three investigated dosages (5, 10 and 20 μg/kg, respectively; $n = 6$ per group) in rats. The r^2 for C_{max} and $AUC_{0-\infty}$ are 0.975 and 0.991 respectively. The good fitting of the linear regression line indicates the linear disposition of rhPTH (1–34) in plasma over the investigated dosage range. Error bars for some time points are too small to be seen.

absolute bioavailability were observed when compared to the previous studies in human (Lindsay et al., 1993; Brixen et al., 2004; Quattrocchi and Kourlas, 2004). On the other hand, relatively longer $t_{1/2\beta}$ of TCA-precipitable peptide were found than those previously reported with rhPTH (1–34) in rats after s.c (Codrons et al., 2003; Frolik et al., 2003) and i.v. administration (Frolik et al., 2003; Codrons et al., 2004). The possible reason for this might due to the different sources of the tested peptide and assay methods utilized. The presence of inter-species and/or inter-strain differences might also contribute to such findings.

Recently, alternate delivery systems including oral delivery are becoming interesting approaches of biologically active protein/peptide drugs (Brown, 2005). A preclinical study reported on oral delivery of rhPTH (1–34) (Leone-Bay et al., 2001) in rats showed an essentially linear dose–response relationship after oral administration of rhPTH (1–34) at dosages of 100, 200 or 400 μg/kg, with the AUC of 12,891, 37,779 and 65,054 pg h/ml, respectively. The relative bioavailability of oral delivery to s.c. injection was 5%. Taking the different administration routes and dosages into account, our results in the pharmacokinetic study were highly comparable to such findings.

Extensive studies have been performed by various researchers to examine the pharmacodynamic and toxicities of rhPTH (1–34) in humans and animal models. Almost all the clinical trials used the dosage at 20 or 40 μg/day (Finkelstein and Arnold, 1999; Neer et al., 2001; Body et al., 2002; Finkelstein et al., 2003; Orwoll et al., 2003; Zanchetta et al., 2003). Based on the balance of skeletal benefits and adverse events, the preferred dose of rhPTH (1–34) was selected to be 20 μg/day (∼0.3 μg/kg/day) rather than 40 μg/day (Neer et al., 2001; Body et al., 2002). Therefore, the recommended dose of the FDA-approved use of rhPTH (1–34) in clinical setting is 20 μg/day by once-daily s.c. injection (Quattrocchi and Kourlas, 2004). However, the dosages used in animal models were much higher than those used in humans. In rat studies, the used dosages to achieve anabolic effects on bone varied from 5 to 200 μg/kg/day (Mosekilde et al., 1995; Li et al., 2001; Zhou et al., 2001; Andreassen and Cacciafesta, 2004; Lotinun et al., 2004) by once-daily s.c. injection. The dosages used in the present studies were chosen mainly according to the results of our pharmacodynamic and toxicological studies in rats on the tested rhPTH (1–34) in order to keep the results consistent and comparable. The tested peptide was found to be well tolerated and induce dose-dependently beneficial effects in the structural architecture and mechanical strength of the bones at the dosages of 5–40 μg/kg/day by once-daily s.c. injection in rats for 8 weeks (data not shown). Similarly, previous studies in rats showed that rhPTH (1–34) enhanced the fracture healing and increased the mechanical strength of fracture calluses in a dose-dependent manner at doses of 5–200 μg/kg/day (Andreassen et al., 1999; Holzer et al., 1999; Nakajima et al., 2002). However, once-daily s.c. injection of rhPTH (1–34) at 2 μg/kg/day failed to show significant effect on the mechanical strength of fracture calluses in rats (Nakajima et al., 2002). On the other hand, safety concern for rhPTH (1–34) was indicated in the previous study by the increased risk for osteosarcoma in rats even at 5 μg/kg/day by once-daily s.c. injection for 17 months (Vahle et al., 2002). Therefore, it was reasonable for us to choose the 5, 10 and 20 μg/kg/day as effective and safe dosages in our experiments.

3.2. Tissue distribution of rhPTH (1–34)

Tissue distribution of ^{125}I-rhPTH (1–34) was investigated following a single s.c. administration to rats at 10 μg/kg dosage. The data were expressed as a percent of injected dosage per gram tissue/organ or ml of fluid (%ID/g or %ID/ml) to compare the distribution of different tissues (Fig. 3). However, since the densities of the various tissues/organs are not identical, the concentrations in tissues/organs were further compared with that in plasma at all time points to compare the elimination rates more approximately (Fig. 4).

The results indicated that the TCA-precipitable peptide underwent a rapid and wide distribution in the tissues/organs throughout the whole body within the time course examined. Half an hour following s.c. administration, most of the tissues analyzed contained a significant amount of radioactivity. The TCA-precipitable radioactivity showed substantial disposition in lung, kidneys, spleen, thyroid and liver, which is similar to the

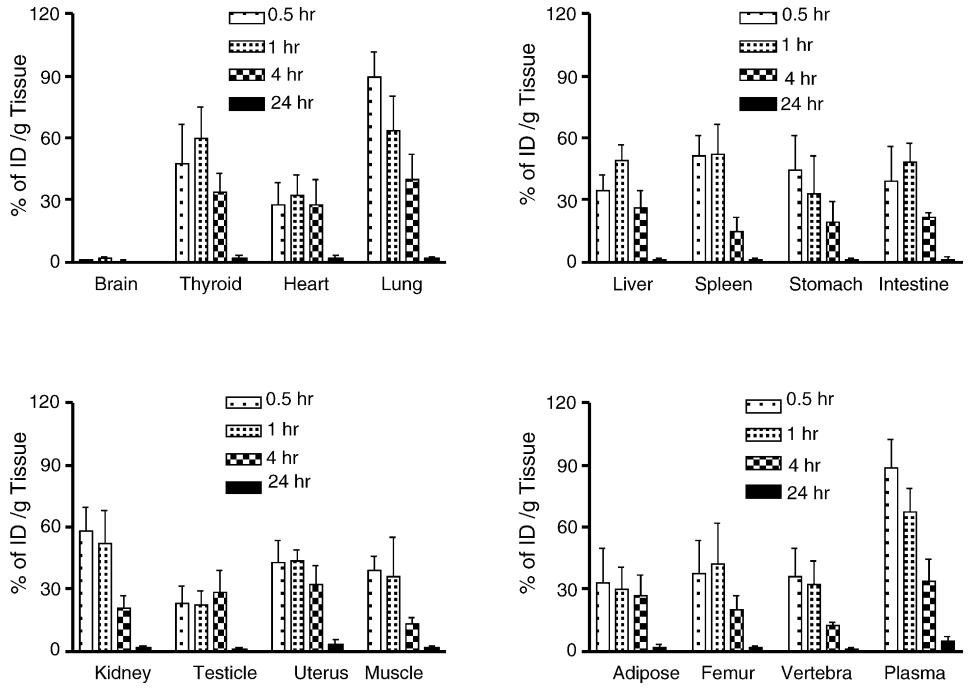

Fig. 3. The tissue and organ distribution of total radioactivity at 0.5-, 1-, 4- and 24-h time points after a single s.c. administration of ^{125}I-rhPTH (1–34) at 10 μg/kg in rats ($n = 6$ per time point), expressed as percents of the injected dose (ID) per gram tissue (1 ml of plasma is assumed to be 1 g in the weight). The tissues/organs were individually homogenized in 1 ml of ice-cold TCA (10%, v/v) immediately. The homogenates were then centrifuged and the TCA-precipitated pellet was used for counting in the gamma-counter. The recovery of ^{125}I-rhPTH (1–34) after TCA precipitation was proved to be more than 95% for all the tested tissues/organs in the preliminary validation experiments.

previous report on the distribution of ^{125}I-labeled hPTH (1–84) after i.v. injection in rats (Neuman et al., 1975). The highest radioactivity levels, $90 \pm 12\%$ and $89 \pm 13\%$ ID/g tissue were detected in lung and plasma, respectively, followed by kidneys ($59 \pm 12\%$) and spleen ($52 \pm 10\%$). For the clearance organs, kidneys and liver, kidneys initially absorbed much more labeled peptide than liver did, but the counts in kidneys peaked early (0.5 h postdosing) and decreased rapidly. However, the radioactivity in liver reached the maximum until 1 h, and decreased at a lower rate as compared to kidney. The amount of radioactivity detected in plasma and kidneys was found to decrease rapidly with time, followed by peak excretion in urine. There

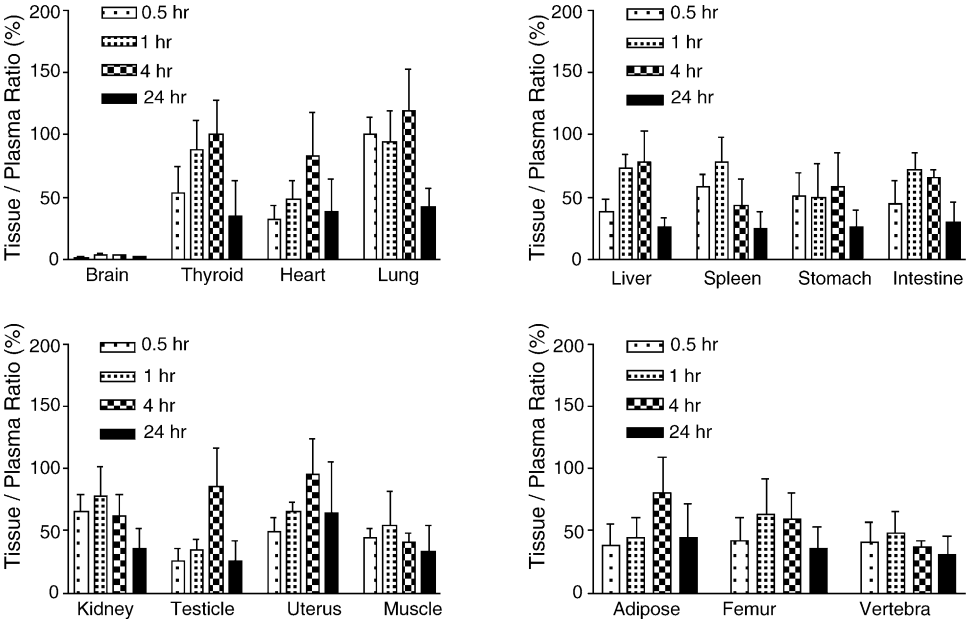

Fig. 4. The tissue/organ-to-plasma ratios of the radioactivity distribution at 0.5-, 1-, 4- and 24-h time points after s.c. administration of ^{125}I-rhPTH (1–34) at 10 μg/kg in rats ($n = 6$ per time point), expressed as tissue/plasma ratio (%) (1 ml of plasma is assumed to be 1 g in the weight when computing the ratio of tissue/organ to plasma).

was no marked increase in liver radioactivity with the time postdosing. As this study consisted of short-term single-dose experiments, however, we cannot conclude whether the circulating immune complexes could be developed during long-term repeated administration. Further studies are needed to explore the multiple dose pharmacokinetics and disposition of the peptide in rats and the possibility of antibody generation can be then investigated.

As expected, the skeletal tissues, i.e. femur and vertebra, were detected considerable and sustainable distribution of radioactivity. Substantial disposition of radioactivity were also observed in gastrointestinal tract at 0.5 h postdosing. TCA-precipitable radioactivity was also detectable in heart at 0.5 h postdosing. But the levels were found to be low (less than 30% ID/g tissue) in all animals, approximated that in skeletal muscle and adipose. Notably, reproductive tissues also absorbed considerable TCA-precipitable radioactivity. The accumulated TCA-precipitable radioactivity in brain was extremely low, as only 1% and 3% ID/g tissue was detected in brain at 0.5 and 1 h postdosing, respectively.

The amount of TCA-precipitable radioactivity sharply decreased with time in all the tissues examined. The amount of the radioactivity remaining in the body by 24 h postdosing was approximately 8%, calculated by totaling the urinary, fecal, and biliary excretion and subtracting from 100%. At 24 h following administration, only 5.2% and 2.2% ID/g was detected in plasma and lung, compared to 89.3% and 89.8% at 0.5 h postdosing, respectively. Moreover, only an average of 2.3% ID/g tissue was detected in all the rat tissues/organs examined at 24 h postdosing. Thyroid ($101 \pm 27\%$ at 4 h postdosing) and lung ($101 \pm 13\%$ at 0.5 h postdosing, and $119 \pm 35\%$ at 4 h postdosing) showed a tissue-to-plasma ratio slightly greater than 100%. In view of the large variability, however, such data did not suggest the accumulation of the peptide in these organs at the time points. The brain-to-plasma ratios were extremely low, which indicate that ^{125}I-rhPTH (1–34) cannot penetrate the blood-brain barrier (BBB) to an appreciable extent. These findings suggested that ^{125}I-rhPTH (1–34) was not likely to accumulate in the tissues/organs examined following a single s.c. administration.

These distribution properties revealed in our studies might be related to the distribution of PTH/PTHrP receptors. Urena et al. (1993) and Tian et al. (1993) reported that PTH/PTHrP receptor mRNAs are widely distributed in a variety of rat tissues, with highly expressed transcripts found in PTH target tissues, namely, kidney and bone (Urena et al., 1993). The findings in our study that rhPTH (1–34) were widely distributed to tissues and organs are basically consistent with these previously reported investigations. The concentrations of rhPTH (1–34) in the different tissues were also consistent with the expression levels of PTH/PTHrP receptor mRNA, such as kidney, testis and heart (Urena et al., 1993).

3.3. Excretion of rhPTH (1–34)

The excretion data after a single s.c. administration of 10 μg/kg ^{125}I-rhPTH (1–34) to intact and bile duct cannulated rats are illustrated in Figs. 5 and 6. Interestingly, more than

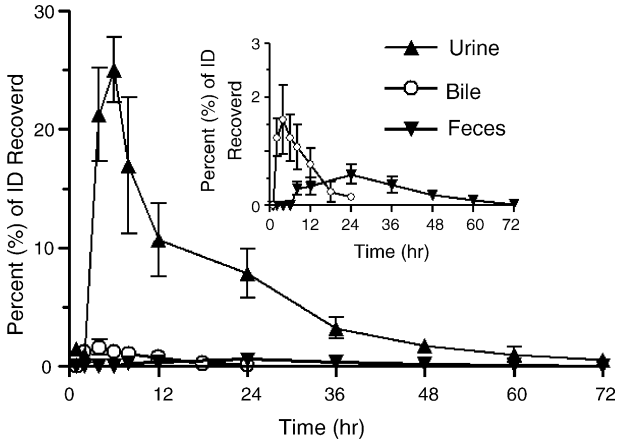

Fig. 5. The time-course profiles for excretion of total radioactivity from urine (including cage rinse), feces and bile following a single s.c. injection of ^{125}I-rhPTH (1–34) at the dosage of 10 μg/kg to intact or bile-duct cannulated rats ($n = 6$ per time point). ID, injected dose.

95% of the radioactivity excreted in urine, feces and bile was detected in the TCA supernatant, instead of in pellet (data not shown). This finding suggests that rhPTH (1–34) might be enzymatic degraded into low molecular fragment, which were not TCA-precipitable. Additionally, the free ^{125}I may account for the radioactivity in the supernatant. After single s.c. administration in rats, excretion of radioactivity was rapid and almost complete. The cumulative excretion of rhPTH (1–34) reached 92% of the administered radioactivity at 24 h, and was only marginally more, i.e. 99% at 72 h. Urinary excretion was the dominant route of elimination following s.c. administration, as 91% and 2% of administered radioactivity were recovered in urine and feces, respectively, in intact rats over 72 h. When ^{125}I-rhPTH (1–34) was administered to bile-duct cannulated rats, 6% of the dosed radioactivity was excreted into bile up to 24 h postdosing, which exceeded the amount of radioactivity excreted in the feces. Therefore, fecal excretion of radioactivity was mainly due to biliary excretion rather than the unabsorbed dose. Such findings might also suggest the possible presence

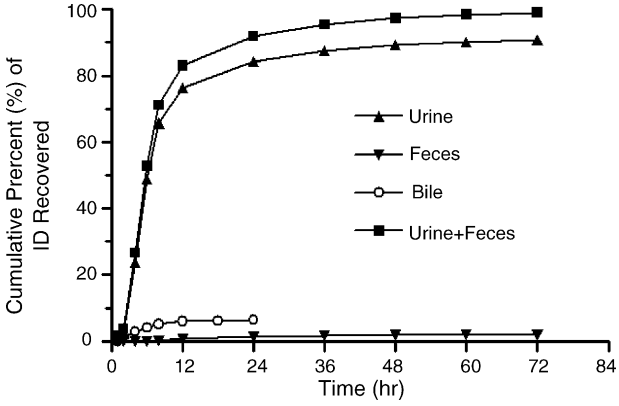

Fig. 6. The cumulative total radioactivity recovered from urine (including cage rinse), feces, and bile during the period examined (72 h postdosing for urine and feces, and 24 h postdosing for bile) following a single s.c. injection of ^{125}I-rhPTH (1–34) at the dosage of 10 μg/kg in rats ($n = 6$ per time point). ID, injected dose.

of the entero-hepatic circulation of the degraded fragments of the intact peptide. Fig. 5 also shows the excretion rate of total radioactivity in urine, feces, and bile respectively, which suggests that the excretion peaked during 4–6 h postadministration through urinary route.

Previous metabolic studies demonstrated that rhPTH (1–34) is predominantly eliminated by kidney and liver (Daugaard et al., 1994). Kidneys and bone are the primary target tissues of hPTH (Mannstadt et al., 1999). Furthermore, kidneys, by the way of proteolytic enzymes such as protease and peptidase, represent the critical organ for the elimination, re-uptake and catabolism of proteins in the size range (below 60 kDa) of rhPTH (1–34) (Modi, 1994; Tang et al., 2004). It has also been reported that the tissue distribution of other proteins such as humanized anti-IgE antibody (Fox et al., 1996) and recombinant human interleukin-11 (Takagi et al., 1995) is higher in kidneys and liver. These findings may reflect residual organ blood and possible metabolism and clearance of the studied protein by these organs (Fox et al., 1996). hPTH (1–84), the parent analogue of rhPTH (1–34), is primarily metabolized and eliminated in kidneys (Hruska et al., 1981) and liver by Kupffer cells and hepatocyte (Segre et al., 1981), as well as limited proteolysis by endopeptidases in other tissues such as blood, lungs and muscle (Modi, 1994). Maier et al. (1998) reported that such elimination patterns of PTH (1–84) might also apply to other human peptide hormones and could generally provide some insights into the elimination kinetics of human peptide hormones. The present pharmacokinetic and tissue distribution studies proved that rhPTH (1–34) followed the similar disposition pattern to that of PTH (1–84).

Kidneys play a more important role than liver does in the elimination of TCA-precipitable peptide. Thus, monitoring of the renal function may become necessary and dose adjustment is needed in patients whose kidney metabolic functions are impaired. Previous reports indicated that the extended exposure at low level of rhPTH (1–34) was more important for the pharmacodynamic effect (Putney and Burke, 1998), and the presumed clinical administration route for rhPTH (1–34) is multiple intermittent s.c. administration (Uzawa et al., 1995). Therefore, further experiments to study the multiple-dose pharmacokinetics and disposition of rhPTH (1–34) following intermittent s.c. administrations will be required.

4. Conclusion

To our knowledge, this is the first report to evaluate the pharmacokinetics, tissue distribution, and excretion of rhPTH (1–34) in rats. Following a single s.c. administration, the peptide experienced a rapid and considerable absorption, and was eliminated by first-order processes. The findings indicated linear disposition of rhPTH (1–34) at the examined s.c. dosage range, with C_{max} and $AUC_{0-\infty}$ being proportional to the administered dosages in rats. The s.c. administered TCA-precipitable radioactivity was widely distributed to most of the tissues/organs examined and sharply decreased with time. Therefore, the accumulation of rhPTH (1–34) in tissues and organs appeared to be low. The major elimination route of rhPTH (1–34) was urinary excretion. Lack of accumulation of radioactivity in feces, however, indicates little hepatic elimination or the hepatic elimination followed by entero-hepatic recirculation of the peptide's degradation products. The present pharmacokinetic study of rhPTH (1–34) in rats will provide helpful information for the design of pharmacodynamic animal models investigating the concentration–response relationships of this novel anabolic agent, especially when it is used in combination with other agents.

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