Kidney & Blood Pressure Research

Kidney Blood Press Res 2016;41:507-518

DOI: 10.1159/000443453
Published online: August 04, 2016

Accepted: June 13, 2016

Original Paper

Pharmacokinetic and Pharmacodynamic Characteristics of Subcutaneously Applied PTH-1-37

Wolf-Georg Forssmann[a] Hanns-Christian Tillmann[c] Dieter Hock[a]
Kristin Forssmann[c] Corrado Bernasconi[c] Ulf Forssmann[a] Rudolf Richter[a]
Berthold Hocher[d,e,f] Andreas Pfützner[b]

[a]IPF PharmaCeuticals GmbH, Hannover; [b]Institute for Clinical Research and Development, IKFE GmbH, Pfützner Science & Health Institute, Mainz, Germany; [c]Biomit Inc, Basel, Switzerland; [d]Department of Basic Medicine, Medical College of Hunan Normal University, Changsha, China; [e]Institute of Nutritional Sciences, University of Potsdam, Potsdam; [f]IFLb GmbH – Institut für Laboratoriumsmedizin, Berlin, Germany

Key Words
Pharmacokinetics

Abstract
Background/Aims: Parathyroid hormone (PTH) derivatives exert pronounced renal and osteoanabolic properties when given intermittently. The current study was performed to assess the pharmacokinetic and pharmacodynamic properties as well as safety of subcutaneously applied PTH-1-37 after repeated dosing in healthy subjects. ***Methods:*** This randomized, double-blind, dose-escalating, placebo and active comparator controlled study was conducted in 33 healthy postmenopausal women. Subjects were allocated to one of five treatment options: 10, 20, or 40 µg PTH-1-37, 20 µg PTH-1-34 or placebo, administered as once daily subcutaneous doses for three days. Plasma drug concentrations and serum levels of endogenous PTH-1-84, and calcium as markers of biological activity were monitored during the treatment. ***Results:*** PTH was absorbed rapidly from the subcutaneous tissue with a median t_{max} of 30 minutes for 20 and 40 µg of PTH-1-37. t_{max} was 45 minutes for 20 µg PTH-1-34. Elimination half-lives were estimated as 76 ± 34 min and 70 ± 13 min for 20 µg and 40 µg PTH-1-37 (mean ± SD), and 78 ± 34 for 20 µg PTH-1-34. Both PTH fragments (PTH-1-37 and PTH-1-34) increased serum calcium. For PTH-1-37 the effect on serum calcium was dose-dependent. Suppression of endogenous PTH-1-84 was seen after the application of both PTH-1-37 and PTH-1-34. During the study period, the subjects experienced no unexpected or serious adverse events. ***Conclusions:*** PTH-1-37 is rapidly absorbed after s.c. injection, has a short plasma elimination half-life, and does not accumulate during multiple dosing. Biological activity was demonstrated by rising serum calcium and decreasing endogenous PTH-1-84 in

Prof. Dr. Berthold Hocher
University of Potsdam, Institute of Nutritional Science, Arthur-Scheunert-Allee 114-116
14558 Nuthetal, Potsdam (Germany)
Homepage: http://www.uni-potsdam.de/eem, Email: hocher@uni-potsdam.de

blood plasma. The study drugs were well tolerated and safe. Our investigation presents data that PTH-1-37 is an excellent drug candidate for intervening with syndromes of dysregulation of calcium metabolism.

Introduction

Parathyroid hormone (PTH) is one of the major hormones modulating calcium and phosphate homeostasis and hence bone metabolism. PTH is synthesized in the chief cells of the parathyroid gland as a 115 amino acid polypeptide called pre-pro-PTH, which is cleaved within parathyroid cells at the N-terminal portion first to pro-PTH (90 amino acids) and then to PTH (84 amino acids). The latter is the major storage, secreted, and biologically active form of the hormone. PTH acts via stimulation of PTH receptors, G-protein coupled receptors, generating cAMP as second messenger when activated [1-3]. The N-terminal 34 amino acids of the 84-amino-acid parathyroid hormone are sufficient for the known endocrine biological activities of this peptide hormone. The naturally occurring hPTH-1–37 fragment as well as the hPTH-1–34 fragment maintain normocalcemia in blood via adenylate cyclase activation. The involvement of the phosphatidylinositol hydrolysis signaling pathway is also postulated for this function. The amino acids His14 to Phe34 are suggested to represent the receptor binding region, whereas the very N-terminal part of the peptide is required for stimulation of the cAMP-dependent signal transduction pathways [4, 5]. In particular, it was shown that N-terminal PTH fragments exhibit an influence on cell proliferation of skeletal tissues and on bone metabolism, thus playing meanwhile playing an important role in osteoporosis therapy. In women with postmenopausal osteoporosis, PTH increases bone mineral density more than anti-resorptive agents, and its use markedly reduces the incidence of new spine and non-spine fractures [6-8].

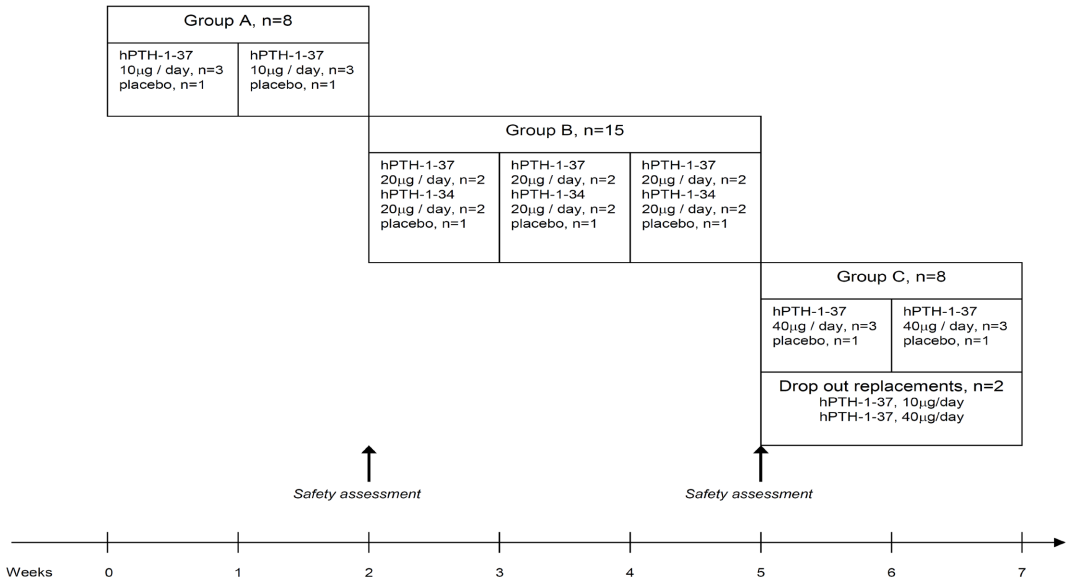
These studies were performed with the FDA-approved PTH fragment PTH-1-34. This drug is approved for the treatment of patients with advanced stages of osteoporosis and vertebral compression fractures, patients with a very high risk of fractures and low bone formation as well as patients with unmanageable hypoparathyroidism. Hypoparathyroidism in patients with end-stage renal disease is rare but associated with a very high morbidity and mortality. The morphological and biochemical features of this condition are known as low bone turnover disease in end-stage renal disease characterized by an absence of PTH or inadequately low PTH concentrations Low bone turn over disease is associated with a high fracture risk and an increase mortality – mainly cardiovascular diseases. Treatment of these CKD patients with biological active PTH fragments might be a suitable hormone replacement therapy superior to current approaches with bisphosphonates [6-8].

In a large randomized, double-blind placebo-controlled trial, PTH-1-34 given as daily subcutaneous dosis of 20 µg has been shown to decrease the incidence of vertebral fractures in elderly women by 65 % [9]. Studies in men with reduced bone mass, albeit of smaller size than trials in women, similarly showed beneficial effects of once daily s.c. PTH on bone mineral density and changes in biochemical markers of bone turnover as well as fracture incidence [10, 11].

In contrast to continuously elevated levels of PTH, which leads to net bone resorption, intermittent s.c. PTH administration with short-lasting plasma peaks can now be considered as an established bone building therapeutic concept [12].

As was demonstrated for example with natriuretic peptides [13, 14] the pharmacodynamics as well as the pharmacokinetics of peptide drugs may be modified by adding amino acids. PTH-1-34 is well studied with this respect [6-10]. However, the pharmacodynamics

Table 1. Study design and time-course of the study



and pharmacokinetics of the naturally occurring PTH-1-37 is less well characterized. Our study was thus performed to characterize the pharmacokinetic profile, pharmacodynamic effects, and tolerability of subcutaneously applied human PTH-1-37 in healthy postmenopausal women compared to placebo and PTH-1-34.

Table 2. Treatment groups and attribution to dosing

Treatment option	Group A	Group B	Group C
Dose	10 μg	20 μg	40 μg
PTH-1-37	n = 6	n = 6	n = 6
PTH-1-34		n = 6	
Placebo	n = 2	n = 3	n = 2

Methods and Subjects

Study Design

The study was approved by the local ethical committee. This was a randomized, double-blind, placebo and active comparator controlled, repeated dose, escalating dose study conducted in a single center over a study period of 11 weeks. Table 1 illustrates the study design, the time course of the study, and the disposition of subjects.

During the screening visit, inclusion and exclusion criteria were evaluated and subjects were randomized to one of the treatment options in the respective study group (Table 2). Subjects of study groups A and C were randomized in a 6 : 2 ratio (PTH-1-37 or placebo). In study group B, subjects were randomized in a 6 : 6 : 2 ratio (PTH-1-37, PTH-1-34, or placebo). Medication was administered in-house in the morning of each of the three consecutive study days subcutaneously into the abdominal wall.

Groups entered the study in the sequence A - B - C. The next higher dosing group was initiated after the lower dosing group had concluded at least the 3 in-house treatment days and a safety evaluation had been performed. An independent physician not involved with other aspects of the study assessed safety. Subjects were seen for a follow-up visit one day after the last of the three treatment days. A conclusion visit was performed one week later to record AEs and obtain blood samples for determination of PD parameters.

Subjects

After review and approval by the responsible ethics committee, the study was performed in accordance with "Good Clinical Practice", the Declaration of Helsinki, and all applicable regulations. After written informed consent was obtained from each of the individuals, 33 healthy postmenopausal women

who satisfied all inclusion requirements during their screening visit were enrolled into the study. The study population's demographic characteristics are listed in table 1. The study drug PTH-1-37 was synthesized according its amino acid sequence and tertiary structural properties for clinical use as recently described [15]. The product was prepared under GMP conditions. For active comparison, we used the marketed PTH-1-34 peptide.

Study assessment

Intensive sampling for analysis of drug concentrations was performed on the 3 treatment days. Blood samples were obtained in relation to administration of the study medication (t = 0) at t = - 15, 15, 30, 45, 60, 90, 120, 180, 360, and 1440 minutes on each day. For simplicity reasons the 1440 min sample post-dosing sample was taken as the –15 minute sample of the next treatment day.

Samples for determination of serum cAMP, calcium, and PTH-1-84 were drawn at t = - 15, 30, 60, 90, 120, 180, 360, and 1440 minutes. Biochemical markers of bone turnover were determined immediately before the first as well as 1 and 7 days after the last dosing.

Bioanalytical methods

PTH-1-34 and PTH-1-37 were measured using the Immunoradiometric Assay Kit for PTH-1-34 from Immutopics International (San Clemente, CA, USA). In a pre-study evaluation, this test had shown the best analytical recovery in human serum spiked with the study drug. This assay is known to detect biologically inactive PTH that has lost the first three amino acids [16]. Human PTH-1-84 was measured by using the Active PTH-Intact Elisa from Diagnostic Systems Laboratories Deutschland (Sinsheim, Germany). This assay exhibits no cross-reactivity with human PTH-1-34 or PTH from other species.

Serum calcium concentrations were determined photometrically using the Roche Diagnostics in-vitro diagnostic system in the central laboratory (IKFE, Mainz, Germany). Serum osteocalcin concentrations were measured using a commercially available, validated radioimmunoassay from Immutopics International (San Clemente, CA, USA). Serum osteoprotegerin concentrations were determined by means of a commercially available validated ELISA (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria). Bone-specific alkaline phosphatase (BSAP) concentrations were determined using a commercially available, validated ELISA (Metra Biosystems, Mountain View, CA, USA). Serum alkaline phosphatase was measured photometrically using the Olympus Diagnostics in-vitro diagnostic system in the central laboratory (IKFE, Mainz, Germany).

Urinary N-telopeptide concentrations were determined by using a commercially available, validated ELISA (Ostex International Inc, Seattle, WA, USA). Similarly, urinary deoxypyridinoline concentrations were also measured using a commercially available, validated ELISA (Metra Biosystems, Mountain View, CA, USA).

PK and statistical analysis

The pharmacokinetic evaluation for PTH-1-37 and PTH-1-34 including t_{max}, c_{max} , elimination half-life ($t_{1/2}$), and AUCs was performed using standard non-compartmental methods the software program WinNonlin Pro version 4.1 (Pharsight Product, Certara, Princeton, NJ, USA). Other statistical analyses were performed with SAS version 8 (SAS Institute Inc, Cary, NC, USA).

Assays for determination of PTH plasma levels were standardized with a lower limit of quantification of 25 pg/ml for PTH-1-34 and of 27.1 pg/ml for PTH-1-37, the difference owing to the difference in molar mass of the two peptides (4118 Dalton for PTH-1-34 and 4401 Dalton for PTH-1-37). This standardized sensitivity, however, was just insufficient to yield results that could be used in non-compartmental PK analyses following 10 µg of PTH-1-37. PK parameter estimates for this dose group are therefore presented also using assay read-outs between the upper quartile of the mean measured predose PTH level and the LLOQ (8.3 to 27.1 pg/ml). After post-hoc evaluation of plasma concentration-time curves, these assay readings were considered to be of sufficient reliability considering the exploratory character of this study.

All calculations to determine an area under the plasma concentration-time curve (AUC) were done using the trapezoidal method. The $t_{1/2}$ was calculated as ln2/z, where z is the terminal elimination rate constant. Intraindividual variability was estimated by determination of the coefficient of variation at t = 60 min after administration of study medication on the three consecutive treatment days. Exposition following s.c. PTH-1-37 compared to s.c. PTH-1-34 was calculated from the $AUC_{0-\infty}$ for plasma PTH-1-37 concentrations by dividing the respective AUCs after adjustment for differences in molar mass.

Table 3. Baseline characteristics of included subjects

	study population (n = 33)	hPTH-1-37 10 µg/day (n = 7)	hPTH-1-37 20 µg/day (n = 6)	hPTH-1-37 40 µg/day (n = 7)	hPTH-1-34 20 µg/day (n = 6)	Placebo (n = 7)
Age (years median)	57	61	54	53	64	56
(range)	(47-71)	(50-71)	(52-59)	(50-64)	(54-68)	(47-66)
Weight (kg)*	73.2 ± 9.5	71.2 ± 12.1	69.7 ± 10.3	73.4 ± 8.0	81.7 ± 8.8	70.9 ± 4.5
BMI (kg/m^2)*	27.0 ± 2.8	26.4 ± 3.4	26.8 ± 3.3	26.9 ± 3.0	29.4 ± 1.7	25.9 ± 1.6
Postmenopausal (years median)	10	11	10	4	13	11
(range)	(2-28)	(7-19)	(2-28)	(3-19)	(4-25)	(3-26)
hPTH-1-84 baseline pg/ml*	41 ± 16	49 ± 19	37 ± 19	41 ± 10	37 ± 16	41 ± 16

*(mean ± SD)

Table 4. Pharmacokinetic parameters of different doses of s.c. PTH-1-37 and PTH-1-34

parameters	Dose (µg)	c_{max} (pg/ml)	t_{max} (min)	$t_{1/2}$ (min)	$AUC_{0-\infty}$ ng*ml/min)
PTH-1-37	10	30.2 ± 3.5	70.7 ± 11.4	95.6 ± 11.8	5.8 ± 0.673
PTH-1-37	20	84.2 ± 11.6	42.5 ± 6.8	95.9 ± 17.5	12.9 ± 1.4
PTH-1-37	40	135.9 ± 13.6	43.6 ± 3.7	71.0 ± 5.5	17.8 ± 1.3
PTH-1-34	20	115.19 ± 4.9	50 ± 2.9	75.9 ± 10.9	17.7 ± 1.7

All values presented as means ± SE of the mean. c_{max} = maximal concentration; t_{max} = time to maximal concentration; $t_{1/2}$ = terminal elimination half life; $AUC_{0-\infty}$ = Area under the concentration time curve, extrapolated to infinity.

Analysis of variance models were used to compare levels of biochemical markers of bone turnover across the five treatment options one and 7 days after the last administration of study medication and to explore dose-response relationships. Means for every measurement point were calculated for serum cAMP and calcium, as well as endogenous plasma PTH-1-84. These means were used in repeated measures ANOVA models to investigate potential differences between the five treatment options in these parameters over time. A two-tailed *p*-value was used, with the required level of significance being $p < 0.05$.

Results

Demographic characteristics

The women with a median age of 57 years (range: 47 - 71) included in the study were well-balanced across treatment groups with respect to demographic parameters (Table 3). There were no statistically significant differences between groups for median age, average weight and BMI, as well as years since menopause. Mean baseline endogenous PTH-1-84 levels varied slightly between groups and were lowest in the PTH-1-34 group. The women in this group also had the highest median age and the highest mean BMI. However, there was no significant correlation between endogenous PTH-1-84 levels and these potentially predictive factors across the entire study population.

Pharmacokinetics

Both investigated PTH fragments showed fast absorption in the subcutaneous tissue. T_{max} following PTH-1-37 was reached for all dose groups at a median of 45 minutes after dosing and 60 minutes after dosing of PTH-1-34. Plasma levels of PTH fragments had receded to baseline levels within 360 min after administration of study medication. The 20 µg dose showed a $t_{1/2}$ of 76 ± 34 min (mean ± SD) for PTH-1-37 and 78 ± 18 min for PTH-1-34. Descriptive statistics of the pharmacokinetic parameters of the different doses of PTH-1-37 and the 20 µg dose of PTH-1-34 are given in Table 4.

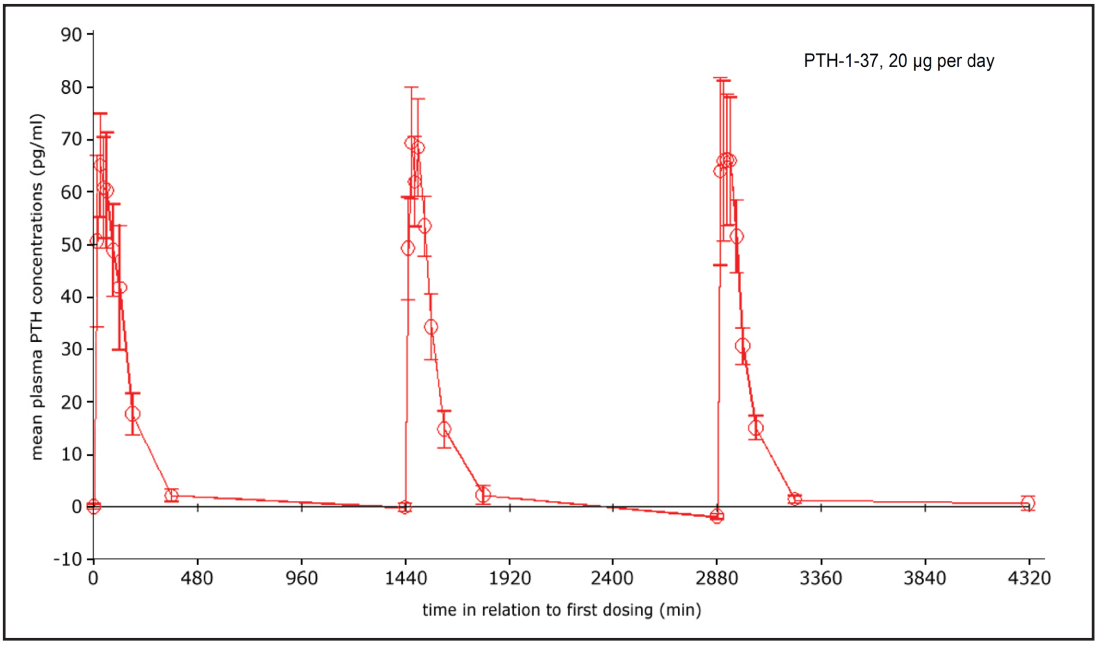


Fig. 1. Mean plasma PTH concentrations (± SE) following 20 µg PTH-1-37, summarized for all subjects (n = 6) over each period of the three treatment days are displayed.

The summarized plasma concentration-time profile following once daily dosing of 20 µg for three days is given in Figure 1. No accumulation was observed for any of the PTH peptides over the three treatment days. Intraindividual variability (CV %) at t = 60 minutes after dosing was 18 % for the 10 µg dose of PTH-1-37, 16 % for the 20 µg dose and 15 % for the 40 µg dose with 13 % for the 20 µg dose of PTH-1-34. Interindividual variability at t = 60 minutes was slightly higher at 24 % for the 10 µg dose of PTH-1-37, 34 % for the 20 µg dose and 22 % for the 40 µg dose with 11 % for the 20 µg dose of PTH-1-34.

Mean plasma concentrations were calculated for respective time points summarizing the 3 treatment days. In Figure 2 the resulting plasma concentration-time profiles for the different doses of PTH-1-37, PTH-1-34 and placebo are shown. Total exposure was estimated by calculating AUCs extrapolated from t = 0 to infinity. AUCs approximated linear dose-dependency following the different doses of PTH-1-37 (Table 4). Exposure following 20 µg of PTH-1-37 was 14.6 ± 1.9 min*ng/ml compared to 18.1 ± 2.8 min*ng/ml following 20 µg of PTH-1-34 which is 80 % measured on the basis of the AUC extrapolated to infinity.

Pharmacodynamics

Serum calcium and endogenous PTH-1-84 were measured as markers of immediate PTH biological activity (Figures 3 and 4), whereas levels of other serum parameters (BSAP, Osteocalcin, NTX, DPD) were assessed as indirect biochemical markers for bone turnover (Table 5).

Serum calcium showed a clearly dose-dependent increase following PTH administration with similar efficacy for 20 µg PTH-1-37 and PTH-1-34 (Figure 4). Endogenous PTH-1-84 was suppressed by all doses of PTH. However, no clear dose-dependency could be established for this effect. 10 µg of PTH seemed to be almost as effective as 40 µg whereas 20 µg PTH-1-37 of both, PTH-1-34, and PTH-1-37 result in a less pronounced effect.

Osteoprotegerin, bone-specific alkaline phosphatase, and osteocalcin as well as urinary N-telopeptide were evaluated as specific markers of bone turnover. These biochemical markers of bone turnover did slightly but not significantly change from baseline throughout the study.

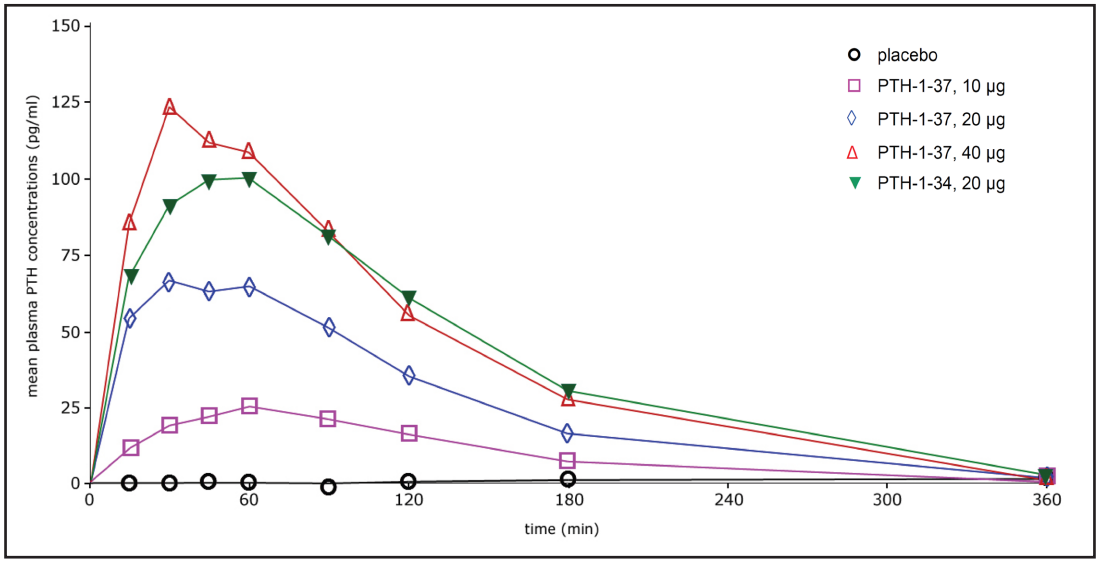


Fig. 2. Mean plasma PTH concentrations (± SE) summarized for all three treatment days by medication are displayed.

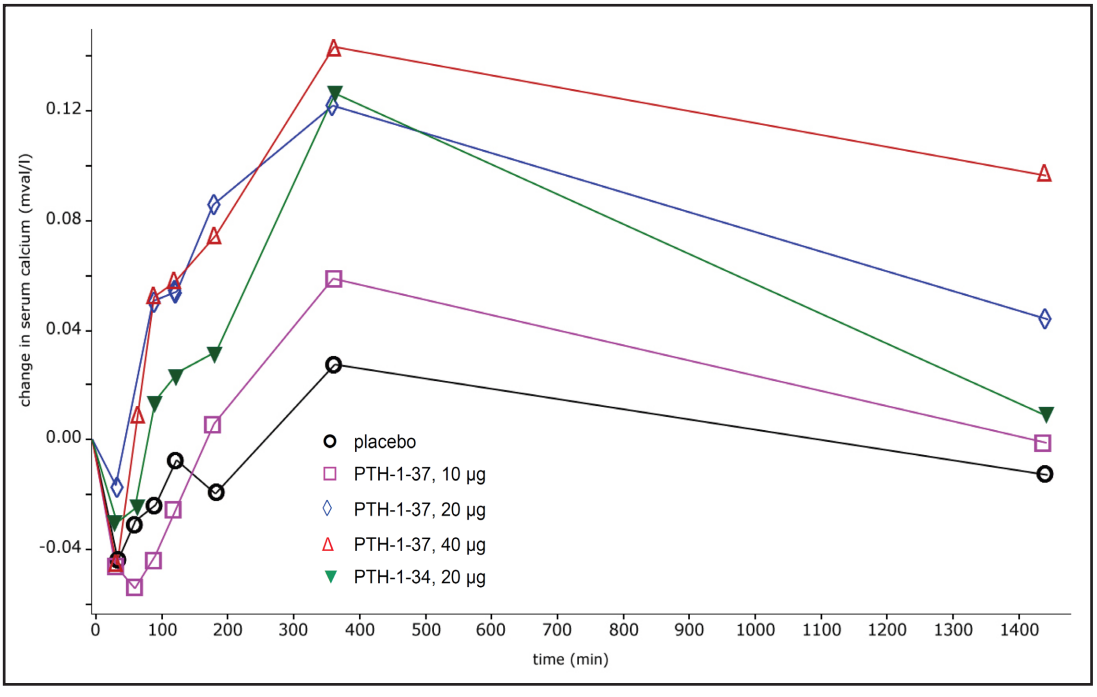


Fig. 3. Mean changes in serum calcium concentrations summarized for all three treatment days by medication are displayed.

Tolerability

Both PTH-1-37 and PTH-1-34 were well tolerated at all doses (see Table 6). No severe and no unexpected adverse events occurred. In particular, no macroscopically visible inflammatory reaction at the injection site was noted. Recorded adverse reactions with some

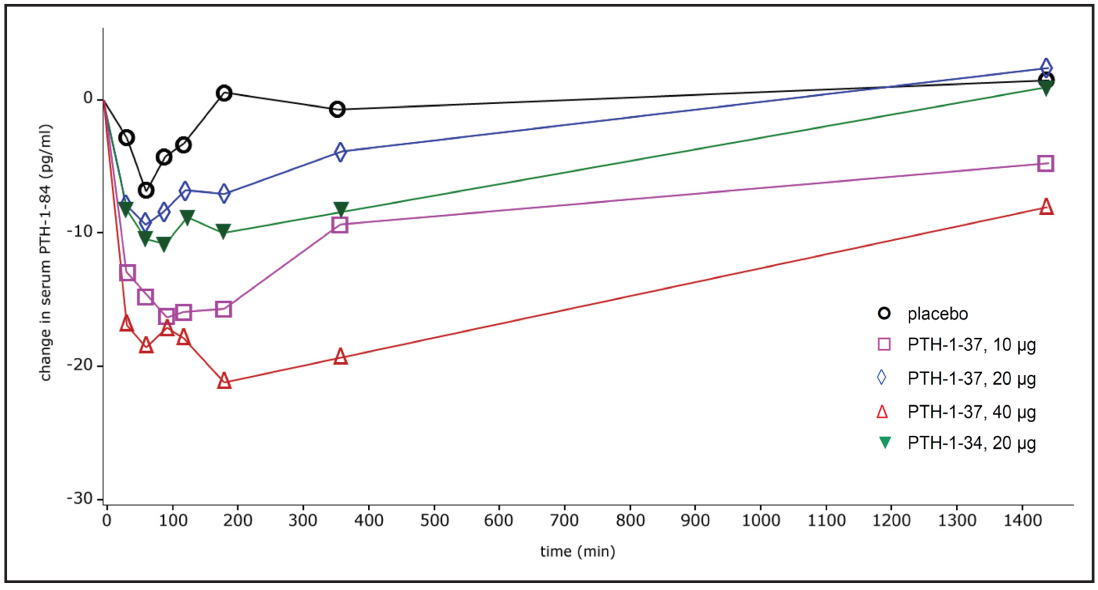


Fig. 4. Mean changes in serum PTH-1-84 concentrations summarized for all three treatment days by medication are displayed.

Table 5. Changes in biochemical markers of bone turnover

	hPTH-1-37 10 µg/day (n = 7)	hPTH-1-37 20 µg/day (n = 6)	hPTH-1-37 40 µg/day (n = 7)	hPTH-1-34 20 µg/day (n = 6)	Placebo (n = 7)
BSAP	-1 %	8 %	17 %	1 %	1 %
Osteocalcin	-5 %	19 %	3 %	3 %	0
NTX	-86 %	0	-28 %	-34 %	-46 %
DPD	-87 %	70 %	-21 %	-9 %	-55 %

Values are given as % change from baseline. Measurements immediately before first and 7 days after last dosing. Bone specific alkaline phosphatase (BSAP) and Osteocalcin as markers of bone formation, N-telopepide (NTX) and deoxypyridinoline (DPD) as markers of bone resorption.

Table 6. Incidence of adverse reactions classified as possibly related to study drug

Symptom	hPTH-1-37 10 µg/day (n = 7)	hPTH-1-37 20 µg/day (n =6)	hPTH-1-37 40 µg/day (n =7)	hPTH-1-34 20 µg/day (n =6)
Nausea	2	0	2	0
Xerostomia	2	0	0	0
Headache	1	0	2	0
Constipation	1	0	0	0
collapse (likely orthostatic)	0	0	1	0
flush	0	0	0	1

likelihood of being related to study medication were transient, mild in severity, and hardly ever required treatment. A listing of all adverse events possibly or probably related to study medication broken down by dose and body system is provided in Table 5.

Discussion

This double-blind placebo and active comparator controlled study demonstrated that PTH-1-37 is rapidly absorbed after s.c. injection, has a short plasma elimination half-life, and does not accumulate during multiple dosing. Biological activity was clearly evident as seen by a rise of serum calcium. The study duration was most likely too short to show distinct effects on markers of bone metabolism. In this short-term study, the PTH peptides were safe and well tolerated.

The pharmacokinetics of PTH-1-37 was clearly established as shown by the demonstrated dose dependency for maximal PTH-1-37 concentrations after s.c. injection of PTH-1-37 (c_{max}). The time course of the rise and fall of PTH-1-37 is short (short pulse of PTH-1-37). This is clearly a desirable property of any PTH drug candidate, since a short pulse is known to be associated with beneficial effects on bone turnover without causing a clinically important elevation of mean plasma calcium concentrations [7-9]. The pharmacokinetic profile of PTH-1-37 is thus in line with data reported on the pharmacokinetics of PTH-1-34 s.c. an approved drug for the treatment of postmenopausal osteoporosis, see data presented in this study and reported data on the marketed PTH-1-34 peptide [9]. Similar data is seen with PTH-1-84 s.c. [17]. The bioavailability of PTH-1-37 s.c. seems to be slightly lower compared to PTH-1-34 as indicated by differences in the area under the concentration time curve ($AUC_{0-\infty}$) of both peptides (Table 4). The differences in the bioavailability are most likely due to differences in the C-terminus of the peptides; since these are the only differences between the two peptides analysed in this present study (the used solvents were identical). The molecular mechanisms why differences in the C-terminus of the peptides cause differences in the bioavailability of the PTH fragments are not yet known. We have, however, to keep in mind that the sample size of this phase 1 study is small. Therefore, we only present descriptive statistics as is usually done in early studies in drug development. A final conclusion on differences in the bioavailability requires larger studies. This needs to be kept in mind when comparing our data to reports on PTH-1-34 bioavailability after pulmonary or oral application as well as data on s.c. PTH-1-84 bioavailability. These forms of application seem to have a lower bioavailability [18-20].

The N-terminus of the entire mature 84 amino-acid PTH molecule is responsible for its biological action on the PTH receptor. The amino acids His14 to Phe34 are suggested to represent the receptor binding region, whereas the very N-terminal part of the peptide is required for stimulation of the cAMP-dependent signal transduction pathways [4, 5]. In line with these findings our data shows a dose-dependent increase in calcium after s.c. injection of PTH-1-37. This fits in with reports on PTH-1-34 s.c. and the PTH-1-34 data in this present study. In human this peptide also stimulates a transient rise in calcium [7-9].

It is of note that we also saw a fall in endogenous human PTH-1-84 after subcutaneous injection of PTH-1-37 and PTH-1-34. To the best of our knowledge, this present paper is the first report showing that in human the injection of a PTH receptor ligand causes a fall in endogenous human PTH-1-84. The data is reliable from an analytical method point of view, since the human PTH-1-84 assay is a sandwich assay ensuring the detection of only intact whole PTH-1-84 molecules and the signal is generated only when one antibody binds to the N-terminus of the PTH molecule and the other binds to the C-terminus of the entire PTH-1-84 molecule [21-23]. PTH-1-37 lacking the C-terminus of the entire PTH-1-84 molecule can thus not generate a signal in the assay system we use. It is interesting that despite the fall of endogenous human PTH-1-84, the injection of PTH-1-37 as well as PTH-1-34 causes a transient rise in plasma calcium. This indicates that the C-terminal deletion of PTH fragments (PTH-1-37 as well as PTH-1-34) are more bioactive PTH receptor ligands as compared to the entire human PTH-1-84. This hypothesis needs to be proven in further studies for example by classical receptor binding studies or by modeling of the interaction of the PTH receptor with the entire human mature PTH 1-84 molecule and with the PTH fragment PTH-1-37. The molecular pathway why PTH-1-34 or PTH-1-37 decreases endogenous PTH-1-84 is not

known, but we suggest that this is due to a transient rise in calcium after s.c. injections of the PTH fragment. Rising calcium is a known suppressor of endogenous PTH secretion in the parathyroid gland [1-5].

Neither PTH-1-34 nor PTH-1-37 cause significant changes in biochemical markers of bone formation such as NTX and DPD, most likely due to the low numbers of patients in this phase 1 trail. .Trends, however, point into the expected direction. This is most likely due to the short study period and the small size of the study. Others reported that these parameters were affected after long-term treatment with PTH-1-34 [7-9]. In our present study, PTH-1-34 exhibits pharmacodynamic and pharmacokinetic properties comparable to PTH-1-37. Thus it is very likely that long-term treatment with PTH-1-37 would also have the beneficial effects on bone turnover/biochemical markers of bone formation known for PTH-1-34.

Both PTH-1-37 and PTH-1-34 are well tolerated at the investigated dosages. There was no local reaction detected at the injection site of the peptides. Besides a transient rise in calcium which is attributed to the mode of action of PTH-1-37 and PTH-1-34 (see above and introduction), there was no specific drug-related adverse event. This is in line with extensive safety reports on the FDA approved PTH-1-34.

Secondary hyperparathyreodism is often seen in patients with end-stage kidney disease [24-28]. However, there are meanwhile well established therapeutic options to cope with this condition such as reducing phosphate reupsorption by phosphate binders, vitamin D treatment, treatment with calimimetitics such as cinacalcet and finally removal (partially or total) of the parathyroid gland [29]. However, the opposite condition is also observed in CKD. Low bone ture-over disease due to absence of functionally active PTH is a rare but severe complication of CKD associated with both high fracture rates and mortality rate. So far we do not have good therapeutic options for these patients. Administration of active PTH peptides might be an option. However, this needs to be proven in adequately designed phase 2 and 3 trails.

Once again, the study duration and the number of participating subjects is a clear study limitation. On the contrary, this study shows the value of native human PTH-1-37 (hemoparatide) for clinical developments in several indications due to the safety, tolerability and putative bioactive parameters. Larger phase 2 and 3 studies are planned to prove definitively these properties for PTH-1-37.

Conclusion

Our double-blind, placebo and active comparator controlled ascending dose phase 1 study shows that PTH-1-37 is rapidly absorbed after s.c. injection, has a short plasma elimination half-life, and does not accumulate during multiple dosing. PTH-1-37 is well tolerated. No safety concerns rise from this phase 1 study. Biological activity is clearly evident as seen by rising of serum calcium. Larger long-term studies are required to establish beneficial effects on bone health in postmenopausal women and other syndromes.

Disclosure Statement

The authors state that they have nothing to disclose.

Acknowledgements

We thank Mirjam Löbig and the IKFE laboratory team for the excellent laboratory work, the study nurses and physicians at IKFE clinic as well as the Biomit coworkers for the support in running this clinical study. PTH-1-37.

References

1 Kumar R, Thompson JR: The regulation of parathyroid hormone secretion and synthesis. J Am Soc Nephrol 2011;22:216-224.
2 Souberbielle J-CP, Roth H, Fouque DP: Parathyroid hormone measurement in CKD. Kidney Int 2010;77:93-100.
3 Silver J, Naveh-Many T: Phosphate and the parathyroid. Kidney Int 2009;75:898-905.
4 Marx UC, Adermann K, Bayer P, Forssmann WG, Rösch P: Solution structures of human parathyroid hormone fragments hPTH(1-34) and hPTH(1-39) and bovine parathyroid hormone fragment bPTH(1-37). Biochem Biophys Res Commun 2000;267:213-220.
5 Hock D, Mägerlein M, Heine G, Ochlich PP, Forssmann WG: Isolation and characterization of the bioactive circulating human parathyroid hormone, hPTH-1-37. FEBS Lett 1997;400:221-225.
6 Deal C: The use of intermittent human parathyroid hormone as a treatment for osteoporosis. Curr Rheumatol Rep 2004;6:49-58.
7 Dempster DW, Cosman F, Parisien M, Shen V, Lindsay R: Anabolic actions of parathyroid hormone on bone. Endocrine Rev 1993;14:690-709.
8 Rubin MR, Cosman F, Lindsay R, Bilezikian JP: The anabolic effects of parathyroid hormone. Osteoporos Int 2002;13:267-277.
9 Neer RM, Aranud CD, Zanchetta JR, Prince R, Gaich GA, Reginster J-Y, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH: Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 2001;344:1434-1441.
10 Slovik DM, Rosenthal DI, Doppelt SH, Potts JT Jr, Daly MA, Campbell JA, Neer RM: Restoration of spinal bone in osteoporotic men by treatment with human parathyroid hormone (1-34) and 1,25-dihydroxyvitamin D. J Bone Miner Res 1986;1:377-381.
11 Kaufman J-M, Orwoll E, Goemaere S, San Martin J, Hossain A, Dalsky GP, Lindsay R,Mitlak BH: Teriparatide effects on vertebral fractures and bone mineral density in men with osteoporosis: treatment and discontinuation of therapy. Osteoporos Int 2005;16:510-516.
12 Lindsay R, Nieves J, Hennerman E, Shen V, Cosman F: Subcutaneous administration of the amino-terminal fragment of human parathyroid hormone-(1-34). kinetics and biochemical responses in estrogenized osteoporotic patients. J Clin Endocrinol Metab 1993;77:1535-1539.
13 Kimura K, Yamaguchi Y, Horii M, Kawata H, Yamamoto H, Uemura S, Saito Y: ANP is cleared much faster than BNP in patients with congestive heart failure. Eur J Clin Pharmacol 2007;63:699-702.
14 Charles CJ, Espiner EA, Richards AM, Nicholls MG, Yandle TG: Comparative bioactivity of atrial, brain, and C-type natriuretic peptides in conscious sheep. Am J Physiol 1996;270:R1324-1331.
15 Marx UC, Austermann S, Bayer P, Adermann K, Ejchart A, Sticht H, Walter S, Schmid F-X, Jaenicke R, Forssmann WG, Rösch P: Structure of human parathyroid hormone 1-37 in solution. J Biol Chem 1995;270:15194-15202.
16 Rucinski B, Mann GN, Epstein S: A new rapid and reproducible homologous immunoradiometric assay for amino-terminal parathyroid hormone in the rat. Calcif Tissue Int 1995;56:83-87.
17 Clarke BL, Kay Berg J, Fox J, Cyran JA, Lagast H: Pharmacokinetics and pharmacodynamics of subcutaneous recombinant parathyroid hormone (1-84) in patients with hypoparathyroidism: an open-label, single-dose, phase I study. Clin Ther 2014;36:722-736.
18 Pfützner A, Flacke F, Pohl R, Linkie D, Engelbach M, Woods R, Forst T, Beyer J, Steiner SS: Pilot study with technosphere/PTH(1-34) - a new approach for effective pulmonary delivery of parathyroid hormone (1-34). Horm Metab Res 2003;35:319-323.

19 Leone-Bay A, Sato M, Paton D, Hunt AH, Sarubbi D, Carozza M, Chou J, McDonough J, Baughman RA: Oral delivery of biologically active parathyroid hormone. Pharm Res 2001;18:964-970.
20 Schwietert HR, Groen EWJ, Sollie FAE, Jonkman JHG: Single-dose subcutaneous administration of recombinant human parathyroid hormone [rhPTH(1-84)] in healthy postmenopausal volunteers. Clin Pharmacol Ther 1997;61:360-376.
21 Hocher B, Armbruster FP, Stoeva S, Reichetzeder C, Grön HJ, Lieker I, Khadzhynov D, Slowinski T, Roth HJ: Measuring parathyroid hormone (PTH) in patients with oxidative stress - do we need a fourth generation parathyroid hormone assay? PLoS One 2012;7:e40242.
22 Tepel M, Armbruster FP, Grön HJ, Scholze A, Reichetzeder C, Roth HJ, Hocher B. Nonoxidized, biologically active parathyroid hormone determines mortality in hemodialysis patients. J Clin Endocrinol Metab 2013;98:4744-4751.
23 Hocher B, Oberthür D, Slowinski T, Querfeld U, Schaefer F, Doyon A, Tepel M, Roth HJ, Grön HJ, Reichetzeder C, Betzel C, Armbruster FP: Modeling of oxidized PTH (oxPTH) and non-oxidized PTH (n-oxPTH) receptor binding and relationship of oxidized to non-oxidized PTH in children with chronic renal failure, adult patients on hemodialysis and kidney transplant recipients. Kidney Blood Press Res 2013;37:240-251.
24 Kuczera P, Adamczak M, Wiecek A: Changes of Serum Total and Free Testosterone Concentrations in Male Chronic Hemodialysis Patients with Secondary Hyperparathyroidism in Response to Cinacalcet Treatment. Kidney Blood Press Res 2016;41:1-8.
25 Bohnert BN, Daniel C, Amann K, Voelkl J, Alesutan I, Lang F, Heyne N, Häring HU, Artunc F: Impact of phosphorus restriction and vitamin D-substitution on secondary hyperparathyroidism in a proteinuric mouse model. Kidney Blood Press Res 2015;40:153-165.
26 Artunc F, Nowak A, Müller C, Peter A, Heyne N, Häring HU, Friedrich B: Mortality prediction using modern peptide biomarkers in hemodialysis patients - a comparative analysis. Kidney Blood Press Res 2014;39:563-572.
27 Ishimura E, Okuno S, Okazaki H, Norimine K, Yamakawa K, Yamakawa T, Shoji S, Nishizawa Y, Inaba M: Significant association between bone-specific alkaline phosphatase and vascular calcification of the hand arteries in male hemodialysis patients. Kidney Blood Press Res 2014;39:299-307.
28 Nowak A, Artunc F, Serra AL, Pollock E, Krayenbühl PA, Müller C, Friedrich B: Sclerostin quo vadis? - is this a useful long-term mortality parameter inprevalent hemodialysis patients? Kidney Blood Press Res 2015;40:266-276.
29 Salam SN, Khwaja A, Wilkie ME: Pharmacological Management of Secondary Hyperparathyroidism in Patients with Chronic Kidney Disease. Drugs 2016;76:841-852.