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The calcimimetic AMG 641 abrogates parathyroid hyperplasia, bone and vascular calcification abnormalities in uremic rats

Charles Henley [a,*], James Davis [a], Gerald Miller [a], Edward Shatzen [a], Russ Cattley [b], Xiaodong Li [a], David Martin [a,1], Wei Yao [c], Nancy Lane [c], Victoria Shalhoub [a]

[a] Department of Metabolic Disorders, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[b] Department of Pathology, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[c] Department of Medicine, University of California at Davis, Medical School, Sacramento, California 95817, USA

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ABSTRACT

Calcimimetics and vitamin D sterols reduce serum parathyroid hormone (PTH) in patients with secondary hyperparathyroidism receiving dialysis, a disease state associated with parathyroid hyperplasia, vascular calcification, bone disease, and increased mortality. The aim of this study was to determine the effects of the research calcimimetic AMG 641 (Amgen, Inc., Thousand Oaks, CA) or calcitriol (Sigma Aldrich Corporation, St. Louis, MO) on vascular calcification in a rodent model of progressive uremia with accompanying secondary hyperparathyroidism induced by dietary adenine. Treatment effects on parathyroid gland hyperplasia and bone loss were also investigated. Rats were treated daily with vehicle, calcitriol (10 ng), AMG 641 (3 mg/kg), or no treatment during the 4 week period the animals were fed adenine. The uremia-induced increases in serum PTH levels were significantly attenuated by both AMG 641 (>90%) and calcitriol (~50%). AMG 641 significantly reduced calcium–phosphorus product (Ca × P) and significantly attenuated the development of both parathyroid hyperplasia and vascular calcification. In addition, AMG 641 prevented the defects in trabecular bone volume, trabecular number, and bone mineralization, as well as increases in trabecular spacing in this rodent model of secondary hyperparathyroidism. Calcitriol (10 ng/rat) decreased osteoid surface/bone surface, but had no effects on other bone parameters, or parathyroid hyperplasia (likely due to the lower PTH suppressive effect of calcitriol at the dose used in this study). However, this dose of calcitriol significantly exacerbated vascular calcification. These results suggest that calcimimetics can reduce the development of vascular calcification, parathyroid hyperplasia and bone abnormalities associated with secondary hyperparathyroidism.

1. Introduction

Patients with chronic kidney disease receiving dialysis often develop secondary hyperparathyroidism characterized by increased serum parathyroid hormone (PTH), increased serum phosphorous, decreased serum calcium and calcitriol (Goodman and Quarles, 2008; Drueke et al., 2007). Secondary hyperparathyroidism is accompanied by parathyroid hyperplasia and excessive synthesis and secretion of PTH, which can result in disproportionate bone resorption and other bone disorders, soft tissue and vascular calcification, and significant risk for cardiovascular morbidity and mortality (Block et al., 2004; de Francisco, 2004; Hebert, 2006; Kalantar-Zadeh et al., 2006; Young et al., 2005).

Evidence suggests that reductions in PTH and serum phosphorus may slow or prevent secondary hyperparathyroidism-associated parathyroid hyperplasia, vascular calcification, and renal osteodystrophy. Traditional therapeutic approaches rely on the actions of vitamin D sterols, which, while able to decrease PTH levels, have also been associated with hypercalcemia and vascular calcification in preclinical studies (Henley et al., 2005; Lopez et al., 2006). Calcimimetics (e.g., cinacalcet HCl), pharmacologic agents that act directly at the calcium-sensing receptor in the parathyroid gland to reduce PTH secretion, represent a relatively new therapeutic approach. Evidence suggests that calcimimetics may slow or prevent parathyroid hyperplasia in uremic animals (Colloton et al., 2005; Wada et al., 1997) without inducing vascular calcification (Henley et al., 2005; Lopez et al., 2006). Moreover, preclinical (Wada et al., 1998) and clinical (Lien et al., 2005; Cunningham et al., 2005) evidence suggests that calcimimetics improve bone health, including reducing the incidence of fractures. Some clinical data show that calcitriol may influence bone remodeling and ameliorate osteitis fibrosa (Slatopolsky et al., 1984; Andress et al.,

* Corresponding author. Department of Metabolic Disorders, MS-15-2-A, Amgen Inc, One Amgen Center Drive, Thousand Oaks, CA 91320, USA. Tel.: +1 805 447 7023.
E-mail address: chenley@amgen.com (C. Henley).
[1] Current address: Department of Biology, Nektar Therapeutics, San Carlos, Ca USA.

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1989), although other studies have shown either no or detrimental effects on bone remodeling (Costa et al., 2003; Pahl et al., 1995).

There are currently no reports of calcimimetic effects on the totality of biochemical (PTH, calcium, phosphorus, Ca×P) and pathological parameters of secondary hyperparathyroidism (parathyroid hyperplasia, vascular calcification and renal osteodystrophy) in a rodent model of uremia with secondary hyperparathyroidism. Herein, we investigated whether the research calcimimetic AMG 641 (chemical name: (1R)-N-((6-(methyloxy)-4′-(trifluoromethyl)-3-biphenylyl)methyl)-1-phenylethanamine) could abrogate these characteristic biochemical and pathological changes without exacerbating vascular calcification observed in the adenine-treated uremic rat. AMG 641 is an arylalkylamine with a molecular weight of approximately 400 g/mol, is more potent than cinacalcet, and has approximately a 3-fold longer half-life and a larger volume of distribution. We also investigated the effects of calcitriol on the above parameters utilizing a dose that would significantly lower PTH while avoiding the potentially deleterious effects of hypercalcemia.

2. Materials and methods

Male Sprague–Dawley rats (350–390 g) were purchased from Harlan (Indianapolis, IN). Rats were pair-housed under a 12 h/12 h light/dark cycle and given ad libitum access to standard rat chow (1.2% calcium, 0.9% phosphorus) and water. Experiments were performed under protocols approved by Amgen's Internal Animal Care and Use Committee.

2.1. Adenine-induced uremia and drug administration

Rats were randomly assigned into treatment groups based on the normal distribution of baseline body weights, then fed a diet containing 0.75% adenine (Adenine freebase A8626, Sigma Aldrich, St. Louis, MO) or a control diet with equivalent amounts of calcium (1.1%) and phosphorus (0.9%) (Dyets, Inc, Bethlehem, PA) for 4 weeks. Concurrent with adenine exposure, rats were divided into the following daily treatment groups: normal, non-adenine control ($n=8$), adenine control (no treatment; $n=9$), vehicle for AMG 641 (12% captisol in water p.o.; $n=9$), AMG 641 (3 mg/kg, p.o.; $n=9$), vehicle for calcitriol (0.19% ethanol in phosphate buffered saline, s.c.; $n=10$), or calcitriol (1α, 25-dihydroxycholecalciferol, Sigma Aldrich Corp, St. Louis, MO, 10 ng/rat [~0.025–0.028 μg/kg], s.c.; $n=10$). The dose of calcitriol was chosen since higher doses resulted in significant vascular calcification in subtotal nephrectomized rats (Henley et al., 2005), and we wanted to reduce serum PTH significantly while attempting to avoid overwhelming vascular calcification in the rat uremia adenine model.

2.2. Aorta histopathology

Twenty-four hours following the last dose, rats were carbon dioxide-euthanized. The thoracic aorta was then excised and fixed in aqueous buffered zinc formalin (Z-fix; Anatech Ltd; Battle Creek, MI), paraffin embedded, sectioned longitudinally, and von Kossa stained. A board-certified pathologist blinded to treatment groups evaluated and scored the stained sections within a single session (to avoid drift in scoring outcome). One section per animal was scored on a 0–5 scale: 0 = no calcification, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, and 5 = severe calcification.

2.3. Blood biochemistries

PTH, total serum calcium and phosphorus, ionized calcium, blood urea nitrogen [BUN], and creatinine were determined from blood collected from the retro-orbital sinuses of isoflurane anesthetized rats. Ionized calcium was measured using a Ciba-Corning 634 ISE Ca^{++}/pH Analyzer (Ciba-Corning Diagnostics Corp, Medfield, MA) immediately after collection into heparinized capillary tubes. Blood for the remaining parameters was collected into SST (clot activator) brand blood tubes. Serum was removed and stored at −70 °C. A blood chemistry analyzer (AU 400; Olympus, Melville, NY) was used to determine calcium, phosphorus, BUN, and creatinine, and rat $PTH_{(1-34)}$ immunoradiometric assay kits (Immutopics, San Clemente, CA) were used to determine PTH levels.

2.4. Parathyroid hyperplasia

After sacrifice, the laryngo-tracheal complex was removed, stored in zinc-buffered formalin (2 to 3 days), transferred to 70% alcohol and trimmed. The thyroid and parathyroid glands were dissected from each other, and parathyroid glands were blotted dry on a lint-free Kim wipe (KimberlyClark Corp., Roswell, GA, USA), weighed, and paraffin embedded. Sections (5 μm) were placed onto charged slides (VWR Scientific, West Chester, PA, USA). Increases in the number of proliferating parathyroid cells were determined by immunostaining using a proliferating cell nuclear antigen (PCNA) staining kit (Zymed Laboratories, Inc., South San Francisco, CA, USA). Parathyroid area was determined as previously described (Colloton et al., 2005) using an area-measurement graticle with 0.01 mm^2 grids. Data is expressed as the number of PCNA-positive cells/mm^2.

Total gland weight is expressed as parathyroid gland weight/body weight.

2.5. Bone histopathology and histomorphometry

Approximately 1/3 of the distal femur and 1/3 midshaft femur was prepared using slow speed saw. Undecalcified segments were processed through defatting and infiltration and embedded in methylmathacrylate. Frontal sections of the distal femur were obtained near the middle of the bone using a Leica/Jung 2255 microtome set at 4 μm. Distal femur sections were counter-stained with Von Kossa and tetrachrome bone stains. Histomorphometric evaluations (Bioquant Image Analysis Corporation, Nashville, TN) of the stained slides were performed in a blinded manner with the nomenclature and calculations based on standardized terms and formulae (Parfitt et al., 1987), such as percent bone volume per tissue volume (%BV/TV), percent osteoblast surface per bone surface (%ObS/BS), percent osteoclast surface per bone surface (%OcS/BS),

Table 1
Biochemical markers.

Treatment	No adenine	Adenine	Adenine + AMG 641 vehicle	Adenine + AMG 641 (3 mg/kg)	Adenine + calcitriol vehicle	Adenine + calcitriol (10 ng/rat)
Creatinine mg/dL	0.3 ± 0.01	3.5 ± 0.2[a]	3.5 ± 0.3[a]	2.8 ± 0.1[a]	3.6 ± 0.4[a]	3.3 ± 0.4[a]
BUN mg/dL	18 ± 1	160 ± 12[a]	146 ± 9[a]	125 ± 5[a]	159 ± 10[a]	139 ± 5[a]
iPTH pg/ml	35 ± 3	565 ± 91[a]	656 ± 76[a]	13 ± 3[c]	801 ± 70[a]	396 ± 57[a,b]
Ca mg/dL	10.6 ± 0.1	10.7 ± 0.1	10.8 ± 0.2	9.4 ± 0.3[a,c]	10.8 ± 0.2	11.0 ± 0.2
P mg/dL	7.2 ± 0.2	23.0 ± 2.0[a]	21.1 ± 0.9[a]	21.8 ± 1.0[a]	20.1 ± 1.7[a]	18.7 ± 0.7[a]

Measurements were taken 24 h after treatment administration on treatment day 28. Values are mean ± S.E.M. ($n=8$–10).
[a] $P<0.05$ significantly different from standard chow (no adenine, no treatment).
[b] $P<0.05$ significantly different from adenine + calcitriol vehicle treated group.
[c] $P<0.05$ significantly different from adenine + AMG 641 vehicle.