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Velcalcetide (AMG 416), a novel peptide agonist of the calcium-sensing receptor, reduces serum parathyroid hormone and FGF23 levels in healthy male subjects

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ABSTRACT

Context. Velcalcetide, also known as AMG 416, is a novel, long-acting selective peptide agonist of the calcium sensing receptor. It is being developed as an intravenous treatment of secondary hyperparathyroidism (SHPT) in hemodialysis patients with chronic kidney disease—mineral and bone disorder.
Objective. To assess the safety, tolerability, pharmacokinetics and pharmacodynamics of velcalcetide in healthy male volunteers.
Methods. The study was a double-blind, randomized, placebo-controlled, single-dose, dose-escalation study in healthy males aged 18–45 years conducted at a single center. Each cohort included eight subjects randomized 6:2 to velcalcetide or placebo.
Intervention. Velcalcetide at 0.5, 2, 5 and 10 mg or placebo was administered intravenously.
Outcomes. Measurements included plasma ionized calcium (iCa), serum total calcium, intact parathyroid hormone (iPTH), phosphorus and fibroblast growth factor-23 (FGF23), 1,25-dihydroxyvitamin D, calcitonin and urine creatinine, calcium and phosphorus and plasma pharmacokinetics for velcalcetide. Vital signs, safety biochemical and hematological indices, and adverse events were monitored throughout the study.
Results. Intravenous administration of velcalcetide was well tolerated with no adverse reaction of nausea, vomiting or diarrhea reported. Velcalcetide mediated dose-dependent decreases in serum iPTH at 30 min, FGF23 at 24 h and iCa at 12 h post dose ($P < 0.05$) and in urine fractional excretion of phosphorus and increases in tubular reabsorption of phosphorus. Velcalcetide plasma exposure increased in a dose-related manner and the terminal elimination of half-life was comparable across the dose range evaluated and ranged from 18.4 to 20.0 h.
Conclusion. Single IV doses of velcalcetide were well tolerated and associated with rapid, sustained, dose-dependent reductions in serum PTH. The results support further evaluation of velcalcetide as a treatment for SHPT in hemodialysis patients.

Keywords: AMG 416, calcimimetic, FGF23, parathyroid hormone, velcalcetide

ORIGINAL ARTICLE

INTRODUCTION

Chronic kidney disease–mineral and bone disorder (CKD–MBD) is a systemic disorder of mineral and bone metabolism due to CKD. Secondary hyperparathyroidism (SHPT) is a common feature of CKD–MBD and is characterized by increased serum parathyroid hormone (PTH) levels, hypocalcemia and hyperphosphatemia and decreased serum 1,25-dihydroxyvitamin D ($1,25(OH)_2$ vitamin D) levels [1, 2]. Standard treatment of SHPT in CKD–MBD has relied upon combinations of various vitamin D sterols and dietary phosphate binders to normalize serum calcium reduce hyperphosphatemia and serum PTH. Exogenously administered calcitriol or its bioactive analogues reduce serum PTH levels. However, they promote hypercalcemia and hyperphosphatemia, limiting an effective dose that can be given safely [3]. Thus, standard treatment of SHPT in CKD–MBD is often not sufficiently effective to manage serum PTH levels on a long-term basis.

A major advance in the management of SHPT in dialysis patients was achieved with the introduction of allosteric calcimimetics [4]. These are the first agents that lower serum PTH without increasing the concentrations of serum calcium and

phosphorus [5]. Calcimimetics act as modulators of the calcium-sensing receptor (CaSR) [4]. The CaSR, a G-protein coupled receptor, enables the parathyroid cell and other CaSR-expressing cells involved in calcium homeostasis, such as in kidney and bone, to sense alterations in the level of serum calcium and respond with changes in activity that are directed at normalizing the serum calcium concentration [6, 7]. Presently cinacalcet (Sensipar®) is the only approved calcimimetic for treating dialysis patients with elevated PTH [5]. Although cinacalcet is a significant advance in the management of SHPT, its use has been limited by gastrointestinal (GI) tolerability and poor compliance [5, 8]. Thus, there is a need for other calcimimetic agents that have improved efficacy, better tolerability and increased compliance over cinacalcet.

Velcalcetide (AMG 416) is a novel long-acting peptide agonist of the CaSR [9]. In the 5/6 nephrectomized rat model, velcalcetide treatment reduced plasma PTH levels, markedly reduced ectopic calcification and attenuated parathyroid gland hyperplasia and downregulation of the CaSR, vitamin D receptor and fibroblast growth factor receptor 1 in the parathyroid glands [9]. These preclinical findings supported the development of velcalcetide as treatment for hemodialysis patients with SHPT.

This Phase 1 study was undertaken to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics of increasing single doses of velcalcetide administered by IV bolus injection to healthy adult male volunteers.

ORIGINAL ARTICLE

MATERIALS AND METHODS

Subjects

The clinical study protocol informed consent document and all other appropriate study related documents were reviewed and approved by the Alfred Ethics Committee Melbourne, Australia. Written informed consent was obtained from each subject prior to enrollment. A total of 32 healthy males aged 18–45 years were enrolled. Exclusion criteria included any chronic or acute illness, mental or legal incapacitation, participation in another clinical trial within the last 8 weeks, recent excessive alcohol or illicit drug use and any clinically significant abnormalities upon screening clinical examination or laboratory safety tests.

Study design

The study was a double-blind, randomized, placebo-controlled, single-dose, dose-escalation study in healthy young males conducted at a single center (Nucleus Network, Melbourne, Australia). Four cohorts enrolled eight subjects each and were randomized 6:2 to velcalcetide or placebo. The first cohort was treated with velcalcetide 0.5 mg and the dose was escalated through 2, 5 and 10 mg following review of safety data from the prior cohort. Subjects were admitted to Phase 1 unit 24 h prior to dosing and were observed for 48 h postdose in the Phase 1 unit. All subjects were dosed within a 3.25-h period in the morning allowing pooling of the placebo subjects across cohorts. Velcalcetide or 0.9% saline placebo was administered intravenously over 30–60 s. Adverse events (AE) were monitored through 48 h after study drug administration and serious adverse events were monitored through Day 7. Dose escalation was stopped at the 10 mg dose level.

Hematology, coagulation, chemistry, urinalysis, physical examinations, vital signs and 12-lead ECGs were monitored during the study. Continuous ECG monitoring by telemetry was performed for Cohort 1 only.

Subjects were fasted except for water at least 10 h prior to the collection of blood samples at predose, and at hour 24 and 48 postdose and for 2 h after study drug administration. A standard diet was provided for the duration of the subject's stay on the Phase 1 unit. Meals or snacks were not offered within 2 h prior to hour 12 blood collection.

Blood was obtained prior to dosing and every 2–6 h following dosing for 48 h and processed to serum, and stored at −70°C.

Timed urine collection

Urine was collected for determination of tubular reabsorption of calcium and phosphorus. Timed urine collection was done in 12-h intervals beginning 24 h (± 30 min) prior to study drug administration and continued through 48 h after study drug administration at six 12-h intervals. The following equations were used to calculate tubular reabsorption of calcium and phosphorus: Tubular reabsorption of calcium = [total serum calcium × 0.59 − (Uca × Scr/Ucr)]/(1 − 0.08 log [total Sca × 0.59/(Uca × Sca/Uca)]); and tubular reabsorption of phosphorus = [Sp − (Up × Scr/Ucr)]/(1 − 0.1 log[Sp/(Up × Scr/Ucr)]) [10].

Biochemical methods

All samples were assayed at central laboratories for intact parathyroid hormone (iPTH) using the electrochemiluminescence immunoassay on the Roche Elecsys® analyzer (normal range 15–65 pg/mL), FGF23 using FGF23 ELISA Kit, (Kainos Labs, Tokyo), calcitonin using IMMULITE® 2000 (Siemens Healthcare, Llanberis, Gwynedd, UK) (normal range: 0–8.3 pg/mL) and $1,25(OH)_2$ vitamin D using gamma-B $1,25(OH)_2$ vitamin D radioimmunoassay (Immunodiagnostics Systems Ltd, Scottsdale, AZ) (normal range: 17.5–70.4 pg/mL) according to vendor's instructions. Blood was collected into heparin evacuated tubes and ionized calcium (iCa) analyzed within 10 min utilizing the i-STAT System (Abbott Labs; normal range: 1.12–1.32 mmol/L).

Pharmacokinetics assessments

Blood samples for PK assessment were drawn predose, at 5, 10, 15, 20 and 30 min, and at 1, 1.5, 2, 2.5, 3, 4, 6, 12, 24, 36 and 48 h postdose. Blood samples were collected into K_2EDTA tubes and processed to plasma within 30 min and stored at −70°C. Plasma samples were analyzed for levels of velcalcetide using a validated liquid chromatography/mass spectrometry method. The lower limit of quantification of the assay was 0.2 ng/mL.

Noncompartmental methods of analysis using WinNonlin, version 5.2 (Pharsight Corp, Mountain View, CA), were used to estimate the pharmacokinetic parameters of velcalcetide. The pharmacokinetic parameters of velcalcetide, assessed at

each dose level included: (i) observed maximal plasma concentration (C_{max}), (ii) area under the plasma concentration–time curve from time 0 to ∞ (AUC_{INF}), (iii) total plasma clearance (CL), (iv) steady-state volume of distribution (V_{ss}) and (v) terminal elimination half-life ($t_{1/2}$).

Antibodies to velcalcetide

A validated enzyme-linked immunosorbent assay (ELISA) to assess the presence and concentration of antibodies to velcalcetide in human serum was developed by KAI Pharmaceuticals and the protocol provided to Millipore (St. Louis, MO) who performed the assay.

Statistical analysis

Data from all randomized subjects were included in the analyses. Summaries of subject disposition, demographics and baseline characteristics were examined by dose group.

All reported adverse events were coded to a standard set of terms, using the Medical Dictionary for Regulatory Activities (MedDRA®). A treatment-emergent adverse event (TEAE) was defined as an adverse event that occurred after the start of study medication administration or that was present prior to the start of study medication administration but increased in severity after treatment. Summaries of the incidence of treatment-emergent adverse events were presented by dose group.

Mean percent change in serum iPTH, iCa and phosphorus concentrations were plotted over time for each velcalcetide dose level and for the pooled placebo group. Urine creatinine, phosphorus and calcium concentrations were summarized by dose group using descriptive statistics.

The dose response trend in iPTH, FGF23 and iCa reductions were examined by fitting a first-order regression equation where percent change from baseline is modeled as a function of velcalcetide dose. Due to the variation in serum phosphorus levels, the following statistical analysis was conducted. Treatment differences in phosphorus trend over time was evaluated using the AUC and was examined by analysis of variance with Dunnett–Hsu adjustment. This type of statistic is advantageous from both statistical and biological stand points. Statistically, it simplifies the analyses by creating a single summary response from multiple measurements and also increases the power of testing without sacrificing the information contained in the multiple measurements. Biologically, it provides a means to incorporate both intensity and sensitivity contained in repeated measurements into the statistical analyses [11]. Analysis of percent change in iPTH at 30 min postdose was performed using the analysis of covariance with treatment and baseline value as a covariate with Dunnett–Hsu adjustment.

RESULTS

The demographic and mineral metabolic variables were not different among the treatment and placebo groups. The only exception was the mean baseline $1,25(OH)_2$ vitamin D which was higher in the placebo group compared with the velcalcetide 0.5 mg group ($P < 0.05$) (Table 1), but was not significantly different between velcalcetide treatment groups.

Response in serum iPTH, calcium, phosphorus and FGF23

There were no changes in the placebo group, other than those from diurnal variation (Figure 1). Treatment with velcalcetide at doses of >0.5 mg resulted in dose-dependent decreases in iPTH. The decrease in iPTH in the 2, 5 and 10 mg dose groups was observed 10 min postdose (data not shown). Thirty minutes postdose, serum iPTH was reduced by 3.5, 21.7, 55.4, 69.0 and 72.6% in the placebo, 0.5, 2, 5 and 10 mg dose groups, respectively (Figure 1A). The percent change in

Table 1. Demographic and baseline characteristics for study subjects

	Placebo	Velcalcetide			
		0.5 mg	2 mg	5 mg	10 mg
No. of subjects	8	6	6	6	6
Age					
Mean	22.4	27.8	26.8	24.2	26.0
SD	3.5	8.9	4.9	4.4	6.2
Median	21.5	24.0	26.0	23.0	24.5
Range	18–27	20–43	22–35	20–32	20–34
Race (%)					
White	75	67	100	50	67
Asian	25	33	0	33	33
Other	0	0	0	17	0
BMI (kg/m^2)					
Mean	23.8	24.4	23.8	23.5	24.8
SD	2.6	2.7	1.1	1.8	2.2
Median	23.2	23.6	23.5	23.3	24.8
Range	21–28	21–29	23–26	21–26	21–28
Serum creatinine (mg/mL)					
Mean	0.80	0.88	0.85	0.79	0.83
SD	0.07	0.09	0.07	0.02	0.10
Median	0.79	0.87	0.81	0.80	0.80
Range	0.67–0.93	0.78–0.98	0.80–0.95	0.76–0.81	0.74–1.02
Serum iPTH (pg/mL)					
Mean	38.3	33.5	35.3	36.8	40.7
SD	16.9	19.0	10.6	11.2	22.0
Median	31.5	30.0	35.0	35.5	33.5
Range	24–71	15–68	21–49	24–54	23–84
Plasma ionized calcium (mmol/L)					
Mean	1.22	1.16	1.23	1.25	1.21
SD	0.03	0.03	0.02	0.04	0.05
Median	1.26	1.15	1.23	1.24	1.21
Range	1.16–1.25	1.13–1.21	1.21–1.27	1.21–1.32	1.16–1.26
Serum phosphorus (mmol/L)					
Mean	1.12	1.02	1.13	1.14	1.15
SD	0.16	0.08	0.14	0.12	0.11
Median	1.18	0.98	1.10	1.16	1.18
Range	0.84–1.31	0.96–1.14	0.98–1.30	0.99–1.32	0.98–1.27
Serum $1,25(OH)_2$ vitamin D (pg/mL)					
Mean	57.0	41.9	43.4	45.9	46.3
SD	12.5	6.3	9.5	9.5	14.1
Median	54.0	40.6	40.3	47.4	40.2
Range	39–73	35–51	35–59	35–57	33–66
Serum FGF23 (pg/mg)					
Mean	31.6	24.2	32.3	30.6	36.9
SD	5.4	6.5	11.6	7.1	12.2
Median	33.6	23.7	26.8	29.1	34.7
Range	25–37	14–33	21–50	22–40	23–55

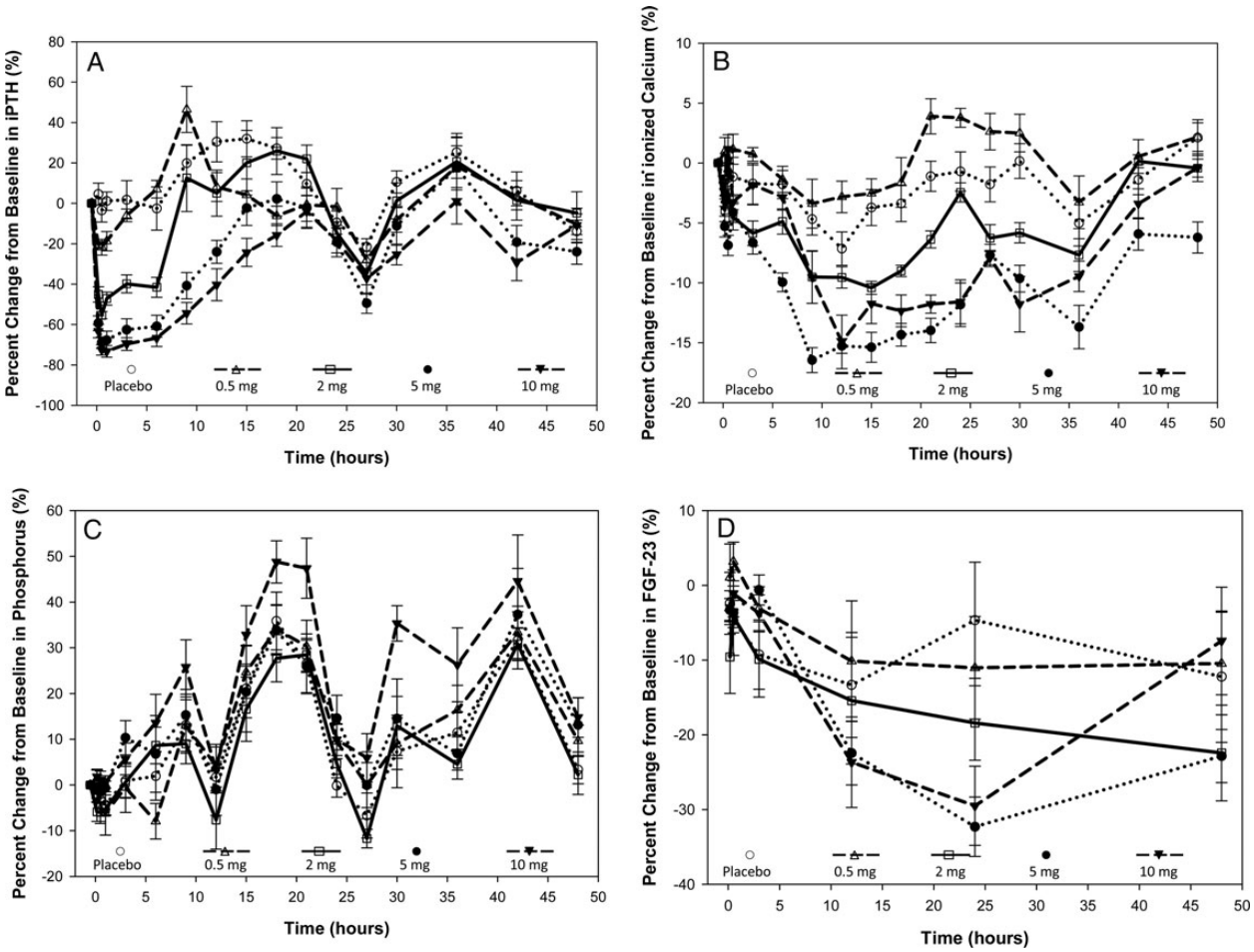

FIGURE 1: Percent changes from baseline in serum iPTH (**A**), plasma iCa (**B**), serum phosphorus (**C**) and serum FGF23 (**D**) between the velcalcetide treatment groups and the placebo over the course of the study. The percent change in iPTH at 30 min was significantly different from the placebo for all the velcalcetide dose groups ($P < 0.01$ for the 0.5 mg group; $P < 0.0001$ for the 2, 5 and 10 mg groups). Velcalcetide mediated a significant ($P < 0.05$) dose-dependent reduction in plasma iCa at 2, 5 and 10 mg at the 12 h time point. The changes in serum phosphorus levels were not significantly different from the placebo ($P < 0.076$). Velcalcetide (5 mg and 10 mg) mediated significant reductions in FGF23 compared with the placebo ($P < 0.05$). Data are shown as mean±SEM.

iPTH at 30 min was significantly different from the placebo for all the velcalcetide dose groups ($P < 0.01$ for the 0.5 mg group; $P < 0.0001$ for the 2, 5 and 10 mg groups). The long-term response in serum iPTH followed the diurnal rhythm and, gradually attenuated by 24 h (Figure 1A) and 48 h, the value was back to the placebo control level.

Velcalcetide at 0.5 mg and placebo were associated with no change in iCa (Figure 1B). Velcalcetide mediated a significant ($P < 0.05$) dose-dependent reduction in plasma iCa at 2, 5 and 10 mg at the 12 h time point. The response was present at 6 h postdose, was maximal between 15 and 18 h postdose and returned to baseline by 48 h postdose (Figure 1B).

Serum phosphorus levels varied diurnally (Figure 1C) and changes were not different in the placebo group and the velcalcetide 0.5, 2 and 5 mg dose groups. Compared with placebo, the serum phosphorus AUC in the 10 mg dose group tended to be higher but the difference was not significant ($P = 0.076$).

Velcalcetide treatment was associated with a dose-dependent decrease in serum FGF23 that was most apparent at the 24-h postdose (Figure 1D; $P < 0.05$). The 5 and 10 mg doses of velcalcetide mediated robust changes in FGF23 were significantly different from the placebo ($P < 0.05$). Following dosing with velcalcetide, serum 1,25$(OH)_2$ vitamin D concentrations did not differ from placebo (change from baseline: −3.0, −0.1, 2.3, −0.4 and −2.0 pg/mL for placebo, velcalcetide 0.5, 2, 5 and 10 mg, respectively). Neither did calcitonin levels change significantly.

Response in renal tubular reabsorption of phosphorus and calcium

Velcalcetide mediated a dose-dependent increase in tubular reabsorption of phosphorus at the 0–24 h (Figure 2A). At this time period, velcalcetide at 10 mg significantly ($P < 0.05$) increased tubular reabsorption of phosphorus by ~50%. Phosphorus reabsorption returned toward baseline in the 24–48 h time period.

Velcalcetide or placebo had no effect on fractional excretion of calcium (Figure 2B). At 10 mg, there was a trend to decrease in tubular reabsorption of calcium for both the 0–24 and 24–48 h time periods (Figure 2B), but the trend was not significant.

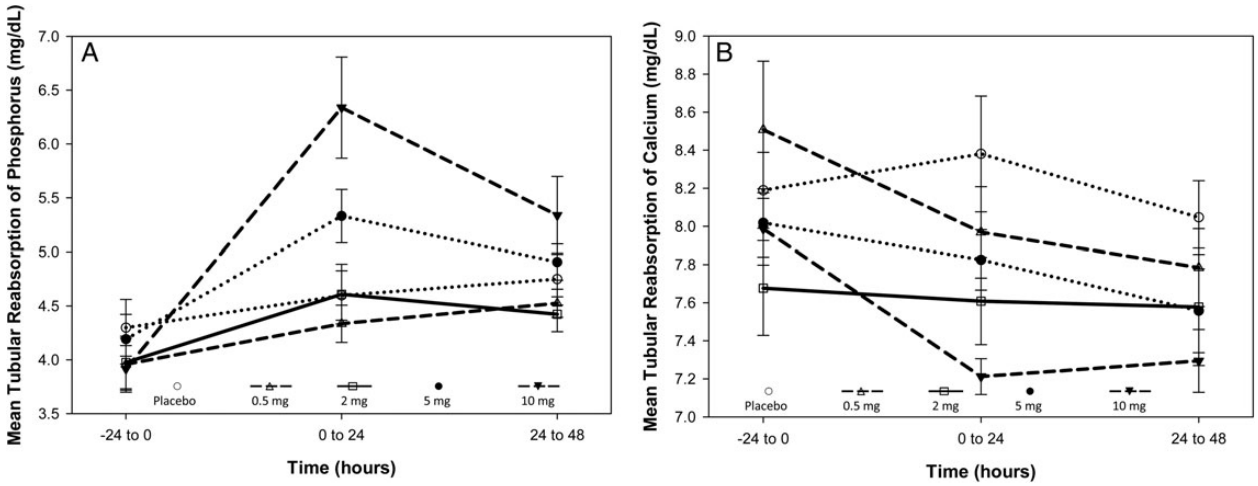

FIGURE 2: Mean tubular reabsorption of phosphorus (**A**) and calcium (**B**) for the velcalcetide treatment groups and placebo. At the 0–24 h time point, velcalcetide at 10 mg significantly ($P < 0.05$) increased tubular reabsorption of phosphorus. Values are mean ± SEM.

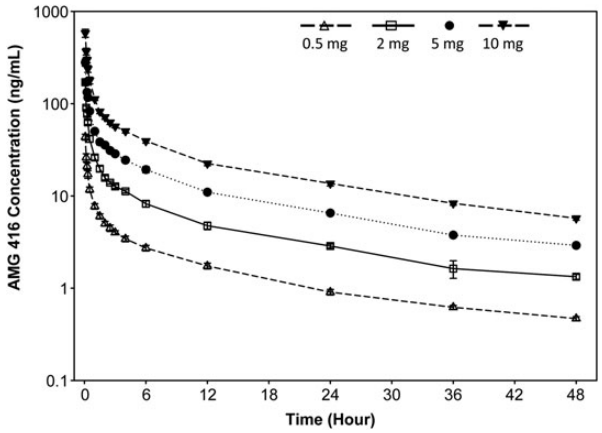

FIGURE 3: Mean velcalcetide plasma concentrations (ng/mL) time course following single intravenous doses of velcalcetide to healthy male subjects. Values are mean ± SEM ($n = 6$ per time point).

Velcalcetide pharmacokinetics

Following single intravenous administration, plasma concentrations of velcalcetide increased with increasing doses across the dose range of 0.5–10 mg. Similarly, mean C_{max} and AUC_{INF} values increased in a dose-related manner (Figure 3 and Table 2). The plasma concentration–time courses of velcalcetide showed multicompartmental disposition characteristics. Terminal elimination of half-life values were comparable across the dose range evaluated and ranged from 18.4 to 20 h. Clearance and volume of distribution remained reasonably constant over the dose range evaluated with mean parameter values ranging from 5.34 to 8.07 L/h and 112–159 L, respectively. Interindividual variability was low with a coefficient of variation of ~11% based on the AUC_{INF} values.

Adverse events

Seven subjects reported eight TEAE, five in the velcalcetide and three in the placebo group (Table 3). Three TEAEs (feeling hot, abdominal discomfort and paresthesia) all of which were mild in intensity, were considered related to study medication (Table 3). Three TEAEs were reported in two subjects treated with placebo (abdominal pain, mild lethargy and diarrhea). The percentage of subjects reporting TEAEs following administration of velcalcetide were comparable with the percentage of subjects reporting TEAEs following administration of placebo. There were no increases in the number of TEAEs or the number of subjects who reported TEAEs as velcalcetide dose increased. TEAEs were mild ($n = 7$) or moderate ($n = 1$) in intensity. All TEAEs resolved without sequelae prior to completion of the study. No subject discontinued due to adverse events. Antibodies to velcalcetide were not detected in serum from subjects treated with velcalcetide at all dose levels.

DISCUSSION

The results of the Phase 1 study indicate that administration of velcalcetide, a CaSR agonist, by IV bolus in doses ranging from 0.5 to 10 mg was safe and well tolerated in healthy males with no antidrug antibody formation occurring. A single dose of velcalcetide lowered serum iPTH within 30 min of administration in a dose-dependent manner with the response largely attenuated by 24 h. These changes in iPTH were followed by corresponding changes in serum calcium and phosphorus homeostasis. Serum iPTH concentrations decreased within 10 min after IV doses of velcalcetide reaching a nadir by 1 h and returning towards baseline by 24 h. This dose–response was maintained within the diurnal variation pattern in serum iPTH. Further, it was observable although much diminished, during the following second 24 h period over which subjects were studied. These results indicate that velcalcetide has a very rapid and prolonged effect on the CaSR in the parathyroid gland and, incidentally, also suggest that the mechanism responsible for diurnal variation in iPTH is not CaSR mediated. In contrast, cinacalcet, a reported allosteric calcimimetic [4], when given orally showed a nadir in inhibition of iPTH secretion at 4 h

Table 2. Summary human plasma velcalcetide pharmacokinetic parameters sorted by cohort and dose

Cohort	Dose (mg)	Subject	C_{max} (μg/L)	AUC_{INF} (h × μg/L)	CL (L/h)	V_{ss} (L)	$t_{1/2}$ (h)
1	0.5	Geometric mean (CV% geometric mean)	42.9 (18.9)	93.6 (13.0)	5.34 (13.0)	112 (18.0)	19.4 (6.9)
2	2	Geometric mean (CV% geometric mean)	168 (20.2)	293 (11.3)	6.83 (11.3)	136 (17.5)	20.0 (10.5)
3	5	Geometric mean (CV% geometric mean)	273 (25.1)	620 (11.5)	8.07 (11.5)	159 (12.7)	18.5 (13.2)
4	10	Geometric mean (CV% geometric mean)	567 (26.3)	1280 (9.4)	7.80 (9.4)	150 (20.4)	18.3 (13.1)

Parameter definitions: AUC_{INF}, area under the plasma concentration–time curve from 0 to ∞; CL, total plasma clearance; CV, Coefficient of variation; C_{max}, maximum observed plasma concentration; $t_{1/2}$, terminal elimination of phase half-life; V_{ss}, steady-state volume of distribution.

Table 3. Adverse events

	Placebo	Velcalcetide			
		0.5 mg	2 mg	5 mg	10 mg
Subjects dosed	8	6	6	6	6
Subjects with AE	2 (25%)	0 (0%)	1 (17%)	2 (33%)	2 (33%)
AE preferred term					
Feeling hot	0 (0%)	0 (0%)	1 (17%)	0 (0%)	0 (0%)
Abdominal pain	1 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Abdominal discomfort	0 (0%)	0 (0%)	0 (0%)	1 (17%)	0 (0%)
Back pain	0 (0%)	0 (0%)	0 (0%)	1 (17%)	0 (0%)
Paresthesia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Epistaxis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Lethargy	1 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Diarrhea	1 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

postdose with a return to base line by 12 h in patients with primary hyperparathyroidism with normal renal function [12].

Plasma iCa concentrations also decreased in a dose-dependent manner, but the decreases followed several hours after the changes in iPTH and reached a nadir 10–15 h postdose. Like iPTH, the dose-dependent changes in iCa were maintained during the pattern of diurnal variation in iCa and were observable during the second 24 h period over which the subjects were studied. The effect of velcalcetide treatment on serum calcium was most likely largely due to its effect on reducing PTH secretion, although a direct effect on the CaSR in the kidney, bone and other tissues cannot be excluded. The PTH-mediated effect probably involved both bone and kidney. The decrease in PTH decouples bone resorption from formation causing a net positive flow of calcium to bone from serum, and decreases tubular reabsorption causing an increased loss of calcium from serum to urine. No data were collected to address an effect of bone, but tubular reabsorption of calcium tended to decrease over the 24 h of the study as has been shown with cinacalcet [13].

Serum phosphorus varied considerably over the 48-h observation following a diurnal pattern that paralleled the changes in serum iPTH. Increases in serum phosphorus were observed in all groups following dosing with the peaks occurring 15–20 and 35–45 h postdose. There were no apparent differences from placebo in serum phosphorous AUC over the 48-h observation period for the velcalcetide at 0.5, 2 and 5 mg dose groups. There was a trend toward an increase in serum phosphorous AUC in the velcalcetide 10 mg treatment group. These changes were associated with and probably largely due to an increase in tubular reabsorption of phosphorus. The latter was most likely due to decreases in serum iPTH. However, as discussed below, it may also reflect the observed decrease in FGF23 which is a potent regulator of renal phosphorus reabsorption [14, 15]. An increase in serum phosphorus due to increased reabsorption of phosphate is a prominent feature of cinacalcet treatment in hyperparathyroid patients with normal renal function [13] and decreased renal function [16].

In this study, serum FGF23 decreased by over 30% at 24 h after the 10 mg dose of KAI. The mechanism underlying the decrease in FGF23 is not clear from the design of this study. It could be due to decreased PTH secretion, since *in vitro* and *in vivo* studies have shown that PTH acts directly on bone cells to increase FGF23 secretion and messenger RNA expression [17] and that surgical parathyroidectomy in secondary hyperparathyroidism of chronic renal failure results in a reduction in serum FGF23 levels [17]. A direct effect of velcalcetide on secretion of FGF23 by the osteoblast/osteocyte is also possible since the increased serum FGF23 present in hemodialysis patients is reduced with cinacalcet [18, 19] and also in the chronic renal failure model in the rat [20]. The importance of a direct effect of velcalcetide on FGF23 secretion lies in the potential pathological role of increased FGF23 in the high

morbidity occurring in chronic renal disease. Recently, several clinical studies of CKD patients have demonstrated an association between high serum FGF23 levels and progression of CKD [21], vascular calcification [22], left ventricular hypertrophy [23, 24] and increased mortality [25]. It is noteworthy that velcalcetide had little or no effect on $1,25(OH)_2$ vitamin D levels in the present study. This may indicate an integrated effect of a decrease in PTH, which would be expected to lead to a decrease in $1,25(OH)_2$ vitamin D, and a decrease in FGF23 which would be expected to lead to an increase in $1,25(OH)_2$ vitamin D [2, 26]. This lack of effect has also been shown with cinacalcet in patients with primary hyperparathyroidism [13].

The plasma pharmacokinetics of velcalcetide were well characterized in the current study and indicated that plasma velcalcetide increased in a dose-related manner. Velcalcetide disposition was dose-independent across the dose range evaluated and terminal half-life in healthy male subjects was ∼19 h. In contrast, cinacalcet's initial half-life is ∼6 h, the terminal half-life ranges from 30 to 40 h and steady state is achieved within 7 days. It should be noted that the pharmacokinetic profile of cinacalcet is not affected by varying degrees of renal impairment but food can change the bioavailability of cinacalcet [27]. In contrast, the clearance of velcalcetide is prolonged with decreased renal function and leads to more prolonged effects in this patient group [28].

As an IV product, the luminal GI exposure to velcalcetide is expected to be considerably lower than that observed with oral calcimimetics. To the extent, the poor GI tolerability is related to high local exposure to oral calcimimetics, velcalcetide may result in fewer GI adverse events [8]. Consistent with the hypothesis nausea, a frequent adverse event associated with oral calcimimetics was not observed in this study, despite significant biological effect on serum iPTH and calcium. In addition, because velcalcetide is a peptide, it is expected that it will not pass the blood–brain barrier and adversely affect the central nervous system which is rich in CaSR [29, 30].

In summary, intravenous doses of velcalcetide, a novel long-acting selective peptide agonist of the CaSR was generally safe and well tolerated in healthy male subjects. Dosing with velcalcetide resulted in dose-dependent reductions in iPTH. Consistent with the observed changes in iPTH, calcium levels were reduced in a dose-dependent manner lagging the nadir in iPTH by several hours. Velcalcetide was associated with a dose-dependent reduction in serum FGF23 but did not significantly reduce 1,25 vitamin D or calcitonin. The IV route of administration, long half-life and GI tolerability suggest velcalcetide may provide a novel therapeutic alternative for controlling increased PTH secretion in hemodialysis patients with CKD–MBD.

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CONFLICT OF INTEREST STATEMENT

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ORIGINAL ARTICLE

Collapsing glomerulopathy superimposed on diabetic nephropathy: insights into etiology of an under-recognized, severe pattern of glomerular injury

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ABSTRACT

Background. Collapsing glomerulopathy (CG) represents severe podocyte injury with massive proteinuria, rapid progression and relative resistance to therapy. It is associated with multiple etiologies, including obliterative arteriopathy in transplants. However, its association with diabetic nephropathy (DN) has not been reported.

Methods. Renal biopsies performed in diabetic patients for either increasing proteinuria or deteriorating renal function, or both, were retrospectively reviewed. The clinicopathologic features and immunohistochemical staining of podocytes were analyzed.

Results. Of 534 patients with DN, 26 human immunodeficiency virus (HIV)-negative patients were found to have CG superimposed on DN (5% DN cases). At the time of biopsy, their mean serum creatinine was 3.8 mg/dL and proteinuria was 9.8 g/24 h. Renal biopsy showed CG in 2–30% (mean 16% of glomeruli), with segmental (2%) and global (33%) glomerulosclerosis. DN classification was Class IV-12, III-8, IIb-4 and IIa-2. Vascular sclerosis was moderate (44%) and severe (56%). Extensive arteriolar hyalinosis with >50% luminal stenosis was seen in 85% of cases. Markers of podocyte differentiation were lost, consistent with other types of CG. Cytokeratin was focally positive in 70% and VEGF overexpressed in 43%. Follow-up on 17 patients: 13 developed end-stage renal disease (ESRD) in 7 months from the time of biopsy. The development to ESRD in these patients was more rapid than diabetic controls without CG ($P = 0.005$). The remaining four, 5–24 months follow-up, had an increase in creatinine with stable proteinuria.