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Bone formation by minimodeling is more active than remodeling after parathyroidectomy

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Bone formation using the process known as minimodeling forms only lamellar bone in the absence of prior bone resorption even in uremic patients. In patients undergoing parathyroidectomy for secondary hyperparathyroidism, we compared the contribution of minimodeling to remodeling during the change in bone volume. Iliac bone biopsies were used to measure parameters related to minimodeling and remodeling before, at 3 to 4 weeks and 10 to 12 weeks after parathyroidectomy. Osteoblast surface due to minimodeling was greater than the entire bone osteoblast surface before and at 10 to 12 weeks after parathyroidectomy, but not 3 to 4 weeks after surgery. Minimodeling significantly increased osteoid volume 3 to 4 weeks after parathyroidectomy. The rate of change of osteoid volume by minimodeling was greater than that of osteoid volume during the first 3 to 4 weeks after surgery, indicating osteoid formation was more active at the minimodeling surface than at the entire bone surface. Furthermore, higher mineral apposition rates at the minimodeling sites than at remodeling sites yielded increased minimodeling bone volume at 10 to 12 weeks after surgery. Our results show that bone formation by minimodeling is more active than by remodeling and accounts, in part, for the increase of bone volume following parathyroidectomy.

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Bone formation is sometimes seen in the absence of previous bone resorption, a process referred to as minimodeling.[1,2] Previous reports have related the cancellous minimodeling observed in uremic patients with hypoparathyroidism to direct activation of osteoblasts[3] (Yajima A, Ogawa Y, Tominaga Y *et al.* Minimodeling in chronic and postparathyroidectomy hypoparathyroidism. *J Am Soc Nephrol* 2004; **15**: 511A (abstract)). In the process of minimodeling, bone formation occurs from a quiescent surface,[3,4] and the resultant newly formed bone is normal lamellar in texture.[1–4] As it has been reported that bone volume (BV)-referent minimodeling BV (Ml.BV) (Ml.BV/BV) in cancellous bone is greater in uremic patients with secondary hyperparathyroidism than in nonuremic subjects, minimodeling might reduce the rate of bone loss in these patients.[4] Minimodeling surface (Ml.S)-referent osteoblast surface (Ob.S) (Ob.S/Ml.S) is reported to be greater than the entire bone surface (BS)-referent osteoblast surface (Ob.S/BS) in patients with secondary hyperparathyroidism,[4] indicating that bone formation is more active at the minimodeling sites in these patients. Ml.BV/BV is reported to be greater in uremic patients with hypoparathyroidism than in those with secondary hyperparathyroidism,[3] suggesting that Ml.BV/BV might increase markedly after treatment for secondary hyperparathyroidism. Although the mean BV at minimodeling sites is reported to account for less than 1% of the trabecular BV, and minimodeling sites were less than 2%, on average, of the entire cancellous bone surface, the mean osteoid volume at sites of minimodeling accounts for approximately 10% of the entire osteoid volume, and the labeled surfaces at minimodeling sites constitute approximately 25–50%, on average, of the entire labeled surface in healthy individuals.[2] These results suggest that minimodeling seems to be important in new bone formation. Therefore, we investigated changes of the relative osteoid volume associated with minimodeling in comparison with those associated with remodeling after parathyroidectomy. We also conducted tetracycline-based analysis of the dynamic parameters at the minimodeling sites as compared with those at the remodeling sites. Thus, we investigated the contribution of minimodeling to the formation of new bone after parathyroidectomy in patients with secondary hyperparathyroidism.

RESULTS

Serum bone metabolic markers

Serum intact parathyroid hormone (iPTH) levels decreased at 3–4 weeks after parathyroidectomy (as measured in Group I, $n = 15$) and the suppression was sustained until 10–12 weeks (as measured in Group II, $n = 7$). Serum levels of bone resorption markers, including tartrate-resistant acid phosphatase and deoxypyridinoline, decreased significantly at 3–4 weeks after parathyroidectomy, and the decreases were sustained until 10–12 weeks. Serum levels of bone formation markers, including carboxy-terminal propeptide of type I procollagen (PICP) and total alkaline phosphatase (total ALP), showed a temporary but significant increase at 3–4 weeks after parathyroidectomy (Group I), as reported previously,[5] although 10–12 weeks after surgery, a tendency toward decrease of the serum PICP and significant decrease of the serum total ALP was observed (Group II). No significant changes were observed in the serum calcium (Ca) levels at either 3–4 or 10–12 weeks after parathyroidectomy, probably because of the initiation of oral administration of alfacalcidol (1α-hydroxyvitamin D_3; Chugai Pharmaceutical Co. Ltd, Tokyo, Japan) at doses of 0.5–2.0 μg/day with intravenous Ca gluconate or oral Ca carbonate. Serum phosphorus (P) levels were significantly decreased at 3–4 weeks after parathyroidectomy (Group I), but not at 10–12 weeks (Group II) (Table 1).

Bone histomorphometry

The remodeling and minimodeling parameters were also measured both before and at 3–4 weeks (Group I) and at 10–12 weeks (Group II) after parathyroidectomy. The histomorphometric parameters, including (1) osteoclast surface (Oc.S/BS), (2) eroded surface (ES/BS), (3) fibrosis volume (Fb.V/TV), (4) osteoblast surface (Ob.S/BS), (5) osteoid volume (OV/BV), (6) osteoid surface (OS/BS), and (7) osteoid thickness (O.Th), were measured at the entire bone surface, including the sites of remodeling and those of minimodeling.[6] The histomorphometric parameters of bone resorption, including Oc.S/BS, ES/BS and Fb.V/TV, which were extremely high before parathyroidectomy, decreased significantly at 3–4 weeks after surgery. Ob.S/BS had already decreased at 3–4 weeks after parathyroidectomy in Group I, although we previously reported a temporary increase of Ob.S/BS at 1 week after parathyroidectomy.[5,7] The decrease was sustained until 10–12 weeks after surgery. The other parameters of bone formation, including OV/BV and OS/BS, which were also markedly increased before parathyroidectomy, showed further increase at 3–4 weeks, and the increases were sustained until 10–12 weeks after surgery (Tables 2 and 3).

In terms of the static minimodeling parameters, significant decrease of Ob.S/Ml.S was observed at 3–4 weeks after parathyroidectomy and the decrease remained sustained until

Table 1 | Serum bone metabolic markers before (A) and at 3–4 weeks after (B) parathyroidectomy (n=15), and those before (A)′ and at 10–12 weeks after (C) surgery (n=7)

	iPTH (pg/ml)	TRAP (U/l)	DPD (pmol/ml)	PICP (ng/ml)	Total ALP (U/l)	Ca (mg/100 ml)	P (mg/100 ml)
(A)	1229.6 ± 447.1	23.5 ± 8.9	48.4 ± 43.3	265.1 ± 79.8	725.2 ± 492.4	10.0 ± 0.9	5.2 ± 1.5
(B)	26.7 ± 13.7	7.3 ± 3.0	9.8 ± 4.2	699.5 ± 450.8	1027.0 ± 540.2	11.1 ± 1.8	2.9 ± 1.7
P-value	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.005$	$P < 0.001$	$P = 0.099$	$P = 0.004$
(A)′	1395.4 ± 812.3	24.6 ± 9.4	76.0 ± 98.8	540.3 ± 681.0	1023.9 ± 641.0	9.7 ± 1.1	5.4 ± 1.7
(C)	31.0 ± 21.6	10.6 ± 5.0	7.4 ± 2.9	199.3 ± 109.5	355.1 ± 112.3	8.6 ± 1.6	3.8 ± 1.5
P-value	$P = 0.028$	$P = 0.028$	$P = 0.028$	$P = 0.063$	$P = 0.028$	$P = 0.063$	$P = 0.076$
Normal range	10-65	5.5-17.2	unknown	30-182	85-255	8.4-10.4	2.5-4.5

Ca, calcium; DPD, deoxypyridinoline; iPTH, intact parathyroid hormone; P, phosphorus; PICP, carboxy-terminal propeptide of type I procollagen; total ALP, total alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase.
The differences in the serum levels of the bone metabolic markers between (A) and (B) and between (A)′ and (C) were analyzed by Wilcoxon's matched pairs test.
Values are expressed as mean ± s.d.

Table 2 | Static histomorphometric variables before (A) and at 3–4 weeks after (B) parathyroidectomy in Group I (n=15), and those before (A)′ and at 10–12 weeks after (C) surgery (n=7) were measured

	Oc.S/BS (%)	ES/BS (%)	Fb.V/TV (%)	OS/BS (%)	O.Th (μm)
(A)	4.5 ± 3.8	27.0 ± 10.6	5.8 ± 4.8	52.8 ± 18.3	14.3 ± 7.6
(B)	0.1 ± 0.5	1.8 ± 1.5	0.6 ± 1.4	83.0 ± 16.4	20.0 ± 9.1
P-value	0.001	0.001	0.001	0.001	0.001
(A)′	3.8 ± 2.9	26.1 ± 10.4	7.5 ± 8.2	51.9 ± 19.2	14.3 ± 5.4
(C)	0.2 ± 0.4	4.0 ± 2.2	0.6 ± 1.7	80.4 ± 19.1	22.9 ± 13.2
P-value	0.028	0.028	0.028	0.028	0.052
Normal range	0.7 ± 0.7	4.0 ± 2.0	0	14.3 ± 6.3	9.3 ± 2.1

ES/BS, eroded surface; Fb.V/TV, fibrosis volume; Oc.S/BS, osteoclast surface; OS/BS, osteoid surface; O.Th, osteoid thickness.
The differences in the histomorphometric variables between (A) and (B) and between (A)′ and (C) were analyzed by Wilcoxon's matched pairs test.
Values are expressed as mean ± s.d.

Table 3 | Static histomorphometric variables, including OV/BV, Ob.S/BS, and minimodeling-related parameters before (A) and at 3–4 weeks after (B) parathyroidectomy (*n*=15), and those before (A)′ and at 10–12 weeks after (C) surgery (*n*=7)

	Ml.BV/TV (%)	Ml.BV/BV (%)	OV/BV (%)	Ml.OV/BV (%)	Ml.OV/Ml.BV (%)	Ob.S/BS (%)	Ob.S/Ml.S (%)
(A)	0.43 ± 0.30	2.00 ± 1.20	12.29 ± 9.75	0.29 ± 0.23	14.21 ± 7.47	20.8 ± 9.9	48.0 ± 25.1
(B)	0.64 ± 0.64	2.57 ± 2.10	19.02 ± 9.39	1.04 ± 0.79	42.00 ± 8.36	14.3 ± 12.6	12.6 ± 12.2
P-value	0.221	0.427	0.011	0.002	< 0.001	0.031	< 0.001
(A)′	0.50 ± 0.38	2.14 ± 1.72	13.21 ± 9.38	0.27 ± 0.23	12.87 ± 2.49	20.7 ± 12.2	43.7 ± 20.9
(C)	2.38 ± 1.23	9.47 ± 4.18	22.0 ± 16.05	2.76 ± 3.37	18.63 ± 15.58	1.2 ± 1.6	7.1 ± 5.8
P-value	0.028	0.028	0.028	0.028	0.345	0.028	0.028
Normal range	Unknown	0.64 ± 1.10	1.9 ± 1.1	0.15 ± 0.33	21.5 ± 8.1	4.4 ± 3.2	Unknown

Ml.BV/TV, tissue volume-referent minimodeling bone volume; Ml.BV/BV, bone volume-referent minimodeling bone volume; Ml.OV/BV, bone volume-referent minimodeling osteoid volume; Ml.OV/Ml.BV, minimodeling bone volume-referent minimodeling osteoid volume; Ob.S/BS, osteoblast surface; Ob.S/Ml.S, minimodeling surface-referent osteoblast surface; OV/BV, osteoid volume.
The differences in the histomorphometric variables between (A) and (B) and between (A)′ and (C) were analyzed by Wilcoxon's matched pairs test.
Values are expressed as mean ± s.d.

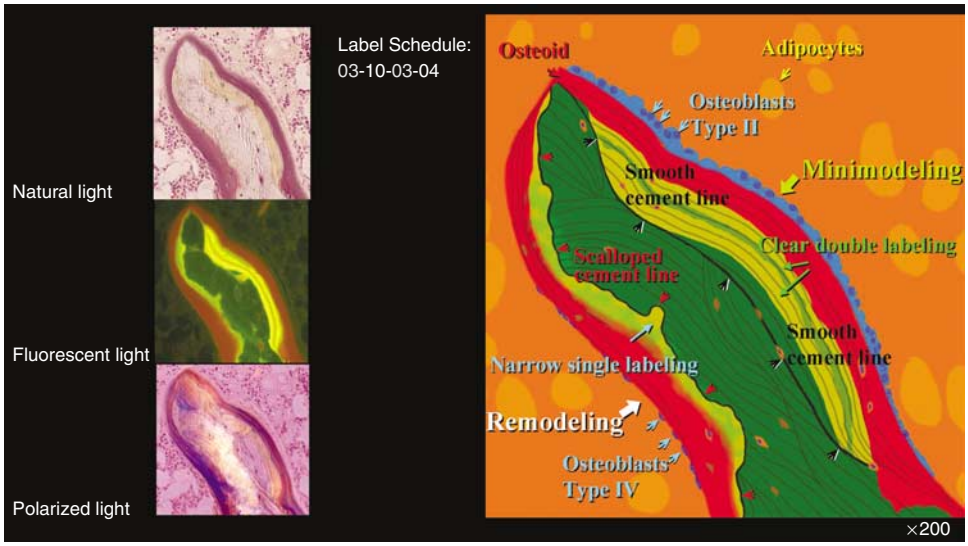

Figure 1 | Tetracycline labeling at 4 weeks after parathyroidectomy at the sites of remodeling and minimodeling. Single and vague labeling was seen more frequently at the sites of remodeling, associated with a scalloped cement line indicating previous bone resorption. Double and clear labeling was seen more frequently at the sites of minimodeling. These were not associated with a scalloped cement line, indicating the absence of previous bone resorption.

10–12 weeks after surgery. Ob.S/Ml.S was significantly greater than Ob.S/BS before parathyroidectomy (48.0 ± 25.1 vs 20.8 ± 9.9%, $P = 0.002$) (Group I), but not at 3–4 weeks after parathyroidectomy (12.6 ± 12.2 vs 14.3 ± 12.6%, $P = 0.532$) (Group I), although Ob.S/Ml.S was greater than Ob.S/BS again at 10–12 weeks (7.1 ± 5.8 vs 1.2 ± 1.6%, $P = 0.028$) (Group II) (Table 3). Although type II osteoblasts were most abundant at the minimodeling sites before parathyroidectomy, a greater abundance of type IV osteoblasts was found at these sites at 3–4 weeks and at 10–12 weeks after parathyroidectomy.

Osteoid seams associated with the minimodeling sites were lamellar,[2,4] but those at the remodeling sites were a mixture of woven and lamellar osteoid seams (Figure 1).[7,8] Significant increase of Ml.BV-referent minimodeling osteoid volume (Ml.OV/Ml.BV) from 14.21 ± 7.47 before parathyroidectomy to 42.00 ± 8.36% ($P < 0.001$) was observed during the first 3–4 weeks after parathyroidectomy, whereas no significant change of Ml.BV/BV was observed at this time point in Group I (2.00 ± 1.20 before parathyroidectomy vs 2.57 ± 2.10% at 3–4 weeks after parathyroidectomy: $P = 0.427$) (Table 3). Ml.OV/Ml.BV was greater than OV/BV at 3–4 weeks after parathyroidectomy (42.00 ± 8.36 vs 19.02 ± 9.39%, $P = 0.001$). To elucidate the important contribution of minimodeling-mediated bone formation to the total increase of BV after parathyroidectomy, the rate of changes of OV/BV (ΔOV/BV) was compared with that of Ml.OV/Ml.BV (ΔMl.OV/Ml.BV) during the first 3–4 weeks after parathyroidectomy. ΔMl.OV/Ml.BV was greater than ΔOV/BV (3.22 ± 1.46 vs 2.16 ± 1.52, $P = 0.020$), indicating that osteoid formation at the minimodeling sites was greater than that at the entire bone surface. No significant difference

Table 4 | Dynamic parameters, including MAR, sLS/BS, dLS/BS, LS/BS, and BFR/BS values at the sites of remodeling and minimodeling at 3–4 weeks after parathyroidectomy (*n*=13)

	MAR (μm/day)	sLS/BS (%)	dLS/BS (%)	LS/BS (%)	BFR/BS (mm^3/mm^2/year)
Remodeling site	0.307 ± 0.177	18.0 ± 9.5	0.9 ± 1.7	18.9 ± 9.7	0.012 ± 0.009
Minimodeling site	0.467 ± 0.179	6.3 ± 4.4	1.1 ± 1.6	7.4 ± 5.0	0.008 ± 0.009
P-value	0.003	0.003	0.611	0.004	0.142

BFR/BS, bone surface-referent bone formation rate; dLS/BS, bone surface-referent double labeled surface; LS/BS, bone surface-referent labeled surface; MAR, mineral apposition rate; sLS/BS, bone surface-referent single labeled surface.
The differences in the dynamic parameters between the sites of remodeling and those of minimodeling were analyzed by Wilcoxon's matched pairs test.
Values expressed as mean ± s.d.

between ΔMl.OV/Ml.BV and ΔOV/BV (1.54 ± 1.44 vs 1.75 ± 1.37, $P = 0.612$) was found during 12 weeks after parathyroidectomy. In terms of the dynamic parameters, mineral apposition rate (MAR) was compared between the sites of minimodeling and those of remodeling after parathyroidectomy. Only slight double-labeling could be observed on cancellous surface at both the sites of remodeling and minimodeling. Among the 13 patients of Group I in whom tetracycline was administered at 3–4 weeks after parathyroidectomy, double-labeling was found in only seven patients, with only single-labeling in the remaining six patients. MAR and BS-referent bone formation rate (BFR/BS) were decreased and single-labelings were abundant after parathyroidectomy.[9] But because mineralization was not sustained even in patients with only single-labeling, we measured MAR and BFR/BS, as indicated in Materials and Methods. When MAR and BFR/BS were measured separately at the sites of remodeling and minimodeling, MAR was greater at the minimodeling sites than at the remodeling sites (0.467 ± 0.179 vs 0.307 ± 0.177 μm/day, $P = 0.003$) after parathyroidectomy, which is concomitant with the more frequent double-labeling at the minimodeling sites than that at the remodeling sites (Table 4; Figure 1). In contrast to MAR, BFR/BS was not greater at the minimodeling sites than at the remodeling sites (0.008 ± 0.009 vs 0.012 ± 0.009 mm^3/mm^2/year, $P = 0.142$), as BS-referent labeled surface (LS/BS) was greater in the latter than in the former (18.9 ± 9.7 vs 7.4 ± 5.0%, $P = 0.004$). Analyzed in further detail, BS-referent single-labeled surface was greater at the remodeling sites than at the minimodeling sites (18.0 ± 9.5 vs 6.3 ± 4.4%, $P = 0.003$), whereas no significant difference of BS-referent double-labeled surface was observed between the remodeling and minimodeling sites (0.9 ± 1.7 vs 1.1 ± 1.6%, $P = 0.611$). But the ratio of BS-referent double-labeled surface to LS/BS was greater at the minimodeling sites than at the remodeling sites in all the seven patients in whom double labeling was found. The observation of seven bone biopsy specimens taken at 10–12 weeks after parathyroidectomy revealed a significant increase of Ml.BV/BV from 2.14 ± 1.72 to 9.47 ± 4.18% ($P = 0.028$) at the minimodeling sites (Table 3).

DISCUSSION

By conducting serial bone biopsies before and after parathyroidectomy, we examined the contribution of bone formation by minimodeling to the increase of bone mineral density, in comparison with that of remodeling. We compared the changes of Ob.S/Ml.S with those of Ob.S/BS after parathyroidectomy to assess the importance of minimodeling-mediated bone formation in these patients. Before parathyroidectomy, Ob.S/Ml.S was greater than Ob.S/BS. The number of type II and type III osteoblasts increased at 1 week after parathyroidectomy at the entire bone surface,[7] leading to a further increase of osteoid tissue. Ml.OV/Ml.BV was greater than OV/BV at 3–4 weeks after parathyroiderctomy, and ΔMl.OV/Ml.BV was also greater than ΔOV/BV during the first 3–4 weeks after surgery, indicating that osteoid formation was activated to a great degree at the minimodeling sites than at the remodeling sites after parathyroidectomy. Osteoid formation by type II and type III osteoblasts appeared to be more active at the minimodeling sites,[7,10] because these osteoblasts, which play important roles in the formation of osteoid tissue, are abundant at the minimodeling sites before parathyroidectomy. However, by 4 weeks after parathyroidectomy, Ob.S/Ml.S decreased to the level comparable with Ob.S/BS, as reflected by the abrupt decrease in the number of active type II and type III osteoblasts.[7] In turn, the number of flattened (type IV) osteoblasts, which play important roles in bone mineralization,[10] was increased at 3–4 weeks after surgery more at the minimodeling sites.

Although Ml.OV/Ml.BV was greater than OV/BV at 3–4 weeks after parathyroidectomy ($P = 0.001$), MAR was significantly greater at the minimodeling sites than at the remodeling sites. Therefore, the amount of osteoid seams mineralized by 12 weeks after surgery would be greater at the minimodeling sites than at the remodeling sites, explaining the significant increase of Ml.BV/BV during the 12 weeks after parathyroidectomy. The greater gain of mineralized BV at minimodeling sites is considered to be caused by the greater MAR attributable to the increase of type IV osteoblasts at both 3–4 weeks and 10–12 weeks after parathyroidectomy, with the result that there was no difference between Ml.OV/Ml.BV and OV/BV at 10–12 weeks after parathyroidectomy ($P = 0.237$). Owing to the absence of bone resorption, greater increase of osteoid volume, and greater MAR at the minimodeling sites (Tables 2–4), the increase of mineralized BV at the minimodeling sites, in part, contributed to the increase of bone mineral density after parathyroidectomy even though LS/BS was significantly small (Table 4). As the normal ranges of bone histomorphometric parameters might differ depending on the gender, ethnicity

and race,[6] one of the limitations of this study is that the normal ranges of histomorphometric parameters except for minimodeling parameters used in this study represent those for postmenopausal Caucasian female[11,12] and Caucasian male[13] subjects, but not for Japanese subjects.

An increase of osteoid formation early after parathyroidectomy was followed by the relatively rapid mineralization at the minimodeling sites as compared with mineralization at the remodeling sites, as MAR at the minimodeling sites was greater than that at the remodeling sites. In addition, Ob.S/Ml.S was again higher than Ob.S/BS at 10–12 weeks after surgery, probably because of the sustained decrease in the serum PTH.[3] As LS/BS at the minimodeling sites was extremely small, BFR/BS at the minimodeling sites was not greater than that at the remodeling sites. Thus, an increased minimodeling volume in patients in the hypoparathyroid state following parathyroidectomy might, in part, contribute to the increase of lamellar BV as well as that of total BV after parathyroidectomy. It would have been of interest to measure bone mineral density before and at 10–12 weeks after parathyroidectomy to determine if there were any further gains in bone mass during this interval. In addition, because minimodeling is associated with the formation of lamellar bone alone, it may, in part, contribute to the reduction of fracture risk after parathyroidectomy in patients with secondary hyperparathyroidism.[14]

Exercise is important in the maintenance of minimodeling in cancellous bone, as evidenced by the reported relation between an adequate exercise and an increase of minimodeling volume.[3] The patients enrolled in this study began to walk and exercise from the day after parathyroidectomy. When unilateral compression loads on a hollow diaphysis increase sufficiently to strain the bone surface, formation drifts associated with modeling in cortical bone expand the outside diameter of bone.[4] If the compression loads were transmitted to cancellous bone, minimodeling in cancellous bone would also be enhanced. However, the type and intensity of exercise that might induce an increase of minimodeling volume have not been investigated yet.

Although hyperparathyroid bone disease begins with stage 3 chronic kidney disease,[15] some studies have shown that the reduction of bone mineral density in renal dysfunction is not as severe as that estimated from the degree of increase of serum PTH.[16] The present results, together with the previous reports, indicated that minimodeling BV is greater in uremic patients than in normal individuals,[2–4] suggesting that bone formation by minimodeling might attenuate the rate of bone loss caused by renal dysfunction.

In conclusion, bone formation, including osteoid formation and bone mineralization, was more active at the minimodeling sites than at the remodeling sites after parathyroidectomy in patients with secondary hyperparathyroidism, suggesting that minimodeling-mediated bone formation might contribute to the recovery of cancellous BV after parathyroidectomy.

MATERIALS AND METHODS

Patients

Sixteen hemodialysis patients (9 males, 7 females) suffering from severe secondary hyperparathyroidism underwent parathyroidectomy and sequential iliac bone biopsies before and after parathyroidectomy. The mean age of the patients was 56.6 ± 9.2 (39–71) years, with a mean hemodialysis duration of 13.6 ± 7.9 (1–25) years. The causes of the renal failure in the patients were chronic glomerulonephritis ($n = 10$), polycystic kidney disease ($n = 1$), and unknown causes ($n = 5$), but none of the patients suffered from diabetes mellitus. Body mass index of the patients was 20.7 ± 2.6 (17.3–25.8) kg/m^2. Bone histomorphometric data were evaluated in 11 patients in our previous study (data not shown),[5] and an additional five patients were recruited for this study. The condition was refractory to conservative therapy with intravenous calcitriol (1α,25-dihydroxyvitamin D_3; Kirin Brewery Co. Ltd, Tokyo, Japan), maxacalcitriol (22 oxa-1α-dihydroxyvitamin D_3; Chugai Pharmaceutical Co. Ltd, Tokyo, Japan) or falecalcitol (26,26,26,27,27,27-hexafluoro-1,25-dihydroxyvitamin D_3; Taisho Pharmaceutical Co. Ltd, Tokyo, Japan) in all the patients, or the patients suffered from increased soft-tissue calcification resulting from persistent elevation of the Ca $\times$ P product. The use of these medications, therefore, was suspended at 2 months before parathyroidectomy to avoid the surgical complications arising from the prolonged hypercalcemia and/or hyperphosphatemia. In addition, as the effects of Ca and/or P levels on minimodeling have not yet been clarified, these parameters were relatively stable to the extent possible, and the mean value of Ca $\times$ P products was below 70 (mg/100 ml)^2 at the time of parathyroidectomy. Serum iPTH levels were 1262.3 ± 478.0 (770–2180) pg/ml and the Ca $\times$ P product was 54.6 ± 18.2 (21.9–84.2) (mg/100 ml)^2. In addition, the patients who had led a sedentary life were excluded because of the possible effect of physical activity on minimodeling.[3,4] The use of medications known to influence the bone metabolism was limited to Ca carbonate before parathyroidectomy. The Ca concentration in the dialysis water was 2.5 mEq/l. The patients were divided into two groups as follows: Group I ($n = 15$) and Group II ($n = 7$)—of the 16 patients, bone biopsy was conducted before and at 3–4 (mean; 3.8 ± 0.4) weeks after parathyroidectomy in 15 patients (Group I). Six out of 15 patients in Group I underwent a third biopsy at 12 weeks after surgery (Group II). An additional patient who underwent bone biopsy before and at 10 weeks after parathyroidectomy was added to these six patients. Thus, Group II had a total of seven patients who underwent bone biopsy before and at 10–12 (mean; 11.7 ± 0.8) weeks after surgery.

Informed consent was obtained from all the patients after an explanation was provided about both the risks and the outcomes of the bone biopsies and of parathyroidectomy. The Institutional Review Board of Towa Hospital and its affiliated hospital approved of the consent form.

Serum bone metabolic parameters

Serum iPTH (Allegro intact PTH, Nichols Institute Diagnostics, San Clemente, CA, USA) was measured by an immunoradiometric assay,[17] whereas the serum level of deoxypyridinoline (Metra DPD, Quidel Corporation, San Diego, CA, USA) was measured by an enzymeimmunoassay[18] and tartrate-resistant acid phosphatase was measured by colorimetry using nitrophenyl phosphatase as the substrate.[5] Serum total ALP was measured by colorimetry using nitrophenyl phosphate as the substrate,[19] and PICP was measured by radioimmunoassay (PICP ORION, ORION Diagnostica,

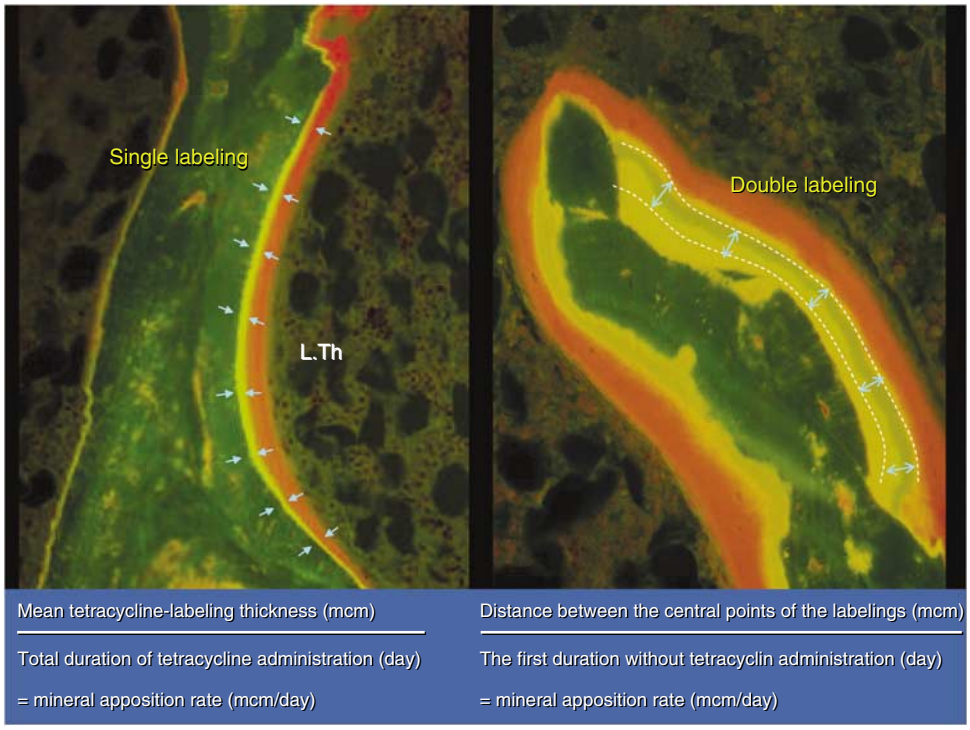

Figure 2 | Measurement of MAR in patients with double labeling and only single labeling. Method to calculate MAR in patients with only single labeling (left figure); the mean of labeled thickness (μm) was divided by the total duration of tetracycline administration (day). Tetracycline hydrochloride (Japan Lederle, Tokyo, Japan) was administered orally for labeling according to the following schedule: tetracycline hydrochloride for 3 days, no agent for 10 days, tetracycline for another 3 days, and no agent for 4 or 5 days. Method to calculate MAR in patients with double labeling (right figure). The distance between the central point of each labeling (μm) was divided by the first duration without tetracycline administration (day). The first duration without tetracycline was 10 days in this patient.

Espoo, Finland).[20] Serum Ca and P levels as well as the serum markers of bone metabolism were measured before and at the time of the second bone biopsy, 3–4 weeks after parathyroidectomy in Group I and 10–12 weeks after parathyroidectomy in Group II. The differences in the serum levels of bone metabolic markers measured before and after parathyroidectomy, that is, 3–4 weeks (Group I, $n = 15$) and 10–12 weeks (Group II, $n = 7$) after parathyroidectomy are shown in Table 1.

Parathyroidectomy

Four or five parathyroid glands were successfully removed under the general anesthesia in most patients and 150 mg of diffuse hyperplastic parathyroid tissue was cut into pieces measuring about 1 mm in diameter and autotransplanted into the adipose tissue of the abdominal wall in the 15 patients.[5,7,9] Serum iPTH levels in these patients fell to 25 pg/ml or less by 1 week after parathyroidectomy. In one patient of Group I, because only three parathyroid glands could be removed, 50 mg instead of 150 mg of parathyroid tissue was autotransplanted, in case one parathyroid gland might have been left unresected. Serum iPTH level in this patient was 212 pg/ml at 1 week and fell to 76 pg/ml at 4 weeks after parathyroidectomy. No surgical complications, including obvious infection such as pneumonia, bacterial infection at the site of neck surgery, blood loss exceeding 50 ml from the wound, or cardiovascular events, were encountered in any patients.

Bone histomorphometry

Transiliac bone biopsy to measure the bone histomorphometric parameters was performed from the left iliac crest before parathyroidectomy and from the right iliac crest at 3–4 weeks after parathyroidectomy (Group I). In Group II, bone biopsy specimens were obtained from the left iliac crest before parathyroidectomy at 10–12 weeks after surgery.

Each bone biopsy specimen was obtained using a trephine (8 mm in inner diameter), fixed in ethanol and stained by the Villanueva method. The histomorphometric dynamic parameters including MAR and BFR/BS were measured at 3–4 weeks after parathyroidectomy in 13 of the 15 patients of Group I because these 13 patients gave us the informed consent for tetracycline administration. Before the second biopsy, tetracycline hydrochloride for labeling was administered for labeling according to the 3, 10, 3, 4 or 5 day schedule.[9] Bone histomorphometric analysis was performed by direct tracing using a digitizer.[4] Histomorphometric parameters were calculated with a computer software (Histomorphometric System, System Supply Co. Ltd., Ina, Japan). A single experienced investigator (Akemi Ito) measured the parameters in each specimen. When histomorphometic analysis was performed by Akemi Ito on 2 specimens for 7 consecutive days to test the reproducibility, the variance of each histomorphometric parameter (BV/TV, OS/BS, BFR/BV) was 0.8–10.8%, which was considerably smaller than that reported previously.[21] The results of the bone histomorphometric analysis were expressed according to the methods of Parfitt *et al.*[6]

The sites of bone formation were classified into those of remodeling and minimodeling sites. Remodeling sites were identified based on the presence of a scalloped cement line indicating previous bone resorption. Minimodeling sites were identified based on the formation of mineralized bone in the absence of previous resorption, that is, the cement line was smooth

(not scalloped) and associated with a quiescent surface on the marrow side.[1–4] The bone formed by minimodeling had a normal lamellar architecture, resembling the adjacent underlying bone. The dividing line between the new bone formed by minimodeling and old bone is known as the 'lamellar separation'. Lamellar bone can be easily detected by microscopy using polarized light.[2] The following bone histomorphometric parameters were calculated: (1) BV/TV, (2) Oc.S/BS, (3) ES/BS, (4) Fb.V/TV, (5) Ob.S/BS, (6) OV/BV, (7) OS/BS, (8) O.Th, (9) Ob.S/Ml.S, (10) Ml.OV/Ml.BV, (11) Ml.OV/BV, (12) Ml.BV/BV, (13) TV-referent Ml.BV (Ml.BV/TV).[2–4,6] MAR, as a dynamic parameter, was also measured. When tetracycline double-labeling was seen, the distance between the central points of the two labelings was divided by the first duration of suspension of the tetracycline administration. When only single clear or vague labeling could be observed, labeled thickness (L.Th; μm) of the single labeling was divided by the sum of the scheduled duration (namely the first duration of tetracycline administration, the first duration of suspension of tetracycline administration, the second duration of tetracycline administration, and the second duration of suspension of tetracycline administration), to calculate MAR (Figure 2). Both MAR and BFR/BS were measured only after parathyroidectomy to determine the relative contributions of both remodeling and minimodeling to the increase of the mineralized BV after parathyroidectomy. The normal values of Tables 2 and 3 were obtained from the previous reports.[2,12]

Statistical analyses

Variables of bone remodeling and minimodeling measured before and after parathyroidectomy were compared (Tables 2 and 3). The values of Ml.OV/Ml.BV and OV/BV measured after parathyroidectomy were divided by those measured before parathyroidectomy to obtain ΔMl.OV/Ml.BV and ΔOV/BV; then, the statistical significance of the difference between ΔMl.OV/Ml.BV and ΔOV/BV was investigated. In addition, the statistical significance of the differences in the dynamic parameters between the remodeling sites and the minimodeling sites was also investigated (Table 4). Statistical analyses were performed by the Wilcoxon's matched pairs test. Differences were considered significant at $P<0.05$.

DISCLOSURE

All the authors declare no competing interest.

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