Chronotherapy of high-dose active vitamin D3 in haemodialysis patients with secondary hyperparathyroidsm: a repeated dosing study

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Aims Renal osteodystrophy is the major complication in patients with end-stage renal failure. Oral or intravenous vitamin D3 (D3) is given to these patients, but severe hypercalcaemia sometimes interrupts this therapy. This study was undertaken to determine whether the effectiveness and safety of D3 also depend on its dosing time during a repeated treatment.

Methods A higher dose (3 μg) was given orally to 13 haemodialysis patients at 08.00 h or 20.00 h for 12 months by a randomized, cross-over design.

Results Three patients were withdrawn due to severe hypercalcaemia after switching from 08.00 h to 20.00 h dosings. The elevation in serum calcium concentration was significantly ($P < 0.001$) greater during the 08.00 h dosing in the remaining ten patients. Mean serum Ca concentration after the trial was 10.92 (95% confidence interval (CI) 10.79, 11.06) and 9.55 mg dl^{-1} (95% CI 9.30, 9.71) by 08.00 h and 20.00 h dosing, respectively. On the other hand, the suppression of the elevated serum parathyroid hormone (PTH) and subsequent increment in bone density were significantly greater during the 08.00 h dosing. Mean PTH concentration after the trial was 414 (95% CI 360, 475) and 220 pg ml^{-1} (95% CI 202, 249) by 08.00 h and 20.00 h dosing, respectively ($P = 0.02$). Mean increment of bone density after the trial was 22 (95% CI 8, 32) and 57 g cm^{-3} (95% CI 43, 83) by 08.00 h and 20.00 h dosing, respectively ($P = 0.04$).

Conclusion These results indicate that a higher dose of oral D3 is more effective and safe after dosing at evening in patients with renal osteodystrophy.

Keywords: dosing time, renal failure, renal osteodystrophy, vitamin D

Introduction

Circadian rhythm is the fundamental biological phenomenon that exists in all living organisms [1]. The occurrence of some diseases such as acute myocardial infarction and stroke also shows circadian variations [2, 3]. Therefore, the advance in chronobiology will provide important clues for understanding physiology and pathophysiology in human subjects. Increasing evidence shows that the effectiveness and toxicity of drugs are affected by the time of dosing. A chronopharmacological approach is desirable to develop a more effective and safe dosage regimen for the treatment of patients. There is precedent for this approach (chronotherapy) in the therapy of several diseases [4, 5].

Serum calcium and phosphate concentrations possess diurnal rhythms, with a peak in the daytime and a nadir at night-time in both nocturnal rodents and human subjects [6, 7]. Although the exact mechanism is not clear, it is speculated to be as follows: lighting stimulates suprachiasmatic nuclei (SCN), in which the circadian clock is located. Such stimuli produce some signals from SCN, which enhance bone resorption and, consequently, increase serum the concentration of calcium and phosphate during the day time [8–13].

Secondary hyperparathyroidism in patients with end-stage renal failure is one of the major complications during long-term maintenance haemodialysis [14]. Oral dosing of a higher-dose active vitamin D3 (D3) (so-called pulse therapy) is sometimes selected to reduce the elevated serum parathyroid hormone concentration as well as the enlarged size of the parathyroid gland, and to

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increase bone density [15–17]. Because D3 increases intestinal absorption of calcium and phosphate, the therapy is sometimes limited by the drug-related hypercalcaemia and hyperphosphataemia [14]. We have previously shown that the hypercalcaemic and hyperphosphataemic effects of D3 after a single dose were varied with its dosing time, and the degree of these adverse effects after dosing in the evening was less than that after dosing in the morning in patients with secondary hyperparathyroidism [18]. We also reported that the repeated dosing of D3 at dark phase was favourable to both increasing the efficacy and reducing the adverse reactions in 5/6 nephrectomized rats [19]. However, to choose the chronotherapy of D3 on a chronic basis in actual patients, it must be determined whether such a chronotoxicological profile of the drug persists after repeated dosing in patients with secondary hyperparathyroidism. In addition, it is interesting to examine whether the effect of D3 on bone density also depends on its dosing time. To address these issues, a higher dose of D3 was given orally three times a week to haemodialysis patients with secondary hyperparathyroidism. The drug was given in the morning or evening for 12 months by a randomized, cross-over design with a 2-month washout period.

Methods

Subjects

Thirteen patients (eight men and five women, mean age 51.1 ± 2.8 years) under the long-term haemodialysis programme at Moka Hospital (Tochigi, Japan) participated in the study. The protocol was approved by the Institutional Review Board of the hospital. All patients gave informed consent before initiation of the study. Clinical profiles of the patients are shown in Table 1. Their urine volume was less than 30 ml day^{-1}. We selected the patients whose serum intact parathyroid hormone (PTH) concentration was >500 μg l^{-1} in each patient. Diameter of parathyroid glands measured by ultrasonography was >1.0 cm in 12 patients. Maintenance haemodialysis (4–4.5 h per session) was performed three times a week (Monday, Wednesday and Friday) in each patient. Although the prescription of dialysis was not altered until the end of the study, the dry weight of the body was changed as appropriate during the study. Their haematocrit was >25% during the whole study period. Seven patients received erythropoietin (epoetin α) for renal anaemia and 11 patients received antihypertensive drugs. Their dosage regimens were changed appropriately during the study. Seven patients already received active D3 (1-α-calcitriol, 0.5–1.0 μg day^{-1}) before entry to this study. After obtaining informed consent from these patients, the D3 analogue was discontinued for 8 weeks just before initiation of the study.

Study protocol

The study was performed by an open-label, randomized, cross-over design with a 8-week washout period. The haemodialysis session was started at 09.00 h. These patients eat breakfast around 07.00–08.00 h and dinner

Table 1 Profiles of patients.

Patient	*Age (gender)*	*Aetiology*	*Time after initiation of haemodialysis therapy (years)*	*Serum creatinine (mg dl^{-1})*	*Serum aluminium (μg l^{-1})*	*Serum β_2-microglobulin (mg l^{-1})*
1	49 (M)	CGN	7	9.5	0.4	25.9
2	57 (M)	CGN	15	11.3	0.7	30.2
3	37 (M)	CGN	9	10.2	0.3	22
4	60 (M)	PCK	19	12.5	0.8	24.5
5	42 (M)	PCK	12	8.9	0.7	22.7
6	51 (M)	CGN	5	8.6	0.3	29.4
7	52 (F)	CGN	7	6.9	0.3	35.1
8	63 (F)	Reflux	12	8.5	0.5	33.2
9	35 (F)	CGN	10	10.3	0.3	30.1
10	50 (F)	PCK	14	11.5	0.3	23.5
11	61 (M)	*CGN*	*9*	*9.6*	*0.2*	*29.5*
12	49 (F)	*CGN*	*5*	*11.7*	*0.2*	*24.2*
13	58 (F)	*PCK*	*14*	*13.5*	*0.7*	*32.5*
Mean ± SE			10.6 ± 1.3	10.2 ± 0.6	0.4 ± 0.1	27.9 ± 1.4
Normal range				0.6–1.3	1.0	1.0–1.9

CGN, Chronic glomerulonephritis; PCK, polycystic kidney; reflux, reflux nephropathy. Three patients (nos 11–13) dropped out because of severe hypercalcaemia when the dosing schedule was switched from evening to morning (see Figure 1).

Table 2 Mean values of serum parameters at initiation of the study.

	Morning trial (n = 7)	*Evening trial (n = 6)*
Calcium (mg dl^{-1})	8.9 ± 0.2	8.6 ± 0.2
Phosphate (mg dl^{-1})	4.1 ± 0.1	3.9 ± 0.1
Intact PTH (pg ml^{-1})	802 ± 115	774 ± 83
ALP (IU l^{-1})	307 ± 38	311 ± 32
Albumin (g dl^{-1})	3.4 ± 0.3	3.6 ± 0.2

Not D3

around 19.00–20.00 h. Patients received 3 µg of 1,25-dihydroxycholecalciferol (Rocaltorol®; Roche, Tokyo, Japan) at 20.00 h on the day of the dialysis (evening trial) or 08.00 h on the next day of the dialysis (morning trial) for 12 months. Seven patients out of 13 received morning dose first. Calcium carbonate (1.5–4.5 g day^{-1}, at 08.00 h, 20.00 h just after meal) was prescribed as phosphate binder and its dose was not changed throughout the study in each patient. Blood samples at just before starting the dialysis session were obtained every month. Serum concentrations of Ca (normal range 8.7–10.1 mg dl^{-1}), P (normal range 2.4–4.3 mg dl^{-1}), intact PTH (normal range 10–65 pg ml^{-1}), and serum alkaline phosphatase (ALP) (normal range 80–260 IU l^{-1}) and albumin (normal range 3.9–4.9 mg dl^{-1}) at the initiation of the study were not significantly different between the two trials.

Measurements

Serum concentrations of calcium and inorganic phosphate were determined by orthocresolphtalein complex method [20], and ammonium molybdate method [21] with an autoanalyser (Autoanalyser 7170; Hitachi, Tokyo, Japan), respectively. Serum ALP [14] was measured by the autoanalyser [22]. Serum intact parathyroid hormone (iPTH) concentration was measured by radioimmunoassay [23]. To evaluate bone density, the quantitative computed tomography (QCT) of fourth lumbar vertebrate with 7 mm slice thickness was performed just before, and at 6 and 12 months after the initiation of the study [24]. A computed tomography value was obtained from a central rectangle of 10×10 pixels of the vertebrate, which was converted to $CaCO_3$ content by comparison with the standard. Each value of $CaCO_3$ content was obtained from the mean value of three continuous slices of the bone. It is reported that the values of trabecular bone volume and bone density obtained by this method have good correlations with those by iliac biopsy [24].

Statistics

Data are shown as the mean ± SE Statistical analysis was performed by two-way ANOVA or paired *t*-test as appropriate. *P*-value <0.05 was regarded as significant.

Results

Hypercalcaemia and hyperphosphataemia were exaggerated in the morning trial

Three out of 13 patients developed severe hypercalcaemia when the dosing schedule was changed from evening to morning and were withdrawn from the study. The individual course of these cases (patients 11–13) are shown in Figure 1a, b and c. Such an exaggerated elevation was not detected in patients who received D3 at 08.00 h in the first year and at 08.00 h in the second year. Serum concentrations of calcium and phosphate in the other ten patients are shown in Figure 2a,b. As shown, the hypercalcaemic and hyperphosphataemic effects of D3 depended on the dosing time, which was deteriorated in the morning trial. Mean serum Ca concentration after the trial was 10.92 (95% confidence interval (CI) 10.79, 11.06) and 9.55 mg dl^{-1} (95% CI 9.30, 9.71) by 08.00 h and 20.00 h dosing, respectively. Serum albumin concentration was not changed throughout the study (data not shown).

Therapeutic effects of D3 were excellent in the evening trial

To evaluate the efficacy of D3 therapy, we monitored serum ALP and iPTH concentrations. As shown in Figure 3a,b, these values were elevated at the initiation of the study and decreased during D3 treatment in both trials. However, the decrements of these parameters were greater in the evening trial. Mean PTH concentration after the trial was 414 (95% CI 360, 475) and 220 pg ml^{-1} (95% CI 202, 249) by 08.00 h and 20.00 h dosing, respectively ($P = 0.02$).

Bone density slightly but significantly increased in the morning and evening trials (Figure 4a). Its increment in the evening trial was significantly greater than that in the morning trial (Figure 4b). Mean increment of bone density after the trial was 22 (95% CI 8, 32) and 57 g cm^{-3} (95% CI 43, 83) by 08.00 h and 20.00 h dosing, respectively ($P = 0.04$). Percent increase of the bone density was 18.4 ± 5.3% and 30.9 ± 5.9%, 08.00 h and 20.00 h dosing, respectively.

Discussion

Secondary hyperparathyroidism, which is frequently observed in patients with chronic renal failure, causes osteoporosis and renal osteodystrophy. The mechanism of

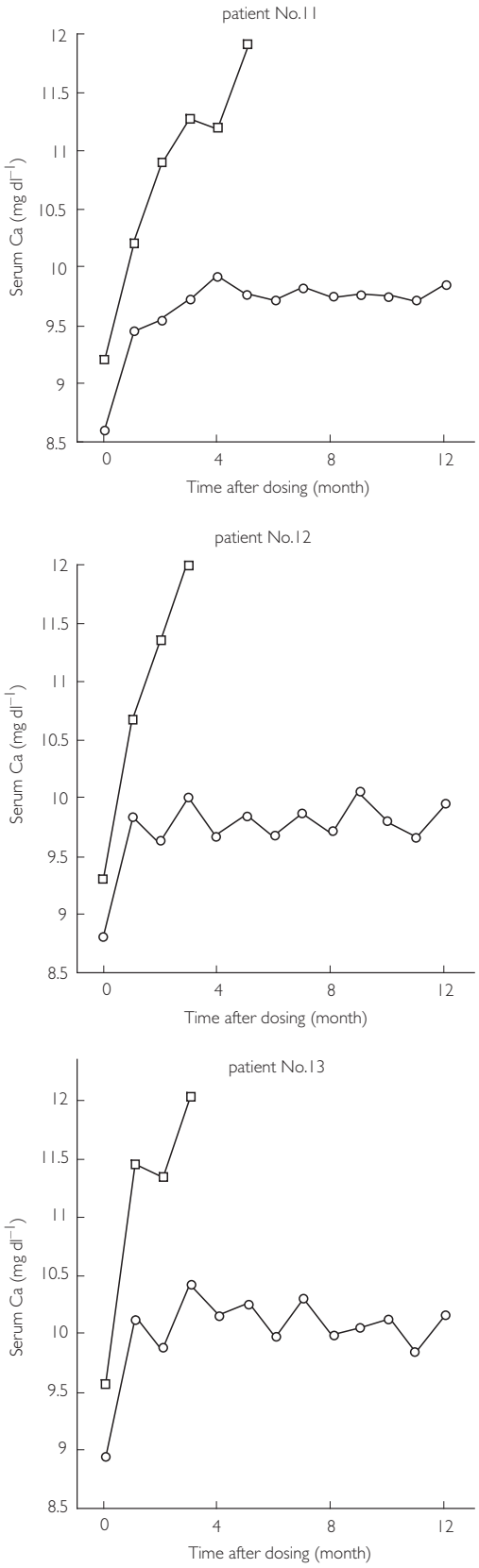

Figure 1 Individual profile of serum Ca concentration in patient 11 (a), 12 (b) and 13 (c) who suffered from severe hypercalcaemia after switching from evening (□-□) to morning (○-○) dosings of D3.

secondary hyperparathyroidsm in these patients is considered to be as follows [14]. Patients cannot excrete enough phosphate in urine, which causes hyperphosphataemia and subsequent hypocalcaemia. Hydroxylation from 25D3 to 1,25D3 in kidney is also impaired in renal failure, which subsequently reduces intestinal Ca absorption and serum Ca concentration. Hypocalcaemia, in turn, stimulates the parathyroid gland to secrete parathyroid hormone, which consequently leads to bone resorption. To treat this condition, a higher dose of D3 is given orally (2–4 μg) or intravenously (1–3 μg) at the end of each haemodialysis session [17, 25]. However, with this therapy, serum calcium concentration needs to be monitored frequently to maintain it within the normal range. When the patient develops hypercalcaemia, the pulse therapy is discontinued until serum calcium concentration returns to normal.

We previously showed that the elevation in serum calcium concentration after a single oral dosing of D3 (2 μg) is greater in the morning than in the evening trials in haemodialysis patients with secondary hyperparathyroidism [18]. In this study, three patients were withdrawn from the trial due to severe hypercalcaemia during the repeated dosing of D3 (3 μg) in the morning. Moreover, the elevation in this parameter in the morning trial was significantly greater than that in the evening trial in the remaining ten patients. Based on these observations, we believe that a higher dose of D3 is safer in the evening than in the morning for the treatment of secondary hyperparathyroidism in haemodialysis patients. Potential mechanisms of the dosing time-dependent difference in the D3-induced hypercalcaemia are: (i) D3 stimulates bone resorption by osteoclasts [26], resulting in the elevation of plasma calcium concentration. We have recently reported that urinary excretion of deoxypyridinolline, a marker of bone resorption, is greater during the repeated dosing of 1-α-(OH) D3, a prodrug of active D3, at an active period than at a resting period in animal model of osteoporosis [18]. Such a dosing time-dependent difference in the sensitivity of osteoclast to D3 may also be involved in the chronotoxic effect of the drug in human subjects; (ii) D3 increases absorption of $CaHPO_4$ from the gut through increasing calcium-binding protein in the intestine [27]. The patients took D3 just after breakfast in the morning trial and after dinner in the evening trial in this study. Therefore, although the calcium content in each meal was not identical, the role of diet in the dosing time-dependent phenomenon of D3 may be small, if any.

The suppressive effect of a higher dose of D3 on serum PTH did not significantly differ between the two trials at 2 months after the initiation of each trial, which is similar to that observed in the single dosing study [19]. However, further repeated treatment with

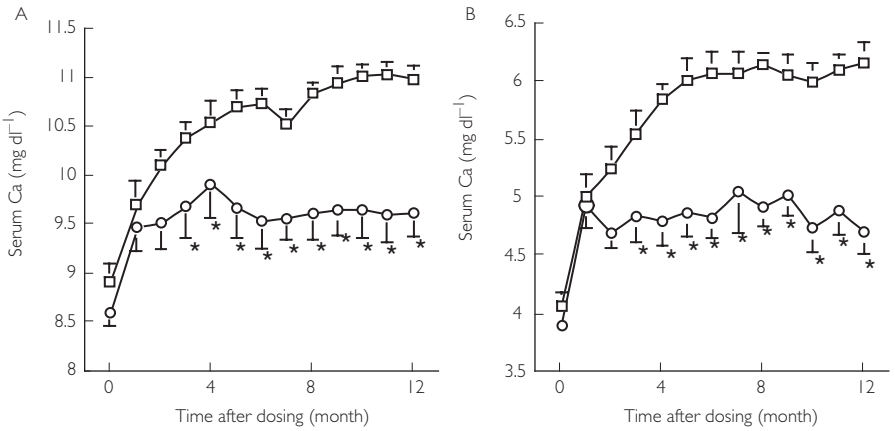

Figure 2 Serum Ca (a) and P (b) concentrations during morning (□-□) and evening (○-○) dosings of D3. Mean ± SE, $n = 10$. ⋆$P < 0.05$ *vs* morning trial.

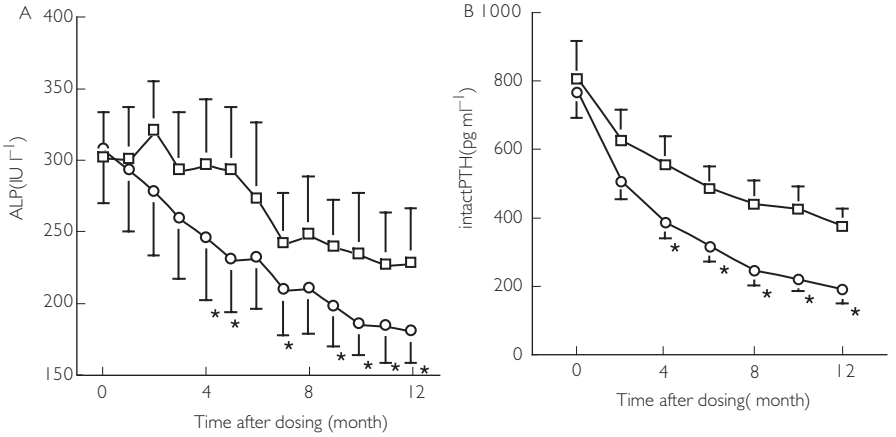

Figure 3 Serum alkaline phosphatase (ALP) (a) and intact parathyroid hormone (PTH) (b) concentrations at morning (□-□) and evening (○-○) dosings of D3. Mean ± SE, $n = 10$. ⋆$P < 0.05$ *vs* morning trial.

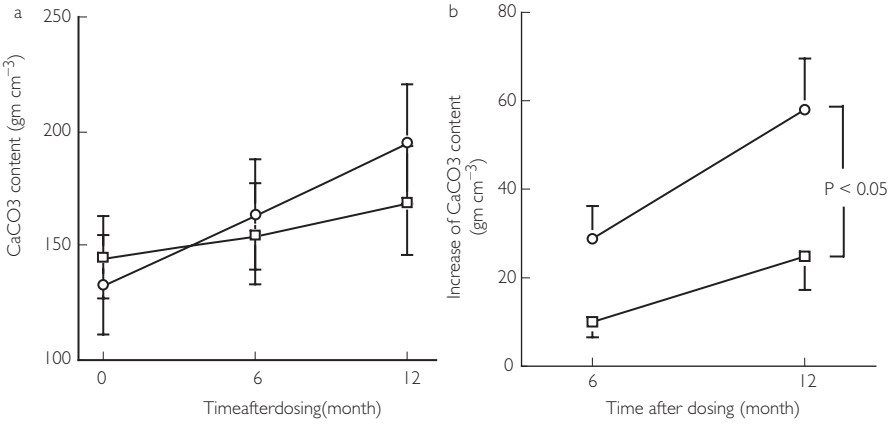

Figure 4 Bone density (a) and its increment from pretreatment level (b) during morning (□-□) and evening (○-○) dosings of D3. Mean ± SE, $n = 10$.

D3 revealed that the evening dosing was more effective for reducing the elevated serum iPTH concentration. Consequently, as expected, the increment of bone density during the repeated treatment with D3 was greater in the evening than in the morning trials in haemodialysis patients with renal osteodystrophy in this study. Thus, this study showed for the first time that a higher dose of D3 is more effective when it is administered in the evening than when it is administered in the morning for the treatment of renal osteodystrophy. A higher dose of D3 reduces the elevated serum PTH concentration and decreases the enlarged parathyroid gland through the direct inhibitory action on the preproparathyroid hormone gene in parathyroid cells [28]. A previous single oral dosing study showed that serum D3 concentrations in the morning and evening trials did not significantly differ at any observation point [18]. Although the pharmacokinetic profiles of D3

were not determined in this study, it is likely that a dosing time-dependent sensitivity to parathyroid gland is involved in the chronoeffectiveness of D3 observed in this study.

The serum Ca concentration before study was slightly but not significantly higher in the morning trial than the evening trial. Because this study was done by randomized cross-over design with an 8-week washout period, we do not consider order effect as the reason. Although the precise reason is not certain, we think that the major reason is the diurnal rhythm of serum Ca concentrations. It is well known that serum Ca and P concentrations peak in the early morning and are at their nadir at night in both humans and rat [6, 11]. We also confirmed this phenomenon in a previous single dosing study with chronic renal failure patients [18]. It is also correct that the serum Ca concentration after initiation of the study was higher in the morning trial. In this case, the lag time between blood sampling and dosing of D3 might have affected the findings. Because $CaCO_3$ was given twice daily at 08.00 h and 20.00 h, there was no lag time between blood sampling and dosing of $CaCO_3$. Also, there was no lag time between dosing of $CaCO_3$ and D3 in the two trials.

In recent years, the prevalence of haemodialysis for the treatment of end-stage renal disease has grown in the USA, European countries and Japan [29]. Unfortunately, many patients on chronic haemodialysis develop renal osteodystrophy. Oral or intravenous D3 is given to these patients, but severe hypercalcaemia sometimes interrupts this therapy. This study shows that the safety and effectiveness of a higher dose of D3 depends on its dosing time, which is superior in the evening. Because a repeated intravenous injection of D3 at evening is inconvenient for most haemodialysis patients, we think that oral dosing is more practical for the chronotherapy of D3. It is correct that increase in rate of bone mineral density might be larger especially during the first year of treatment, and this might affect our results. However, we think such a possibility is low because seven randomly chosen patients received morning dose first and six patients received evening dose first.

In summary, this study showed that a higher dose of oral D3 is more safe and effective after dosing in the evening than after dosing in the morning in patients with renal osteodystrophy. Drug-related hypercalcaemia and hyperphosphataemia were less after dosing at night. Furthermore, the efficacy of the therapy (reduction of serum PTH and ALP concentrations, and increase of bone density) was greater in the evening dosing. These observations will be useful for the treatment of hyperparathyroidism in these patients.

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