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Two siblings with vitamin-D-dependent rickets type II: no recurrence of rickets for 14 years after cessation of therapy

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Abstract. Rickets in a 3-year-old boy and his 1-year-old sister, both with alopecia, was cured by treatment with 50,000 IU of vitamin D_2 daily for 2 years and did not recur within 14 years after cessation of therapy. A diagnosis of vitamin-D-dependent rickets type II was made in these patients at the ages of 20 and 18 years based on the findings that 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] did not inhibit DNA biosynthesis in phytohaemagglutinin-stimulated lymphocytes and that cultured skin fibroblasts showed impaired nuclear uptake and normal cytosol binding of [^{3}H]$1,25(OH)_2D_3$. Surprisingly, the serum $1,25(OH)_2D$ levels of these patients were high and their serum 24,25-dihydroxyvitamin D levels were low, although neither patient showed any symptoms except alopecia. The presence of vitamin D metabolite imbalances in the absence of rickets in these patients might be explained by differences in sensitivity to $1,25(OH)_2D_3$ of bone formation and vitamin D metabolism. In addition, changes of sensitivity to treatment with vitamin D derivatives might be a consequence of differentiation of target cells. From the present findings, it is suggested that in this disease treatment with a sufficient dose of vitamin D derivatives should be initiated in the active phase of rickets.

Key words: Vitamin-D-dependent rickets type II – 1,25-Dihydroxyvitamin D receptor – Alopecia

Introduction

Vitamin-D-dependent rickets type II is a hereditary disease characterized clinically by rickets and is frequently associated with alopecia. This disorder is due to a spectrum of derangements of the 1,25-dihydroxyvitamin D[$1,25(OH)_2D$] receptor-effector system in target organs. The resistance to $1,25(OH)_2D$ has been suggested to be the consequence of structural variations in the receptor molecule [10], since recent studies using monoclonal antibodies against the $1,25(OH)_2D_3$ receptor indicated that all patients tested possessed the receptor protein [16].

Great variety in the clinical features of the patients, in their responses to therapy with vitamin D derivatives and in biochemical findings have been reported [2, 4, 9, 11, 14, 15]. Patients showing spontaneous healing in their teens [10, 11] and others who died in the absence of effective therapy during early childhood [2, 7, 13] have been reported.

This paper reports two siblings with vitamin-D-dependent rickets type II with alopecia, whose rickets was cured by treatment with 50,000 IU vitamin D_2 daily for 2 years. The rickets did not recur within 14 years after cessation of therapy, although the patients showed imbalances of serum vitamin D metabolite concentrations.

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Abbreviations: cyclic AMP = adenosine 3′,5′-cyclic monophosphate; PHA = phytohaemagglutinin; $1,25(OH)_2D$ = 1,25-dihydroxyvitamin D; 24,25 $(OH)_2D$ = 24,25-dihydroxyvitamin D; 25(OH)D = 25-hydroxyvitamin D

Patients and methods

A 41-month-old boy (patient 1) and his 20-month-old sister (patient 2) were admitted to Tokushima University Hospital for evaluation of alopecia and rickets. Their parents are physically normal first cousins. The two patients were born by normal delivery after full-term pregnancy. Their scalp hair began to fall out after birth and alopecia developed at 1–2 months of age. Their development was within normal limits throughout childhood, but a waddling gait and deformity of the lower legs were noted from 16 to 18 months of age.

On admission to hospital, patients 1 and 2 weighed 11.6 kg (−1.5 SD) and 8.0 kg (−2.0 SD) and were 83.7 cm (−2.7 SD) and 76.2 cm (−1.1 SD) in height. Alopecia, genu valgum (patient 1), genu varum (patient 2), Harrison groove, rosary and muscle weakness were observed, and the wrist and ankle joints were thickened.

Before treatment, the serum calcium levels of patients 1 and 2 were 9.0 and 9.6 mg/dl (normal range 8.8–10.8 mg/dl), serum phosphorus levels were 3.7 and 3.5 mg/dl (normal range 4.5–6.5 mg/dl), alkaline phosphatase activities were 35.5 and 37.8 Bodansky units (normal range 0.65–3.3 Bodansky units), and their values for tubular reabsorption of phosphorus were 90% and 83% (normal range 85%–98%), respectively. They both showed generalized aminoaciduria. On radiological evaluation of the skeleton, typical metaphyseal features of rickets with cupping and fraying, which indicated active rickets, were observed in the hand, elbow, shoulder, ankle, knee and hip joints of both patients.

As these patients were suspected as having atypical phosphate diabetes, treatment was started with 50,000 IU vitamin D_2 every other day (Fig. 1a, b). This did not result in any improvement in 2 months, so the dosage was increased to 50,000 IU daily. When this treatment had been continued for 12–18 months, coincidental with improvement in the levels of serum phosphorus and alkaline phosphatase activity, a calcifi-

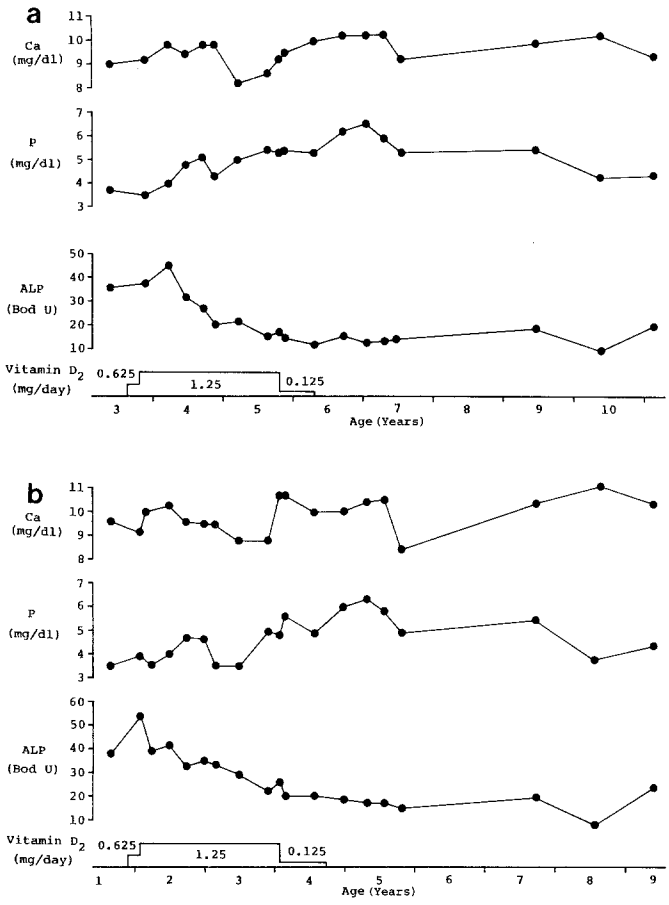

Fig. 1a, b. Sequential serum levels of calcium *(Ca)*, phosphorus *(P)* and alkaline phosphatase activity *(ALP)* of **a** patient 1 and **b** patient 2 before, during and after treatment with vitamin D_2

cation line appeared in metaphyseal areas of their long bones. After about 2 years of therapy, normal levels of serum phosphorus and alkaline phosphatase activity, and normal skeletal radiographs were obtained. The treatment was discontinued after daily administration of 5,000 IU vitamin D_2 for 6 months, when the patients were 6 and 4 years old.

In both patients, serum biochemical data and skeletal radiographs on follow-up for 14 years after cessation of treatment did not reveal any indications of active rickets, although total alopecia persisted. When these patients were 20 and 18 years old, they were re-investigated because of the severe rickets and alopecia observed in their early childhood, and a diagnosis of vitamin D-dependent rickets type II was made.

Cytosol binding and nuclear uptake of $[^3H]1,25(OH)_2D_3$ by cultured skin fibroblasts and the effect of $1,25(OH)_2D_3$ on DNA biosynthesis in phytohaemagglutinin (PHA)-stimulated lymphocytes were investigated as reported previously [18]. The levels of calcium, phosphorus and alkaline phosphatase activity were determined by automated methods. Serum parathyroid hormone (C-terminal fragment) was determined by radioimmunoassay (PTH-C kit: Eiken Immunochemical Laboratory, Tokyo). Urinary adenosine 3',5'-cyclic monophosphate (cyclic AMP) was analysed by competitive protein-binding assay [17]. The maximal tubular reabsorption of phosphate per volume of glomerular filtrate was measured by the method of Walton and Bijvoet [20]. The serum concentrations of 25-hydroxyvitamin D [25(OH)D] and 24,25-dihydroxyvitamin D[24,25$(OH)_2$D] were measured by competitive protein-binding assay and that of $1,25(OH)_2D$ by radioreceptor assay as reported previously [19].

Results

As an easy and rapid diagnostic test for a defect of the $1,25(OH)_2D_3$ receptor-effector system [18], the effect of $1,25(OH)_2D_3$ on the rate of thymidine incorporation into PHA-stimulated lymphocytes was investigated (Fig. 2). Thymidine incorporation into PHA-stimulated lymphocytes of these patients was not inhibited by 10^{-9} to 10^{-7} *M* $1,25(OH)_2D_3$, which caused dose-dependent inhibition of the incorporation of thymidine into PHA-stimulated lymphocytes of normal subjects.

Next we measured the binding of $[^3H]1,25(OH)_2D_3$ in the cytosol of skin fibroblasts and their nuclear uptake of $[^3H]1,25(OH)_2D_3$ (Table 1). The binding capacities and affinities of the cytosol of cultured skin fibroblasts from these patients were normal, but the capacities of these cells for nuclear uptake were decreased to 15% and 31%, respectively, of that of control subjects, although the nuclear affinities were normal.

We then examined the homeostatic parameters of calcium and phosphorus balance of these patients who showed completely normal skeletal radiological findings (Table 2). The serum calcium and phosphorus levels, and alkaline phosphatase activity were normal. The urinary calcium and phosphorus excretion was also within normal limits. Urinary cyclic

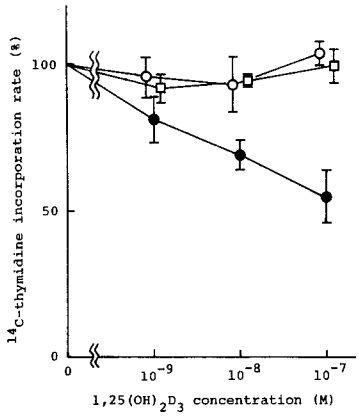

Fig. 2. Effect of the dose of $1,25(OH)_2D_3$ on the rate of thymidine incorporation into PHA-stimulated lymphocytes of six control subjects (●——●), patient 1 (○——○) and patient 2 (□——□). Values are means ± SD for triplicate determinations

Table 1. Binding of $[^3H]1,25(OH)_2D_3$ in cytosol and nuclear uptake of $[^3H]1,25(OH)_2D_3$ by intact skin fibroblasts

	Cytosol binding		Nuclear uptake	
	Capacity (fmol/mg protein)	Affinity (n*M*)	Capacity (fmol/10^7 cells)	Affinity (n*M*)
Patient 1	32	0.32	6.7	0.094
Patient 2	34	0.75	10.7	0.089
Control	31 ± 4	0.52 ± 0.22	70.9 ± 13.6	0.068 ± 0.013
(*n*)	5	5	9	9

Table 2. Biochemical data on patient 1 (20 years old) and patient 2 (18 years old). TmP/GRF = Maximal tubular reabsorption of phosphate per volume of glomerular filtrate

	Patient 1	Patient 2	Normal values
Serum			
Calcium (mg/dl)	9.67	9.50	8.8 – 10.8
Phosphorus (mg/dl)	3.87	3.33	3.0 – 4.5
Alkaline phosphatase (IU/l)	132.00	76.000	40.0 – 110.0
Parathyroid hormone (ng/ml)	0.57	0.43	0.1 – 0.5
25-Hydroxyvitamin D (ng/ml)	7.00	5.00	12.0 ± 1.5
24,25-dihydroxyvitamin D (ng/ml)	0.11	0.16	1.2 ± 0.1
1,25-dihydroxyvitamin D (pg/ml)	100.30	94.10	41.0 ± 3.0
Urine			
Calcium (mg/mg creatinine)	0.062	0.122	<0.22
Phosphorus (μg/mg creatinine)	368.00	338.000	145.00 – 759.00
TmP/GFR	3.92	3.57	2.05 – 5.90
Cyclic AMP (nmol/dl glomerular filtrate)	6.07	8.35	2.81 ± 0.49
Aminoaciduria	–	–	–

AMP was slightly increased, but there was no aminoaciduria. The serum PTH level was slightly increased in patient 1 and normal in patient 2. The serum levels of $1,25(OH)_2D$, 25(OH)D and $24,25(OH)_2D$ were high, low and very low, respectively, in both patients.

Discussion

In these patients a diagnosis of vitamin-D-dependent rickets type II was made on the basis of the findings that $1,25(OH)_2D_3$ did not inhibit DNA biosynthesis in PHA-stimulated lymphocytes and that cultured skin fibroblasts from the patients showed impaired nuclear uptake and normal cytosol binding of $[^3H]1,25(OH)_2D_3$, although the patients had normocalcaemia. Some patients with this disease show normocalcaemia with hypophosphataemia [7, 15] and one patient was reported to have hypophosphataemia and increased serum alkaline phosphatase activity when rickets recurred after cessation of treatment [3]. At 3 and 1 years of age, our patients showed rickets on many joints and alopecia, which suggested marked resistance to $1,25(OH)_2D$ [8, 15]. Moreover they showed generalized aminoaciduria as another indication of secondary hyperparathyroidism during active rickets, although their serum PTH levels were not measured. These findings suggest that the suppression of hyperparathyroidism and normalization of phosphorus were related to recovery from rickets in these patients.

For 14 years after cessation of treatment, these patients maintained normalized serum levels of calcium, phosphorus, PTH and alkaline phosphatase activity. Moreover skeletal radiological findings were also normal, although the circulating $1,25(OH)_2D$ level was high and the 25(OH)D and $24,25(OH)_2D$ levels were low in both patients. The serum $24,25(OH)_2D$ concentrations of these patients were as low as those reported for patients with this disease before treatment [19]. A low level of $24,25(OH)_2D$ may be a specific character of this disease, as previously suggested [19], because $1,25(OH)_2D$ stimulates $24,25(OH)_2D$ synthesis mediated by the receptor for $1,25(OH)_2D$ [5, 6, 8]. 25-Hydroxyvitamin D-24-hydroxylase activity in skin fibroblasts from normal subjects becomes detectable on exposure of cells to $1,25(OH)_2D_3$ at 10^{-9} *M*, and on increasing the $1,25(OH)_2D_3$ concentration it increases to an apparent maximum at 10^{-8} *M* [8, 10], although Griffin and Zerwekh [9] reported that maximal and half maximal responses of the enzyme occurred after exposure to 10^{-10} *M* and 5×10^{-11} *M* $1,25(OH)_2D_3$, respectively.

In contrast, increases in collagen synthesis and alkaline phosphatase activity in osteoblastic MC3T3-E1 cells are stimulated by $1,25(OH)_2D_3$ at 10^{-11}–10^{-10} *M*, which is a physiological concentration range [12]. In addition, in normal skin fibroblasts and MC3T3-E1 the affinities of the cytosol receptor for $1,25(OH)_2D_3$ and the nuclear uptake of $1,25(OH)_2D_3$ are 10^{-11}–10^{-10} *M* [12]. These different sensitivities to $1,25(OH)_2D_3$ of the bone formation system and 24-hydroxylase inducing response may at least partly explain the normal findings in bone but imbalances of serum vitamin D metabolites in these patients.

However, it is still difficult to explain why rickets did not recur in these patients after cessation of therapy although their cells continued to exhibit a genetic defect that caused resistance to the action of $1,25(OH)_2D$. One possible explanation is that the residual $1,25(OH)_2D$ receptor-effector system in target organs of the patients may have been stimulated by the treatment. Recently, physiological concentrations of $1,25(OH)_2D_3$ were found to affect differentiation of various cells in vitro [1, 12]. Thus another possible explanation is that differentiation of target cells may have been important in maintaining calcium and phosphorus homeostasis which resulted in normal mineralization. Liberman et al. [13] reported a patient who seemed to respond strikingly to $24,25(OH)_2D_3$ and in whom normocalcaemia persisted for a long time after all treatment was stopped. Cases of this disease showing a change in the degree of resistance to $1,25(OH)_2D$ [7] or spontaneous healing [10, 11] have also been reported. These previous findings may be due to cellular differentiation during treatment with vitamin D derivatives.

From findings in these patients, we conclude that treatment with a sufficient amount of vitamin D derivatives for an adequate period in the active phase of vitamin-D-dependent rickets type II is essential in its therapy.

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