Effect of 1α-Hydroxyvitamin D_3 on Serum Levels of Thyroid Hormones in Hyperthyroid Patients With Untreated Graves' Disease

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We performed a randomized, open prospective study to determine the effect of 1α-hydroxyvitamin D_3 [1α(OH)D3] on hyperthyroidism in patients with untreated Graves' disease. At the time of entry into the study, 30 patients were randomly assigned to receive a daily dose of 30 mg methimazole (MMI) (group A, n = 15) or the same dose of MMI supplemented with 1.5 μg 1α(OH)D3 (group B, n = 15). These treatment regimens were continued for 24 weeks, and physicians were allowed to adjust MMI dosage during follow-up visits. Blood samples were collected, and serum concentrations of free triiodothyronine (FT_3), free thyroxine (FT_4), T_3, T_4, thyrotropin (TSH), alkaline phosphatase (ALP), and TSH-receptor antibody (TRAb) were determined. During the follow-up periods, all patients became euthyroid. The dose of MMI was not significantly different between these two groups. In contrast, decreases in mean serum FT_3 and FT_4 levels, as well as in mean serum T_3 and T_4 levels, were greater in group B. Correspondingly, the reciprocal increase in the mean TSH level was more prominent in group B. Mean TRAb levels did not differ between the two groups. Mean serum ALP levels in group B were significantly lower than in group A at 24 weeks. Thus, we suggest that concomitant administration of 1α(OH)D3 is useful for treating hyperthyroidism in patients with Graves' disease.

HYPERTHYROIDISM in patients with Graves' disease is due primarily, if not solely, to the production of autoantibodies that bind to the receptors for thyrotropin (TSH) on thyroid cells.[1] Treatment of Graves' hyperthyroidism is directed toward decreasing the serum concentration of thyroid hormones to achieve a eumetabolic state.[1] Initial medical treatment inevitably includes thionamides as antithyroid drugs,[1] which are known to inhibit thyroid hormone biosynthesis[1] and sometimes exert immunomodulatory activity, as reflected by the decrease in serum antithyroid autoantibodies.[2,3] However, the remission rate after antithyroid drug therapy is considered generally unsatisfactory.[4] Moreover, administration of thionamides sometimes causes a variety of adverse reactions, eg, fever, rash, urticaria, arthralgia, and liver dysfunction.[1] Rarely, more serious reactions such as agranulocytosis, aplastic anemia, a lupus-like syndrome, vasculitis, and crescentic glomerulonephritis occur.[1,5-9] Recent studies indicate that although an increased starting dose of methimazole (MMI) might result in long-term remission of Graves' disease, there is some evidence of a dose-dependent increase in the toxicity of thionamide drugs.[10,11] However, alternative medical therapy for hyperthyroidism is not available at this moment.

1,25-Dihydroxyvitamin D_3 [1,25$(OH)_2$D3] is now recognized to have immunomodulatory activity, and has been shown to attenuate immune abnormalities in several animal models of autoimmune diseases, including murine experimental autoimmune thyroiditis.[12,13] Recently, 1,25$(OH)_2$D3 has also been shown to have a direct action on the thyroid in vitro. Treatment of rat thyrocytes with 1,25$(OH)_2$D3 decreases adenylyl cyclase activity, TSH receptor number, cell growth, and iodide accumulation.[14] However, the effects of vitamin D_3 have not yet been clinically explored in patients with Graves' disease.

With these issues in mind, we undertook a randomized, open prospective study to examine whether supplemental administration of 1α(OH)D3 influences the clinical course in patients with untreated hyperthyroid Graves' disease. This study revealed that the combination of MMI and vitamin D_3 resulted in a significant decrease in serum levels of thyroid hormones when compared with MMI alone.

SUBJECTS AND METHODS

Study Design

From 1992 to 1995, we evaluated 45 consecutive patients with untreated Graves' disease (five men and 40 women aged 16 to 67 years) at the Endocrine Clinic of the College Hospital of Asahikawa Medical College. The diagnosis of Graves' disease was made on the basis of clinical features (diffuse goiter, exophthalmos, tachycardia, tremor, and sweating) and laboratory data such as undetectable or very low serum levels of TSH, elevated serum triiodothyronine (T_3) and thyroxine (T_4) levels, and positive TSH-receptor antibody (TRAb). No patients had thyroid cancer or nonthyroidal illness affecting the thyroid or thyroid function. Of 45 patients who fulfilled the entry criteria, 30 agreed to participate in the study and were enrolled after informed consent was obtained.

The patients were randomly divided into two groups at the time of diagnosis of Graves' disease, and the antithyroid drug MMI was started at a dosage of 30 mg daily. Group A (n = 15) received only MMI, and group B (n = 15) received MMI supplemented with 1.5 μg 1α(OH)D3 (Onealfa, Teijin, Tokyo, Japan). During the course of the study, physicians were allowed to adjust the dosage of MMI depending on the patient's status, although 1α(OH)D3 dose was constant. Patients in group B discontinued the use of 1α(OH)D3 24 weeks after the start of therapy. Concomitant drug administration was not allowed throughout the study, except for the β-adrenoreceptor antagonist propranolol to control hyperadrenergic symptoms. Follow-up visits were scheduled at 2- and 4-week intervals for 24 weeks. To limit diurnal variation in the level of TSH, the visit was scheduled at the same time of day. During each visit, venous blood samples were obtained to monitor serum concentrations of FT_3, FT_4, T_3, T_4, TSH, TRAb, alkaline phosphatase (ALP), Ca, and IP. All serum samples from each patient were stored at −70°C before assay.

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Assay and Analysis

Serum levels of thyroid hormones and TSH were measured by commercial assays according to the manufacturers' instructions (FT_4 and FT_3, Diagnostic Products, Los Angeles, CA; T_3 and T_4, Amerlex-M RIA, Eastman Kodak, Rochester, NY; and TSH, Autodelphia TSH Ultra, Wallac, Turku, Finland). Serum concentrations of ALP, Ca, and IP were determined by standard methods using an autoanalyzer. TRAb was determined by a radioreceptor assay (Yamasa, Chiba, Japan); the normal range is less than 10%. All samples were assayed in duplicate in a single assay to limit interassay variance.

Statistical Analysis

Results are expressed as the mean ± SE unless otherwise noted. We used the Student *t* test, Mann-Whitney *U* test, Fisher exact test, and Wilcoxon signed-rank test when appropriate to determine the significance of differences between groups. All tests were two-tailed. Changes in FT_3, FT_4, TSH, and ALP were assessed using repeated-measures ANOVA. Significance was defined as $P < .05$.

RESULTS

The initial demographic characteristics and serum biochemical and serologic features of the two groups are summarized in Table 1. Comparison of the two groups shows that they were well matched with respect to a wide range of variables. All patients were evaluated for the full 24 weeks and became euthyroid at some time during the follow-up period. None complained of any adverse effects. Mean serum levels of FT_3 decreased in both groups 2 weeks after the start of treatment, but the decrease appeared to be significantly greater in group B (Fig 1A). At 6 weeks, serum FT_3 levels reached the normal range in four (26.3%) and 12 (75%) patients in groups A and B, respectively ($P < .05$). Similarly, mean serum FT_4 levels decreased during the treatment period in both groups 2 weeks after the start of treatment. This decrease appeared to be greater in group B (Fig 1B). Relatively rapid normalization in group B was also noted with respect to serum levels of both T_3 and T_4 (Fig 1C and D). TSH serum levels increased from the initial, undetectable levels in both groups, reflecting normalization of thyroid hormone levels. However, the increase was greater in group B: at 8, 12, and 16 weeks, mean serum levels of TSH were significantly higher in group B than in group A (Fig 1E). In parallel with the normalization of serum levels of thyroid hormones and the reciprocal increase in serum TSH levels, MMI doses were tapered in both groups (Fig 2). Although MMI doses were not significantly different between the two groups (Table 1 and Fig 2), it became necessary to decrease the dose in seven patients of group B due to unexpectedly rapid recovery from hyperthyroidism and marked elevation of TSH above the normal range. Mean values for TRAb decreased significantly in both groups by the end of the study (from 49% to 40% in group A and from 52% to 42% in group B), but the mean values did not differ between the two groups at any time during follow-up study (Fig 3). Mean serum ALP levels were initially elevated in both groups, and remained higher than the normal range shortly after the initiation of therapy (Fig 4A). At 24 weeks, group B showed significantly lower ALP values versus both their own initial level and the initial and final level of group A ($P < .05$; Fig 4A). Serum concentrations of Ca were initially elevated, but returned to the normal range after the start of antithyroid therapy in both groups (Fig 4B).

Table 1. Patient Characteristics by Treatment Group

Characteristic	Patients Receiving MMI (group A, n = 15)	Patients Receiving MMI + 1α(OH)D3 (group B, n = 15)	*P*
Mean age (yr)	42.1 ± 1.0	42.5 ± 1.2	.901*
Males			
No.	2	4	.410†
%	13.3	26.7	
Positive for TRAb			
No.	15	15	>.999†
%	100	100	
Positive for thyroid test			
No.	13	13	>.999†
%	86.7	86.7	
Positive for microsome test			
No.	8	6	.494†
%	53.3	66.7	
Time at the start of MMI tapering (wk)	13.2 ± 1.6	10.1 ± 1.3	.159*
Total dose of MMI (g)	4.04 ± 0.32	3.55 ± 0.23	.236*
Patients treated with propranolol			
No.	12	12	>.999†
%	80	80	
Serum levels			
FT_3 (pg/mL)‡	15.33 ± 0.99	15.08 ± 1.21	.633*
FT_4 (ng/dL)	5.37 ± 0.52	5.05 ± 0.37	.648*
T_3 (ng/mL)	3.59 ± 0.25	3.47 ± 0.25	.771*
T_4 (μg/dL)	17.97 ± 1.14	18.25 ± 0.90	.604*
TSH (μIU/L)	0.02 ± 0.01	0.04 ± 0.03	.888*
ALP (IU/L)	448.6 ± 41.8	445.9 ± 40.7	.967*

*Mann-Whitney *U* test.

†Fisher exact test.

‡Normal ranges: FT_3, 2.70-5.60 pg/mL; FT_4, 1.01-1.79 ng/dL; T_3, 0.84-1.56 ng/mL; T_4, 5.70-11.70 μg/dL; TSH, 0.34-3.60 μIU/L; ALP, 96-284 IU/L.

At the end of the study (24 weeks after initiation of antithyroid treatment), 1α(OH)D3 was discontinued in group B, and thereafter all patients were treated with MMI alone. All patients were successfully evaluated for another 24 weeks, and no patient in either group experienced a relapse of hyperthyroidism. At the end of observation, all patients were in a euthyroid state under MMI therapy, and there was no significant difference in mean TRAb values (25% and 24% in groups A and B, respectively). Serum ALP returned to normal concentrations in both groups.

DISCUSSION

In the present study, we sought to clarify whether supplemental administration of 1α(OH)D3 influences the treatment course of untreated hyperthyroidism in patients with Graves' disease. Since serum concentrations of Ca are known to be elevated in hyperthyroidism[1] and the addition of 1α(OH)D3 might cause hypercalcemia, all physicians were advised as to whether the patients were taking MMI alone or MMI and vitamin D_3 after randomization. We found that in patients who were treated with both MMI and 1α(OH)D3, not only FT_3 and FT_4 but also T_3 and T_4 decreased more rapidly compared with the levels in patients treated with MMI alone. Serum levels of TSH showed a reciprocal increase, and the rate of this increase was more rapid

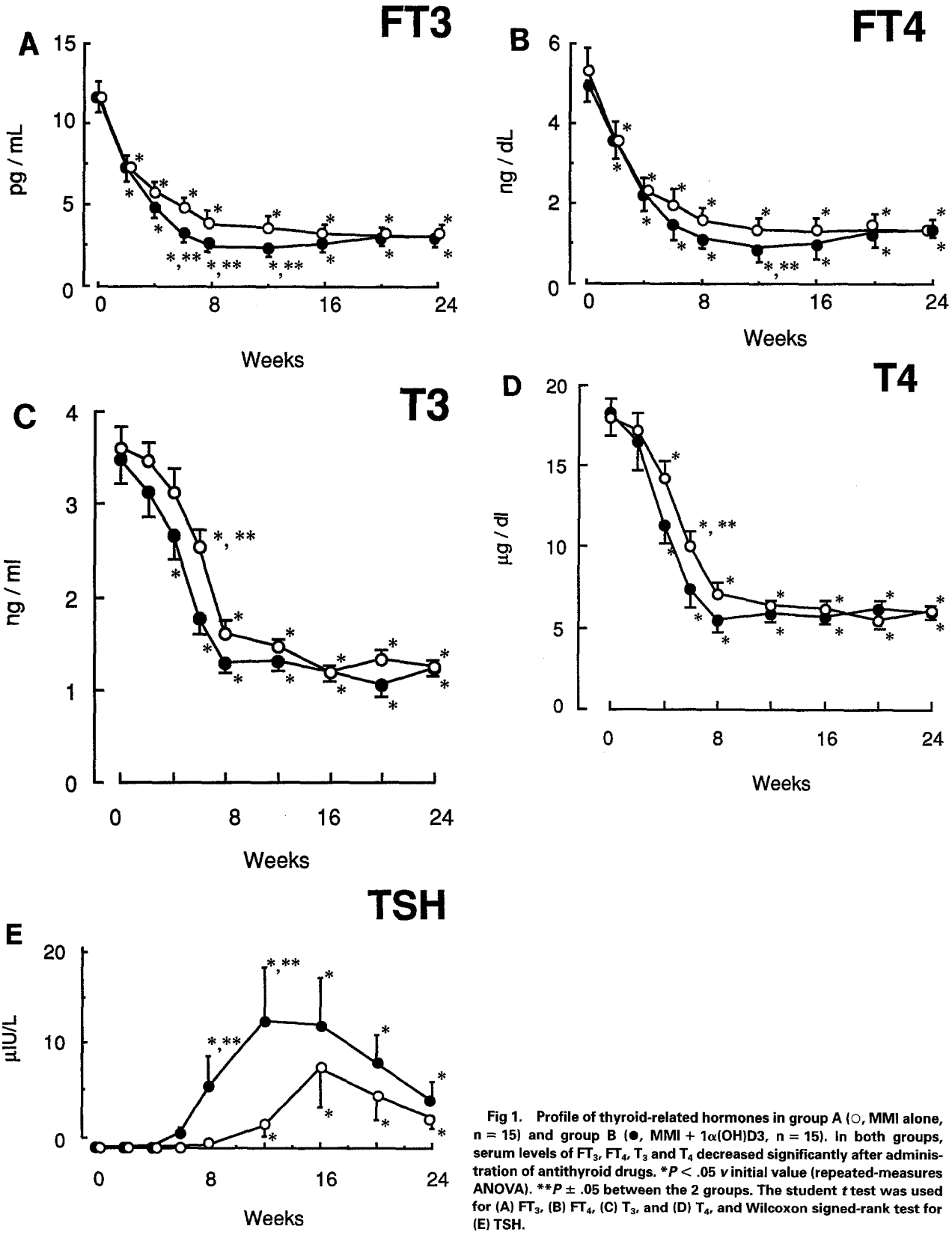

Fig 1. Profile of thyroid-related hormones in group A (○, MMI alone, n = 15) and group B (●, MMI + 1α(OH)D3, n = 15). In both groups, serum levels of FT_3, FT_4, T_3 and T_4 decreased significantly after administration of antithyroid drugs. $*P < .05$ *v* initial value (repeated-measures ANOVA). $**P \pm .05$ between the 2 groups. The student *t* test was used for (A) FT_3, (B) FT_4, (C) T_3, and (D) T_4, and Wilcoxon signed-rank test for (E) TSH.

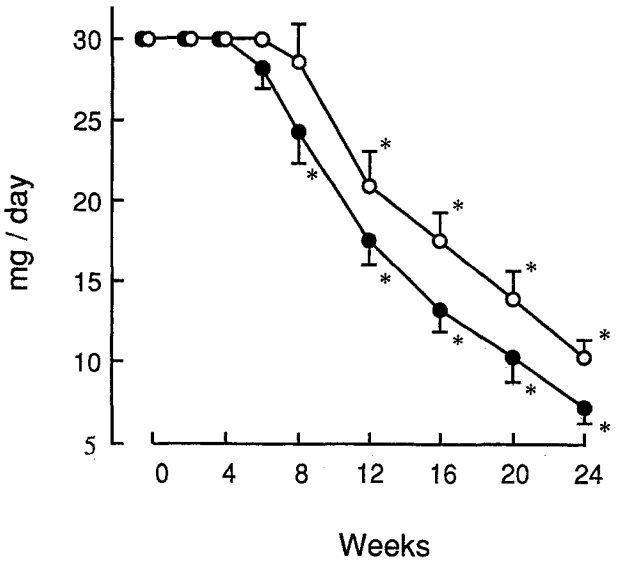

Fig 2. Profile of MMI doses. (○) Group A; (●) group B. There was no significant difference between the 2 groups (Wilcoxon signed-rank test). *$P < .05$ *v* initial doses (Student *t* test).

in group B. The rapid decline of FT_3 and FT_4 in group B may therefore be due to a net decrease in thyroid hormone production by the addition of 1α(OH)D3. Of course, we cannot completely rule out the possibility that the physicians decreased MMI doses more rapidly in patients treated with MMI alone. However, either the total dose of MMI or the mean MMI dose at each visit did not differ significantly between the two treatment groups (Table 1 and Fig 2), strongly indicating that such was not the case. After 16 weeks when all patients were euthyroid, we did not observe any difference in serum concentrations of FT_3, FT_4, T_3, and T_4, possibly due to feedback regulation of TSH secretion.

As described in the introduction, it has recently been shown that $1,25(OH)_2D3$ has immunomodulatory activity. However, TRAb values were not significantly different between the two treatment groups in this study. It therefore appears unlikely that 1α(OH)D3 suppresses the immune system in Graves' disease.

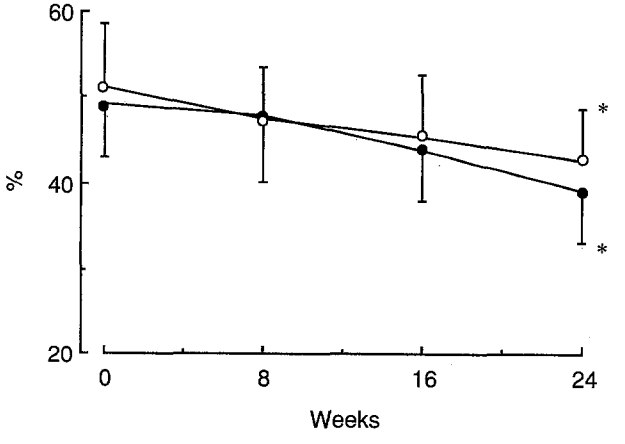

Fig 3. Profile of TRAb. (○) Group A; (●) group B. $P < .05$ *v* initial value (repeated-measures ANOVA). There was no significant difference between the 2 groups (Wilcoxon signed-rank test).

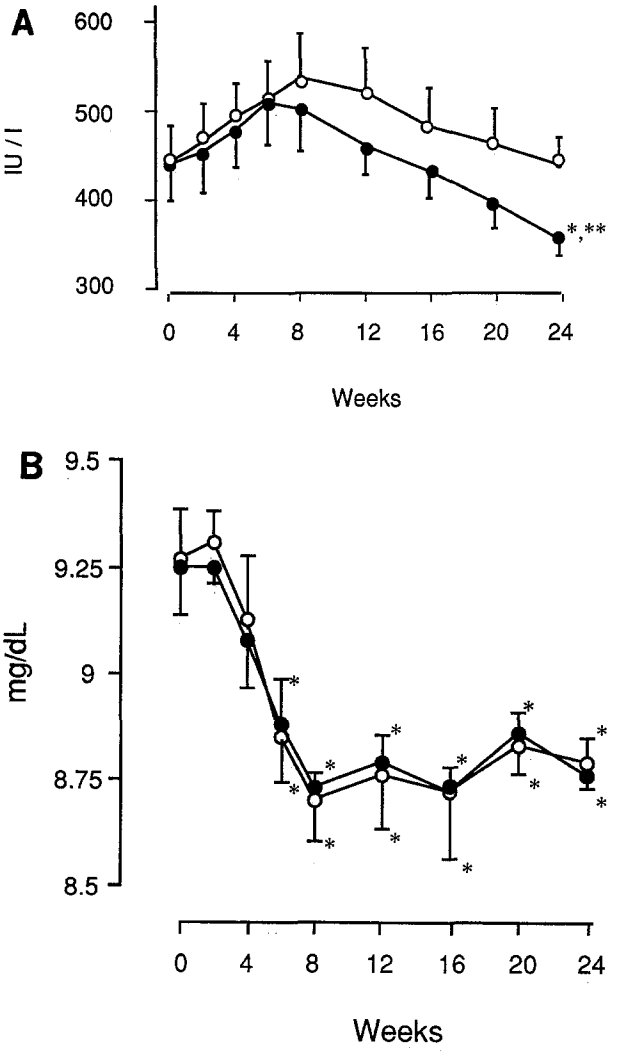

Fig 4. (A) Profile of ALP. (○) Group A; (●) group B. *$P < .05$ *v* initial value (repeated-measures ANOVA). **$P < .05$ between the 2 groups (Student *t* test). (B) Profile of serum Ca. (○) Group A; (●) group B. *$P < .05$ *v* initial value (repeated-measures ANOVA). **$P < .05$ between the 2 groups (Student *t* test).

There may be several possibilities for the mechanism of antithyroid action of 1α(OH)D3. The first is a decrease in biotransformation of T_4 to T_3. The second is an acceleration in the metabolism or a decrease in the biosynthesis of thyroid hormones. At this moment, we are uncertain as to which one is most likely. However, recent studies using cultured thyroid cells strongly suggest direct antithyroid action of $1,25(OH)_2D3$ (see the introduction). In rat thyroid cells, $1,25(OH)_2D3$ specifically binds to the vitamin D receptor, decreasing TSH receptor number, and also suppresses both adenylyl cyclase activity and iodide uptake.[14] To address whether vitamin D_3 alone is effective in patients with hyperthyroidism, we are currently searching for a vitamin D_3 analog that possesses stronger antithyroid activity in an in vitro system.

Calcium metabolism is known to be impaired in Graves' disease.[15] It has been suggested that thyroid hormones stimulate osteoclast activity and bone resorption. Additionally, these hormones increase bone turnover and Ca release from the

bone.[15] It has also been shown that in patients with hyperthyroidism the serum level of $1,25(OH)_2D3$ is decreased, probably due to reduced renal 1α-hydroxylase activity secondary to hypercalcemia.[16] Clinically, osteoporosis is now recognized as one of the complications of Graves' disease, especially in elderly patients.[17] In the present study, serum Ca concentrations were elevated before treatment, as previously reported.[15] However, during and after treatment, these concentrations decreased to normal in a similar fashion in both groups. 1α(OH)D3 may suppress Ca release from the bone, either directly or indirectly via a decrease in serum thyroid hormones, and neutralize the hypercalcemic action of 1α(OH)D3. Thus, administration of 1α(OH)D3 does not always result in hypercalcemia, especially in patients with hyperthyroidism. The significant and rapid decline in serum ALP concentrations in group B appeared to reflect the net effects of 1α(OH)D3 on Ca metabolism.

In summary, we conclude that the decrease in serum thyroid hormones may be accelerated by supplemental administration of 1α(OH)D3. In a case that requires a rapid return to normal of serum thyroid hormone concentrations, such as in the treatment of patients with thyroid storm and those undergoing urgent surgery for other diseases, our regimen may prove useful. To confirm the clinical benefit of this combination therapy, a double-blind control study is being performed. Although we did not observe any adverse drug reactions to either MMI or 1α(OH)D3 in the present study, most possibly due to the small sample size, decreasing the MMI dose by concomitant administration of 1α(OH)D3 might lessen the occurrence of such reactions.[10,11]

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