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Rescue of the phenotype of CYP27B1 (1α-hydroxylase)-deficient mice☆

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Abstract

The treatment of choice for pseudo Vitamin D deficiency rickets (PDDR), caused by mutations in the 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1; 1α-OHase) gene, is replacement therapy with $1,25(OH)_2D_3$. We have previously engineered an animal model of PDDR by targeted inactivation of the 1α-OHase gene in mice (Endocrinology 142 (2001) 3135). Replacement therapy was performed in this model, and compared to feeding with a high calcium diet containing 2% calcium, 1.25% phosphorus, 20% lactose (rescue diet). Blood biochemistry analysis revealed that both rescue treatments corrected the hypocalcemia and secondary hyperparathyroidism. Bone histology and histomorphometry confirmed that the rickets and osteomalacia were cured by both rescue protocols. However, despite the restoration of normocalcemia, the rescue diet did not entirely correct bone growth as femur size remained significantly smaller than control in 1α-OHase$^{-/-}$ mice fed the rescue diet. These results demonstrate that correction of the abnormal mineral ion homeostasis by feeding with a high calcium rescue diet is effective to rescue the PDDR phenotype of 1α-OHase mutant mice. This treatment, however, does not appear as effective as $1,25(OH)_2D_3$ replacement therapy since bone growth remained impaired.

Keywords: Rickets; Vitamin D; 25-hydroxyvitamin D-1α-hydroxylase; CYP27B1

1. Introduction

Pseudo Vitamin D deficiency rickets (PDDR) is a rare autosomal recessive disease associated with mutations in the 25-hydoxyvitamin D-1α-hydroxylase gene (CYP27B1, hereafter referred to as 1α-OHase) [1]. PDDR patients are not able to synthesize $1,25(OH)_2D_3$. Consequently they develop typical symptoms of abnormal mineral ion homeostasis, secondary hyperparathyroidism, growth retardation, hypotonia, rickets, and osteomalacia [2].

We and others have recently reported an animal model of PDDR [3,4]. This mouse model completely recapitulates the features of the human disease, with hypocalcemia, secondary hyperparathyroidism, and bone abnormalities appearing as soon as weaning [3,4].

The treatment of choice for PDDR patients is replacement therapy with $1,25(OH)_2D_3$ [5]. It results in rapid and complete correction of the abnormal phenotype, restoring normocalcemia, eliminating secondary hyperparathyroidism and features of rickets. The restoration of bone mineral content is equally rapid [6] and histological evidence of healing has been documented [5].

Mutations in the Vitamin D receptor (VDR) result in a second form of Vitamin D-related rickets called hereditary Vitamin D resistant rickets (HVDRR). This disease is characterized by symptoms similar to PDDR, with the exception of very high levels of circulating $1,25(OH)_2D_3$ and alopecia [7]. Several laboratories have developed valid mouse models for this type of hereditary rickets [8–10]. Because these animals, like the human patients, are resistant to the activity of $1,25(OH)_2D_3$, rescue of the phenotype has been successfully accomplished using a high calcium, high phosphorus, high lactose diet (2% calcium, 1.25% phosphorus, and 20% lactose) [8,11]. In VDR-ablated mice, the high calcium, high phosphorus, high lactose rescue diet completely normalized blood biochemical and bone histomorphometric parameters in mutant animals, suggesting that the action of $1,25(OH)_2D_3$ on bone is indirect and dependent on the role of Vitamin D in maintaining normal mineral ion homeostasis [8,11].

Hormonal replacement therapy with $1,25(OH)_2D_3$ was performed in the animal model of PDDR, and compared to feeding with a high calcium diet containing 2% calcium,

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1.25% phosphorus, 20% lactose (rescue diet). Our results demonstrate that both rescue regimen were effective to rescue the PDDR phenotype of 1α-OHase mutant mice. Feeding with the rescue diet, however, does not appear as effective as $1,25(OH)_2D_3$ replacement therapy since bone growth remained impaired.

2. Materials and methods

2.1. Animal maintenance

All procedures involving animals were previously approved by the Institutional Animal Care Committee. Fifteen 1α-OHase homozygous null (−/−) males mice and 15 control littermates (heterozygous; +/−) were maintained in a virus- and parasite-free barrier facility and exposed to a 12-h light, 12-h dark cycle.

In order to normalize blood mineral ion levels of the 1α-OHase ablated mice, five 1α-OHase$^{-/-}$ animals received daily subcutaneous injections of $1,25(OH)_2D_3$ (a generous gift of Dr. Milan Uskokovic, Hoffmann-LaRoche, Nutley, NJ). A 'rescue' concentration of 500 pg/g body weight (BW) was administered from 21 days of age until 35 days of age, followed by a 'maintenance' dose of 100 pg/g BW (s.c.) from 35 days of age until they reached the age of 60 days. Injection were given in a vehicle consisting of 0.1% ethanol and 99.9% propylene glycol. Five 1α-OHase$^{+/-}$ animals were treated in the same way as controls. In parallel, groups of five 1α-OHase$^{+/-}$ and 1α-OHase$^{-/-}$ animals were injected with the vehicle alone.

The control and $1,25(OH)_2D_3$-treated groups were fed autoclaved regular rodent chow containing 0.97% calcium, 0.85% phosphorus, 0% lactose, and 4.4 IU Vitamin D/g (#5075, Charles River Laboratories, St-Constant, QC) (normal diet). Five animals of each genotype were fed a γ-irradiated diet containing 2% calcium, 1.25% phosphorus, 20% lactose, and 2.2 IU Vitamin D/g (rescue diet; TD96348, Teklad, Madison, WI) from 21 days of age until sacrifice ($n = 5$ for 1α-OHase$^{-/-}$ and 1α-OHase$^{+/-}$ mice, respectively).

2.2. Serum biochemistry and histomorphometry

Circulating concentrations of calcium, phosphorus, and parathyroid hormone (PTH) were measured as described previously [12]. Bone histomorphometry was performed on a Leica DM-R microscope (Leica Mikrosysteme GmbH, Vienna, Austria) using the Osteomeasure histomorphometry system (Osteometrics Inc., Atlanta, GA, USA) as detailed elsewhere [12]. Statistical analysis was by ANOVA followed by the Dunnett's post-test. $P < 0.05$ was accepted as significant; error bars represent the S.E.M.

3. Results

Treatment with $1,25(OH)_2D_3$ or feeding with the high calcium rescue diet corrected the hypocalcemic status of 1α-OHase$^{-/-}$ mice, restoring normal serum calcium levels comparable to treated or untreated 1α-OHase$^{+/-}$ mice (Table 1). This correction of the circulating calcium level in −/− animals was initiated right from the start of treatment and completed after 2 weeks of treatment (data not shown).

Correlating with the normalization of calcemia, the elevated concentration of serum PTH measured in 1α-OHase$^{-/-}$ mice dropped back to non detectable levels following both rescue treatments, as is observed for treated or untreated 1α-OHase$^{+/-}$ animals (Table 1). No significant differences were highlighted in serum phosphorus levels among all groups (data not shown).

In order to precisely assess bone growth, we compared femur length at sacrifice. There was a significant difference in the length of the femurs between 1α-OHase$^{-/-}$ and 1α-OHase$^{+/-}$ mice fed the normal diet and treated with the vehicle (Control, Fig. 1). This difference was corrected when 1α-OHase$^{-/-}$ mice were treated with $1,25(OH)_2D_3$ (Fig. 1). The treatment with $1,25(OH)_2D_3$ had no effect on the size of the femur of heterozygous control mice. The shorter femur length was not completely corrected when 1α-OHase$^{-/-}$ mice were given the rescue diet (14.3 ± 0.1 mm versus 14.9 ± 0.1 mm for 1α-OHase$^{-/-}$ and 1α-OHase$^{+/-}$ animals fed the rescue diet, respectively, $P < 0.05$). The rescue diet did not affect femur size in 1α-OHase$^{+/-}$ control mice (Fig. 1).

Femurs from all animals in all groups were collected at 60 days of age following 5.5 weeks of treatment. Histomorphometric analysis was performed on Goldner-stained 6 μm sections. Mutant 1α-OHase$^{-/-}$ mice develop rickets from weaning [3,4] and the severity of rickets and osteomalacia is amplified as mice grow (not shown). The severe disorganization of the growth plate in 1α-OHase$^{-/-}$ mice at 8.5 weeks was corrected when they were treated with $1,25(OH)_2D_3$ or when they were fed the high calcium

Table 1
Biochemical data in serum of control or rescue-treated 1α-OHase$^{+/-}$ control littermates (+/−) and 1α-OHase$^{-/-}$ mutant mice (−/−)

	+/− (control)	+/− $1,25(OH)_2D_3$	+/− (rescue diet)	−/− (control)	−/− $1,25(OH)_2D_3$	−/− (rescue diet)
PTH (pg/ml)	N.D.	N.D.	N.D.	1887 ± 307	N.D.	N.D.
Calcium (mmol/l)	2.22 ± 0.06	2.59 ± 0.06	2.34 ± 0.04	1.42 ± 0.13**	2.59 ± 0.07	2.33 ± 0.10

N.D.: not detectable. All values are mean ± S.D.
** $P < 0.01$ vs. +/− control (by ANOVA and Dunnett's post-test).

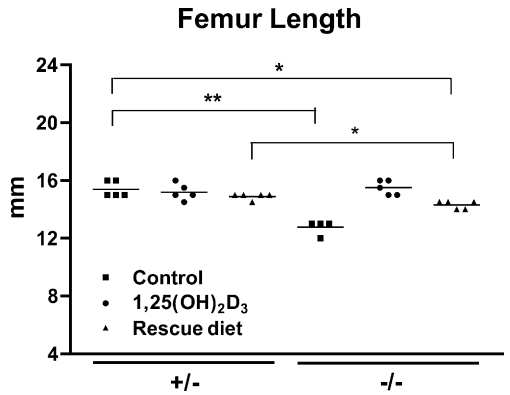

Fig. 1. The hormonal replacement therapy but not the rescue diet corrects femur length in 1α-OHase$^{-/-}$ mutant mice. Femurs were measured at sacrifice (60 days old). 1α-OHase$^{-/-}$ animals had significantly reduced femur lengths, which was corrected by treatment with 1,25$(OH)_2D_3$ but only partially corrected by the rescue diet. The horizontal line represents the mean ($^{*}P < 0.05$; $^{**}P < 0.01$).

rescue diet (not shown). Both rescue regimen restored the normal columnar organization of growth plate chondrocytes and led to normal mineralization of the primary spongiosa (not shown). Control 1α-OHase$^{-/-}$ mice (normal dietary conditions, vehicle-treated) presented with severe osteomalacia: osteoid volume, osteoid surface, and osteoid thickness were increased several-fold, respectively, when compared to control 1α-OHase$^{+/-}$ littermates (Fig. 2). The treatments did not affect osteoid volume or osteoid thickness, but significantly decreased osteoid surface in 1α-OHase$^{+/-}$ animals (Fig. 2). In 1α-OHase$^{-/-}$ mutant mice, both rescue regimen normalized all osteoid-related parameters (Fig. 2). Analysis of the cortex from all groups showed that cortical osteomalacia was completely prevented in 1α-OHase$^{-/-}$ mice by treatment with 1,25$(OH)_2D_3$ or feeding with the high calcium rescue diet (data not shown).

4. Discussion

Replacement therapy with 1,25$(OH)_2D_3$ is the treatment of choice for PDDR [5]. Our results show that in an animal model of PDDR, treatment with the active, hormonal form of Vitamin D also effectively normalized biochemical parameters and cured rickets and osteomalacia. The efficiency of hormonal replacement therapy was compared with feeding of the mutant animals with a high calcium, high phosphorus, high lactose diet. Such a dietary manipulation has been successfully employed to normalize mineral ion homeostasis in VDR-ablated mice [11,13]. Our results show that the rescue diet normalized calcemia and cured rickets and osteomalacia in 1α-OHase$^{-/-}$ animals. Under the conditions used, the only parameter that was not normalized was the overall size of the femur.

The high calcium rescue diet rapidly corrected the hypocalcemia and secondary hyperparathyroidism in 1α-OHase$^{-/-}$ mice. Intestinal calcium absorption is most

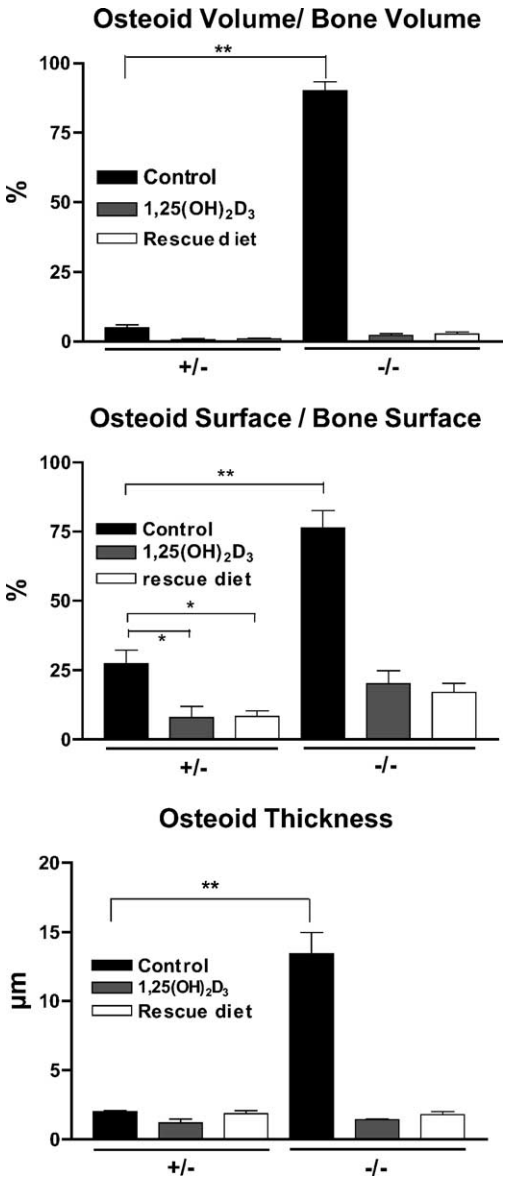

Fig. 2. Histomorphometric analysis of bones from 1α-OHase$^{+/-}$ (+/−) and 1α-OHase$^{-/-}$ (−/−) animals treated with 1,25$(OH)_2D_3$ or fed the high calcium rescue diet (2% Ca^{2+} diet). Both rescue regimen normalized osteoid-related histomorphometric indices.

likely due to passive intake in the absence of circulating 1,25$(OH)_2D_3$. Although 1α-OHase$^{-/-}$ mice fed the rescue diet eventually reached the same weight as control animals, the treatment did not entirely correct bone growth as femur size remained significantly smaller than control. This most likely reflects the high demand for minerals during the period of rapid growth that follows weaning. Passive calcium intake may not be able to adequately meet the demands for calcium during rapid growth spurts.

The results presented here confirm that Vitamin D replacement therapy remains the best therapeutic intervention for the treatment of PDDR. Moreover, 1,25$(OH)_2D_3$ administration to patients is much simpler than overnight intravenous calcium infusions.

References

[1] A.A. Portale, W.L. Miller, Human 25-hydroxyvitamin D-1alpha-hydroxylase: cloning, mutations, and gene expression, Pediatr. Nephrol. 14 (7) (2000) 620–625.
[2] D. Fraser, S.W. Kooh, H.P. Kind, M.F. Holick, Y. Tanaka, H.F. DeLuca, Pathogenesis of hereditary vitamin-D-dependent rickets. An inborn error of vitamin D metabolism involving defective conversion of 25-hydroxyvitamin D to 1 alpha,25-dihydroxyvitamin D, N. Engl. J. Med. 289 (16) (1973) 817–822.
[3] O. Dardenne, J. Prud'homme, A. Arabian, F.H. Glorieux, R. St-Arnaud, Targeted inactivation of the 25-hydroxyvitamin D(3)-1(alpha)- hydroxylase gene (CYP27B1) creates an animal model of pseudovitamin D-deficiency rickets, Endocrinology 142 (7) (2001) 3135–3141.
[4] D.K. Panda, D. Miao, M.L. Tremblay, J. Sirois, R. Farookhi, G.N. Hendy, D. Goltzman, Targeted ablation of the 25-hydroxyvitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction, Proc. Natl. Acad. Sci. U.S.A. 98 (13) (2001) 7498–7503.
[5] E.E. Delvin, F.H. Glorieux, P.J. Marie, J.M. Pettifor, Vitamin D dependency: replacement therapy with calcitriol, J. Pediatr. 99 (1) (1981) 26–34.
[6] F.H. Glorieux, R. St-Arnaud, in: D. Feldman, F.H. Glorieux, J.W. Pike (Eds.), Vitamin D, Academic Press, San Diego, 1997, pp. 755–764.
[7] P.J. Malloy, J.W. Pike, D. Feldman, The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets, Endocr. Rev. 20 (2) (1999) 156–188.
[8] Y.C. Li, A.E. Pirro, M. Amling, G. Delling, R. Baron, R. Bronson, M.B. Demay, Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia, Proc. Natl. Acad. Sci. U.S.A. 94 (18) (1997) 9831–9835.
[9] T. Yoshizawa, Y. Handa, Y. Uematsu, S. Takeda, K. Sekine, Y. Yoshihara, T. Kawakami, K. Arioka, H. Sato, Y. Uchiyama, S. Masushige, A. Fukamizu, T. Matsumoto, S. Kato, Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning, Nat. Genet. 16 (4) (1997) 391–396.
[10] S.J. Van Cromphaut, M. Dewerchin, J.G. Hoenderop, I. Stockmans, E. Van Herck, S. Kato, R.J. Bindels, D. Collen, P. Carmeliet, R. Bouillon, G. Carmeliet, Duodenal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects, Proc. Natl. Acad. Sci. U.S.A. 98 (23) (2001) 13324–13329.
[11] M. Amling, M. Priemel, T. Holzmann, K. Chapin, J.M. Rueger, R. Baron, M.B. Demay, Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses, Endocrinology 140 (11) (1999) 4982–4987.
[12] O. Dardenne, J. Prudhomme, S.A. Hacking, F.H. Glorieux, R. St-Arnaud, Rescue of the pseudo-vitamin D deficiency rickets phenotype of CYP27B1-deficient mice by treatment with 1,25-dihydroxyvitamin D3: biochemical, histomorphometric, and biomechanical analyses, J. Bone Miner Res. 18 (4) (2003) 637–643.
[13] Y.C. Li, M. Amling, A.E. Pirro, M. Priemel, J. Meuse, R. Baron, G. Delling, M.B. Demay, Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor-ablated mice, Endocrinology 139 (10) (1998) 4391–4396.