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ORIGINAL ARTICLE

Takafumi Majima · Yasato Komatsu · Kentaro Doi
Chieko Takagi · Michika Shigemoto · Atsushi Fukao
Takeshi Morimoto · Jerry Corners · Kazuwa Nakao

Clinical significance of risedronate for osteoporosis in the initial treatment of male patients with Graves' disease

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Abstract It has been well established that hyperthyroidism leads to diminished bone mineral density (BMD), and that a previous history of hyperthyroidism remains a risk factor for fractures. However, little is known about how to manage the reduction in BMD caused by hyperthyroidism. The purpose of this study was to evaluate the efficacy of risedronate for the treatment of osteoporosis/osteopenia in patients with Graves' disease (GD). Of 34 Japanese male patients with newly diagnosed GD, 27 with osteoporosis/osteopenia were included in this study. They were randomly divided into two groups by therapeutic regimen. Group A consisted of 14 patients treated with an antithyroid drug and risedronate. Group B consisted of 13 patients treated with the same antithyroid drug only. We used dual-energy X-ray absorptiometry to measure BMD at the lumber spine, femoral neck, and distal radius at baseline, and at 6 and 12 months after the trial. Bone-specific alkaline phosphatase and urinary N-terminal telopeptide of type I collagen normalized by creatinine were significantly more reduced in group A than in group B after both 6 and 12 months. The percentage increases in BMD at the lumbar spine and distal radius were significantly greater in group A than in group B. These beneficial effects of risedronate for patients with osteoporosis/osteopenia caused by GD may lead to a reduced risk of future fractures. We thus conclude that risedronate should be considered for the treatment of decreased bone mass associated with GD.

Key words bisphosphonate · Graves' disease · thyroid hormones · bone mineral density · N-terminal telopeptide of type I collagen

T. Majima · K. Doi · C. Takagi · M. Shigemoto
Department of Endocrinology and Metabolism, Rakuwakai Otowa Hospital, Kyoto, Japan

T. Majima · Y. Komatsu (✉) · K. Nakao
Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan
Tel. +81-75-751-3168; Fax +81-75-771-9452
e-mail: komatsuy@barium.rirc.kyoto-u.ac.jp

A. Fukao
Department of Psychosomatic Medicine, Rakuwakai Otowa Hospital, Kyoto, Japan

T. Morimoto
Department of General Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

J. Corners
Rakuwakai Otowa Hospital, Kyoto, Japan

Introduction

It has been well established that hyperthyroidism leads to diminished bone mineral density (BMD) [1–3] accompanied by an increase in bone turnover in favor of bone resorption [4,5]. After the successful treatment of hyperthyroidism, some improvement of the reduction in BMD has generally been observed [4,6–8]. However, it is not clear whether the reduced bone mass is completely normalized after the attainment of euthyroidism [2]. Some clinical studies have suggested that the reduction in bone mass caused by hyperthyroidism will be completely corrected after several years of continuous euthyroidism [6–8]. However, other studies have shown that the recovery from the reduction in bone mass remains incomplete despite effective treatment for hyperthyroidism [9–14]. Moreover, the risk of fracture for patients with hyperthyroidism has been reported to remain high, even many years after the attainment of euthyroidism [15–17]. This is probably due to the poor bone quality of patients with a past history of hyperthyroidism even if their BMD has apparently recovered as a result of treatment for hyperthyroidism. While the accelerated bone turnover accompanying hyperthyroidism is known to improve rapidly after the beginning of antithyroid treatment [4,5], some investigators have reported slightly but continuously elevated bone turnover 1 year after the attainment of euthyroidism [4,18]. These observations suggest the need for some form of therapeutic intervention, with the increased bone turnover in hyperthyroidism leading to the reduction in bone mass. However, little is known

about how to manage the abnormal bone metabolism in hyperthyroidism.

Likewise, little is known about bone mineral metabolism in male patients with active hyperthyroidism, as Greenspan and Greenspan [1] stated in their review of the published data. In addition, an interpretation of the effects of hyperthyroidism and its treatment on bone metabolism is hampered by the background of involutional osteoporosis in female patients [19]. Our assessment of the potential effects of hyperthyroidism and its treatment on bone metabolism was therefore limited to male patients in an effort to largely eliminate the influence of involutional osteoporosis.

Antiresorptive agents are widely used to treat osteoporosis, Risedronate (RIS), a potent pyridinyl bisphosphonate, has been shown to reduce the risk of both vertebral and nonvertebral fractures by reducing bone turnover, increasing bone mass, and preserving trabecular bone architecture [20–23]. However, there have been no clinical studies to evaluate the utility of RIS for the treatment of osteoporosis/osteopenia in patients with Graves' disease (GD).

The aim of our study was thus to examine and assess the effects of RIS on BMD and bone turnover in male patients with GD, both at diagnosis and prospectively after 6 and 12 months of antithyroid treatment.

Subjects and methods

Subjects

Thirty-four male Japanese patients (mean age 43.7 ± 11.2 years) with newly diagnosed GD, who attended the clinic of Rakuwakai Otowa Hospital between April 2003 and June 2004, were selected for this study. The control group comprised 34 healthy male Japanese volunteers without a past or present history of thyroid disease. All were employees of the Rakuwakai Otowa Hospital, and were similar to the patients in their exercise history and calcium intake. The diagnosis of GD was established on the basis of clinical signs and symptoms, as well as of laboratory finding, including positive thyroid stimulating antibody (TSAb) and/or thyroid-stimulating hormone receptor antibodies (TRAb) and an elevated technetium-99m thyroid scan (>5%). GD in all patients was treated with methimazole (MMI) alone. The initial dose of MMI was 30 mg daily given in three 10-mg doses. During the course of this study, the dose of MMI was adjusted according to the biochemical thyroid status of each patient. Of the 34 patients with GD, seven showed no signs of osteoporosis/osteopenia at the lumbar spine (LS), femoral neck (FN), or distal radius (DR). The other 27 patients had reduced BMD (*T* score below –1 SD) in at least one site (14 in the LS, 8 in the FN, and 22 in the DR). This study involved a 1-year (at baseline, and at 6 months and 12 months after the diagnosis) longitudinal examination of these 27 patients, who were randomly divided into two groups according to their therapeutic regimen. Group A consisted of 14 patients treated with MMI and bisphosphonate (RIS, 2.5 mg/day orally). Group B, the control group, consisted of 13 patients treated with MMI only. Their clinical data at baseline are shown in Table 1.

All subjects completed a questionnaire administered by a doctor or nurse prior to entry into the study, and the results showed that the two subgroups of patients were similar with regard to physical activity and calcium intake. All subjects underwent laboratory blood and urinary tests. We excluded subjects who had a history of fractures and/or of other diseases (liver disease, renal dysfunction, malignancy, diabetes mellitus, hyperparathyroidism, hypercorticoidism, or hypogonadism) and those taking medication (active vitamin D3, bisphosphonates, calcitonin, testosterones, steroids, thyroid hormones, diuretics, heparin, or anticonvulsants) that could influence bone metabolism. Plain X-rays (anteroposterior and lateral views) of the lumbar spine were administered to all patients and controls at baseline, and to all patients at 6 months and 12 months after the diagnosis, and patients with scoliosis, compression fractures, or ectopic calcifications that could interfere with the bone mineral results were excluded. None of the subjects were smokers or substance abusers.

This study was performed in accordance with the recommendations of the Declaration of Helsinki and approved by the Ethical Committee of Rakuwakai Otowa Hospital, and all participants provided informed written consent.

BMD measurements

BMD was measured at the LS (L2–L4), FN, and DR by means of dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500c; Hologic, Waltham, MA, USA) at baseline, and at 6 months and 12 months after the diagnosis in patients, and at baseline only in controls. To eliminate technical discrepancies, the same operator measured all the subjects. The reproducibility was calculated as the coefficients of variation obtained by daily measurements of a standard phantom over a period of 2 years. The CV of our instrument is 0.43% with the standard phantom. Values of BMD at the LS were expressed as the mean of those at the L2–L4. *T* scores and *Z* scores were calculated on the basis of the normal reference values of the age- and sex-matched Japanese group provided by the DXA system manufacturer.

Biochemical measurements

Serum samples were obtained before 0800 hours after overnight fasting, and were immediately processed and kept frozen at –20°C until the assays were carried out. Serum-free T3 (FT3), free T4 (FT4), and thyroid-stimulating hormone (TSH) were measured with the aid of an electrochemiluminescence immunoassay (ECLUSISU; Roche Diagnostics, Tokyo, Japan; normal values: FT3, 2.30–4.30 pg/ml; FT4, 0.90–1.70 ng/dl; TSH, 0.500–5.000 μIU/ml). The minimum detection limit of the TSH assay was 0.005 μIU/ml. TRAb was measured with a

Table 1. Baseline characteristics of examined subjects

	Patients with Graves' disease		Controls ($n = 34$)	Overall P-value
	Group A ($n = 14$)	Group B ($n = 13$)		
Age (years)	43.64 ± 11.0	45.46 ± 13.1	43.8 ± 10.5	0.8850
Height (cm)	169.79 ± 6.0	169.67 ± 5.4	170.1 ± 6.3	0.9740
Weight (kg)	65.79 ± 5.1	64.20 ± 8.5	66.1 ± 5.2	0.6089
BMI (kg/m^2)	22.86 ± 1.9	22.34 ± 1.8	22.9 ± 2.0	0.6550
FT3 (pg/ml)	11.99 ± 4.3**	12.51 ± 4.5**	3.30 ± 0.48	<0.0001
FT4 (ng/dl)	4.71 ± 1.7**	4.90 ± 1.5**	1.34 ± 0.22	<0.0001
TSH (μIU/ml)	<0.005	<0.005	2.17 ± 0.51	–
TRAb (%)	56.68 ± 20.9	47.39 ± 22.5	N.D.	–
TSAb (%)	611.93 ± 299.2	550.00 ± 169.1	N.D.	–
Calcium (mg/dl)	10.01 ± 0.4*	9.99 ± 0.4	9.77 ± 0.31	0.0488
ALP (IU/l)	364.07 ± 49.4**	353.46 ± 57.3**	199.1 ± 37.0	<0.0001
BAP (U/l)	52.82 ± 10.1**	55.73 ± 10.4**	23.8 ± 3.6	<0.0001
UrinaryNTx/Cre (nmolBCE/mmolCr)	157.69 ± 26.7**	151.59 ± 32.2**	34.6 ± 11.2	<0.0001
Lumbar spine				
BMD (g/cm^2)	0.939 ± 0.102*	0.949 ± 0.124*	1.016 ± 0.078	0.0157
T score (SD)	−0.811 ± 1.019*	−0.714 ± 1.236*	−0.035 ± 0.715	0.0157
Z score (SD)	−0.471 ± 0.608**	−0.334 ± 0.830*	0.097 ± 0.501	0.0075
Femoral neck				
BMD (g/cm^2)	0.830 ± 0.137	0.774 ± 0.104	0.841 ± 0.070	0.1028
T score (SD)	−0.263 ± 1.075	−0.703 ± 0.821	−0.170 ± 0.549	0.1028
Z score (SD)	0.055 ± 0.985	−0.291 ± 0.781	0.192 ± 0.492	0.1113
Distal radius				
BMD (g/cm^2)	0.633 ± 0.072**	0.643 ± 0.062**	0.751 ± 0.052	<0.0001
T score (SD)	−1.745 ± 1.136**	−1.582 ± 0.987**	0.129 ± 0.823	<0.0001
Z score (SD)	−1.583 ± 0.964**	−1.418 ± 0.907**	0.045 ± 0.724	<0.0001

Data represent mean ± SD
BMI, body mass index; FT3, free T_3; FT4, free T_4; TRAb, thyroid-stimulating hormone receptor antibodies; TSAb, Thyroid stimulating antibody; ALP, alkaline phosphatase; BAP, bone type alkaline phosphatase; U.NTx, N-terminal telopeptide of type I collagen normalized by creatinine; N.D., not done
* $P < 0.05$ vs. controls, ** $P < 0.01$ vs. controls

radioreceptor assay (Cosmic III, Cosmic, Tokyo, Japan; normal range: 0.0%–15.0%), and TSAb with a bioassay radioimmunoassay (TSAB-Kit-Yamasa; Yamasa, Chiba, Japan; normal range: <180.0%). Serum calcium, phosphate, creatinine, and alkaline phosphatase (ALP) were measured by standard laboratory methods. Bone-specific alkaline phosphatase (BAP) was measured with an enzyme immunoassay kit (Osteolinks-BAP; Sumitomo Pharmaceuticals, Tokyo, Japan; normal range: 13.0–33.9 U/l) as a marker of bone formation. Urinary N-terminal telopeptide of type I collagen normalized by creatinine (U.NTx) was measured in the morning in the second voided urine sample by means of an enzyme-linked immunosorbent assay (Osteomark; Mochida Pharmaceutical, Tokyo, Japan; normal range: 13.0–66.2 nmolBCE/mmolCre) as a marker of bone resorption.

Statistical analysis

The differences between two groups were analyzed with the unpaired t-test, and longitudinal differences in the same group with the paired t-test. Differences among three groups were analyzed with the one-way factorial ANOVA and Fisher's protected least significant difference (PLSD) method. Statistics were calculated using Statview version 5.0 (Abacus Concepts, Berkeley, CA, USA) A P value < 0.05 was considered to be statistically significant.

Results

Table 1 shows a comparison of the baseline values between GD patients and controls. There was no significant differences among the two subgroups of patients (group A and group B) and the control subjects in age, height, weight, and BMI. However, the thyroid hormones, serum calcium, ALP, BAP, and U.NTx levels of the GD patients were significantly higher than those of controls. BMD at the LS and the DR in the GD patients were significantly lower than those of controls. However, BMD at all three sites at the baseline were not different between group A and group B (Fig. 1A).

Table 2 shows the longitudinal characteristics of the patients in group A and group B at the time of diagnosis and at 6 and 12 months after the baseline. FT3, FT4, TRAb, and TSAb significantly decreased after antithyroid treatment in both groups. There was no significant difference in age,

height, weight, body mass index (BMI), thyroid hormones, or thyroid autoantibodies between the two groups at any of the three times. However, BAP and U.NTx were significantly lower in group A than in group B at both 6 months (P = 0.0171 and 0.0012, respectively) and 12 months (P = 0.0079 and 0.0080, respectively) after the baseline, while both BAP and U.NTx decreased significantly compared with the initial values in group A and group B (Table 2).

The percentage values of the decreases (mean ± SD) in U.NTx from the initial value in group A were 72.77% ± 9.5% and 75.65% ± 6.6% at 6 and 12 months, respectively, which were significantly higher than the corresponding values in group B (58.34% ± 17.5% and 66.56% ± 11.5%, respectively; P = 0.0125 and 0.0179, respectively) (Fig. 2). The percentage values of the decreases (mean ± SD) in BAP from the initial value in group A were 26.70% ± 8.9% and 52.22% ± 9.7% at 6 and 12 months, respectively, which were also significantly higher than the corresponding values in group B (11.06% ± 13.2% and 42.88% ± 11.5%, respectively; P = 0.0012 and 0.0311, respectively) (Fig. 2). However, neither BAP nor U.NTx in group A dropped below the normal range at either 6 or 12 months after the baseline.

Table 3 and Fig. 1B show the changes in BMD, T score, and Z score for both groups during the course of this study. While only BMD and the T score at the FN were significantly higher at 6 months than at the baseline in group B, BMD, T score, and Z score at the LS and FN were significantly higher at 6 months, and those at all three sites were significantly higher at 12 months compared with those at the baseline in group A. On the other hand, BMD, T score, and Z score for the two groups at any site were not significantly different at any of the three times.

However, the percentage change in BMD from the baseline was considerably different between group A and group B at both 6 and 12 months (Fig. 3). In group A, the percentage increases (mean ± SD) in BMD at the LS, FN, and DR were 3.53% ± 3.3%, 2.55% ± 1.7%, and 0.63% ± 1.4%, respectively, at 6 months, and 6.07% ± 4.3%, 4.41% ± 2.3%, and 2.41% ± 2.3%, respectively, at 12 months (Fig. 3). In group B, the percentage increases (mean ± SD) in BMD at the LS, FN, and DR were 1.18% ± 2.3%, 1.62% ± 2.5%, and −0.55% ± 3.0%, respectively, at 6 months, and 1.90% ± 3.2%, 2.61% ± 4.5%, and 0.08% ± 3.5%, respectively, at 12 months (Fig. 3). The percentage increases in BMD at the LS were significantly higher in group A than in group B at both 6 and 12 months (P = 0.0428 and 0.0094, respectively), at the FN it was higher, although not significantly so, in group A than in group B, and at the DR it was significantly higher in group A than in group B at 12 months (P = 0.0499), but not at 6 months.

During the course of this study, one patient with RIS and one patient without RIS experienced epigastric discomfort, and one patient with RIS and one patient without RIS experienced diarrhea. Two patients in group A and 2 patients in group B experienced urticaria or general itchiness. However, their symptoms were not so severe that this study had to be discontinued.

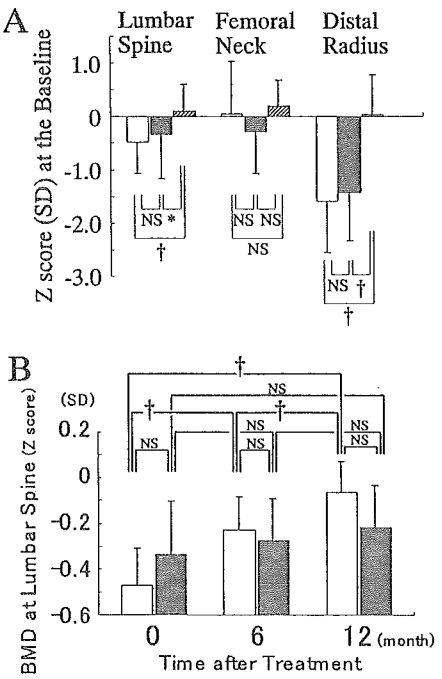

Fig. 1. **A** Comparison of bone mineral density (BMD) at the lumbar spine, femoral neck, and distal radius evaluated at baseline between patients with Graves' disease (group A, *white columns*; group B, *black columns*) and control subjects (*shaded columns*). Each column represents mean ± SD. *NS*, $P \geq 0.05$; *, $P < 0.05$; †, $P < 001$. **B** Z score at the lumbar spine evaluated at baseline and after 6 and 12 months for patients in group A (*white columns*) and group B (*black columns*). Data are shown as mean ± SEM. *NS*, $P \geq 0.05$; †, $P < 0.01$ vs. baseline. All differences between group A and group B were nonsignificant at all three times

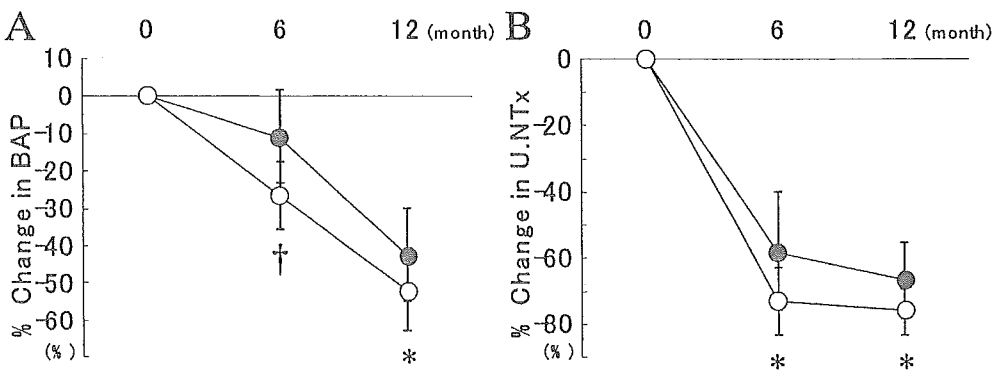

Fig. 2. The percentage change compared with the initial values of BAP (**A**) and U.NTx (**B**) evaluated after 6 and 12 months of treatment. For each figure, *white circles* represent the data of group A, and *black circles* the data of group B. Data are shown as mean ± SD. *, $P < 0.05$ vs. group B; †, $P < 0.01$ vs. group B

Table 2. Longitudinal variables assessed in patients with Graves' disease

	Baseline	6 months	*P*-value[a]	12 months	*P*-value[b]	*P*-value[c]
Group A ($n = 14$)						
Age (years)	43.64 ± 11.0*					
Height (cm)	169.79 ± 6.0*	169.76 ± 6.0*	0.3019	169.74 ± 6.0*	0.2123	0.1648
Weight (kg)	65.79 ± 5.1*	69.56 ± 5.4*	<0.0001	69.26 ± 5.3*	<0.0001	0.3280
BMI (kg/m^2)	22.86 ± 1.9*	24.17 ± 1.8*	<0.0001	24.01 ± 1.9*	<0.0001	0.1840
FT3 (pg/ml)	11.99 ± 4.3*	3.29 ± 0.4*	<0.0001	3.20 ± 0.6*	<0.0001	0.6825
FT4 (ng/dl)	4.71 ± 1.7*	1.21 ± 0.3*	<0.0001	1.11 ± 0.3*	<0.0001	0.3490
TSH (μIU/ml)	<0.005	2.71 ± 1.3*	–	3.27 ± 1.4*	–	0.1247
TRAb (%)	56.68 ± 20.9*	37.81 ± 13.3*	<0.0001	29.91 ± 10.8*	<0.0001	0.0030
TSAb (%)	611.93 ± 299.2	358.57 ± 173.1	0.0003	259.5 ± 92.2*	0.0003	0.0058
Calcium (mg/dl)	10.01 ± 0.4*	9.40 ± 0.3*	<0.0001	9.37 ± 0.2**	<0.0001	0.7271
ALP (IU/l)	364.07 ± 49.4*	274.07 ± 36.4*	<0.0001	226.50 ± 37.0*	<0.0001	0.0002
BAP (U/l)	52.82 ± 10.1*	38.77 ± 8.9**	<0.0001	24.59 ± 3.8†	<0.0001	<0.0001
UrinaryNTx/Cre (nmolBCE/mmolC)	157.69 ± 26.7*	41.66 ± 11.0†	<0.0001	37.53 ± 8.6†	<0.0001	0.0553
Group B ($n = 13$)						
Age (years)	45.46 ± 13.1					
Height (cm)	169.67 ± 5.4	169.60 ± 5.4	0.1205	169.61 ± 5.4	0.8193	0.0876
Weight (kg)	64.20 ± 8.5	67.90 ± 9.6	<0.0001	67.77 ± 8.8	<0.0001	0.6781
BMI (kg/m^2)	22.34 ± 1.8	32.62 ± 2.0	<0.0001	23.60 ± 1.8	<0.0001	0.9199
FT3 (pg/ml)	12.51 ± 4.5	3.14 ± 0.6	<0.0001	3.19 ± 0.5	<0.0001	0.8074
FT4 (ng/dl)	4.90 ± 1.5	1.17 ± 0.3	<0.0001	1.16 ± 0.3	<0.0001	0.8755
TSH (μIU/ml)	<0.005	2.508 ± 1.3	–	2.633 ± 1.4	–	0.7280
TRAb (%)	47.39 ± 22.5	29.9 ± 15.9	0.0006	26.18 ± 13.2	0.0001	0.1257
TSAb (%)	550.00 ± 169.1	359.00 ± 125.7	0.0011	263.23 ± 56.9	<0.0001	0.0010
Calcium (mg/dl)	9.99 ± 0.4	9.54 ± 0.3	0.0010	9.53 ± 0.2	0.0011	0.9254
ALP (IU/l)	353.46 ± 57.3	296.5 ± 42.7	<0.0001	235.9 ± 40.8	<0.0001	0.0001
BAP (U/l)	55.73 ± 10.4	49.64 ± 13.0	0.0179	31.77 ± 8.4	<0.0001	<0.0001
UrinaryNTx/Cre (nmolBCE/mmolC)	151.59 ± 32.2	59.06 ± 13.7	<0.0001	48.32 ± 10.8	<0.0001	0.0047

Data represent mean ± SD
BMI, body mass index; FT3, free T_3; FT4, free T_4, TRAb, thyroid-stimulating hormone receptor antibodies; TSAb, thyroid stimulating antibody; ALP, alkaline phosphatase; BAP, bone type alkaline phosphatase; U.NTx, N-terminal telopeptide of type I collagen normalized by creatinine
P-values[a] for comparisons of the parameters at the baseline and at 6 months, *P*-values[b] for comparisons of the parameters at the baseline and at 12 months, and *P*-values[c] for comparisons of the parameters at 6 and at 12 months
P-values for comparisons of the parameters for group A and group B: * $P > 0.05$; ** $P < 0.05$

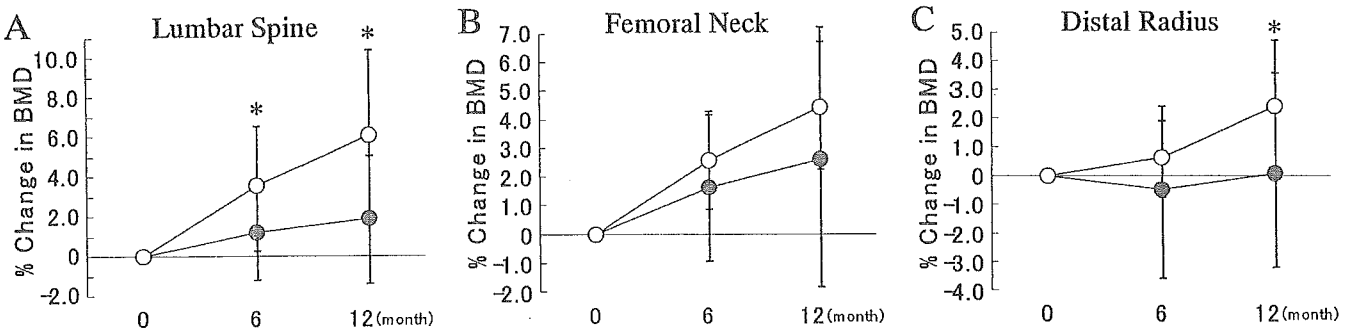

Fig. 3. The percentage change in BMD compared with the initial values at the lumbar spine (**A**), at the femoral neck (**B**), and at the distal radius (**C**) evaluate after 6 and 12 months of treatment. For each figure, *white circles* represent the data of group A, and *black circles* the data of group B. Data are shown as mean ± SD. *, $P < 0.05$ vs. group B

Discussion

It has been suggested that the reduction in BMD in hyperthyroid patients is potentially reversible in association with the cure of hyperthyroidism [2,4,6–8,10]. However, it remains unclear whether the reduction can be completely reversed [2]. Our study showed that 1 year of antithyroid treatment alone, even without any therapeutic intervention for the osteopenia/osteoporosis, increased BMD at the LS and FN by 2.3% and 3.1%, respectively. However, the patients' recovery was deemed unsatisfactory because their *Z*-score was still slightly below zero. On the other hand, BMD at the DR showed no increase during the 1 year of antithyroid treatment. Some longitudinal studies have examined the restoration of bone mass after treatment for hyperthy-

Table 3. BMD, T score, and Z score of patients with Graves' disease at the baseline, at 6 and 12 months

	Baseline	6 months	*P*-value[a]	12 months	*P*-value[b]	*P*-value[c]
Group A ($n = 14$)						
Lumbar spine						
BMD (g/cm^2)	0.939 ± 0.102*	0.970 ± 0.088*	0.0018	0.993 ± 0.085*	0.0002	<0.0001
T score (SD)	−0.811 ± 1.019*	−0.499 ± 0.875*	0.0018	−0.268 ± 0.850*	0.0002	<0.0001
Z score (SD)	−0.471 ± 0.608*	−0.228 ± 0.535*	0.0003	−0.067 ± 0.511*	<0.0001	<0.0001
Femoral neck						
BMD (g/cm^2)	0.830 ± 0.137*	0.850 ± 0.136*	<0.0001	0.866 ± 0.140*	<0.0001	<0.0001
T score (SD)	−0.263 ± 1.075*	−0.101 ± 1.074*	<0.0001	0.021 ± 1.098*	<0.0001	<0.0001
Z score (SD)	0.055 ± 0.985*	0.257 ± 1.023*	0.0002	0.383 ± 1.041*	<0.0001	<0.0001
Distal radius						
BMD (g/cm^2)	0.633 ± 0.072*	0.637 ± 0.073*	0.1134	0.648 ± 0.072*	0.0027	0.0002
T score (SD)	−1.745 ± 1.136*	−1.680 ± 1.155*	0.1133	−1.506 ± 1.140*	0.0027	0.0002
Z score (SD)	−1.583 ± 0.964*	−1.527 ± 0.963*	0.1390	−1.373 ± 0.94*	0.0042	0.0003
Group B ($n = 13$)						
Lumbar spine						
BMD (g/cm^2)	0.949 ± 0.124	0.958 ± 0.107	0.1483	0.965 ± 0.113	0.0624	0.1743
T score (SD)	−0.714 ± 1.236	−0.623 ± 1.074	0.1483	−0.552 ± 1.129	0.0624	0.1743
Z score (SD)	−0.334 ± 0.830	−0.274 ± 0.654	0.3786	−0.217 ± 0.660	0.1835	0.1788
Femoral neck						
BMD (g/cm^2)	0.774 ± 0.104	0.785 ± 0.099	0.0419	0.793 ± 0.100	0.0570	0.1094
T score (SD)	−0.703 ± 0.821	−0.611 ± 0.776	0.0419	−0.552 ± 0.791	0.0570	0.1094
Z score (SD)	−0.291 ± 0.781	−0.228 ± 0.711	0.1818	−0.170 ± 0.688	0.1086	0.1311
Distal radius						
BMD (g/cm^2)	0.643 ± 0.062	0.640 ± 0.067	0.5450	0.644 ± 0.068	0.9243	0.0725
T score (SD)	−1.582 ± 0.987	−1.636 ± 1.061	0.5450	−1.573 ± 1.079	0.9243	0.0725
Z score (SD)	−1.418 ± 0.907	−1.438 ± 0.890	0.8539	−1.379 ± 0.908	0.7635	0.0749

Values are mean ± SD
BMD, bone mineral density
P-values[a] for comparisons of the parameters at the baseline and at 6 months, *P*-values[b] for comparisons of the parameters at the baseline and at 12 months, and *P*-values[c] for comparisons of the parameters at 6 and at 12 months
P-values for comparisons of the parameters for group A and group B: * $P > 0.05$

roidism. Diamond et al. [10] showed that after 1 year of antithyroid therapy, BMD at the LS and FN increased by 6.6% and 1.2%, respectively, while Jodar et al. [11] found that the diminished BMD at the LS and FN increased significantly as early as after 9 months of antithyroid treatment, but remained 5% lower than that of matched controls even at the 18-month follow-up. On the other hand, Toh et al. [9] reported that BMD at the DR in their male patients had decreased 1 year after antithyroid treatment, but returned to pretreatment levels after 2 years, although it remained significantly (16%) lower than that of controls. Their results are in agreement with ours, suggesting that the decrease in BMD is partially, but not completely, reversed in the first year after antithyroid treatment. Jodar et al. [11] maintained that even this small bone mass deficit early after the antithyroid treatment may leave the patients at risk of fracture in later life.

Other studies have suggested that the reduced bone mass can be completely restored after several years of continued euthyroidism [6–8]. However, epidemiological studies have shown that patients with a past history of hyperthyroidism remain at a considerably higher risk of fractures [15–17]. These seemingly inconsistent observations suggest that even complete restoration of BMD with antithyroid treatment cannot always lead to sufficient recovery of bone quality to prevent fractures. Therefore, some therapeutic intervention which can restore not only the bone mass but also the bone quality is needed for osteoporotic/osteopenic patients with GD, and we consider bisphosphonate to be one of the prime candidates.

Bisphosphonates are the most effective antiresorptive agents currently available [21], and RIS has been shown to reduce the rate of bone resorption, increase BMD, and decrease fracture risk in patients with osteoporosis [20–22]. In our study, we found that RIS, in addition to MMI, increased BMD at the LS, FN, and DR by 6.6%, 4.2%, and 2.4%, respectively, during the 1-year treatment, which was significantly more than MMI alone. Two clinical studies have been reported which examined the efficacy of bisphosphonates for bone loss in GD. Lupoli et al. [24] evaluated the effects of alendronate on BMD in female patients with hyperthyroidism. They found that after 12 months of treatment, alendronate significantly increased BMD at the LS in both pre- and postmenopausal female patients compared with the results obtained without alendronate. Fittipaldi et al. [25] investigated the increase in BMD in elderly osteoporotic and hyperthyroid male patients treated with MMI alone vs. alendronate in addition to MMI. The mean changes in BMD at the LS and FN after 12 months of treatment were significantly higher in patients treated with alendronate in addition to MMI (6.2% and 2.1%, respectively) than in those with MMI alone (2% and 1.4%, respectively). Unfortunately, BMD was measured at only a single site in the former study, and the latter did not show the BMD values. Other clinical studies suggest that pamidronate is effective for abnormal bone

metabolism in patients with suppressive doses of thyroid hormone [26]. Studies in rats have also demonstrated that bisphosphonates improve the excessive bone loss caused by hyperthyroidism [27,28]. All these observations agree with ours, and are thus indicative of the potential benefits of bisphosphonates or RIS for osteoporosis/osteopenia in GD.

As far as we know, however, there are no reports in the literature regarding the effect of bisphosphonates on the reduced bone mass at the DR in patients with active hyperthyroidism. As noted above, our study found that RIS caused a significant increase in BMD at the DR as well as at the LS and FN in patients with active hyperthyroidism. Rosen et al. [26] investigated the efficacy of APD on bone metabolism in 55 patients (18 males and 37 females) with thyroid cancer who were being medicated with suppressive doses of levothyroxine. In their study, the administration of pamidronate produced no significant increase in BMD at the DR compared with a placebo, while pamidronate significantly increased BMD at the LS, trochanter, and total hip. However, BMD at the DR treated with pamidronate was significantly increased at 12 and 18 months compared with that at the baseline. In addition, actual increases in radial BMD after bisphosphonate therapy have been observed in postmenopausal osteoporosis [29,30]. These findings are also consistent with ours. Moreover, even if bisphosphonates do not influence BMD at the DR, they are known to reduce the risk of fracture at the DR by reducing cortical porosity [29,31,32], indicating the potential benefit of bisphosphonates for reduced bone mass at the DR in hyperthyroid patients as well.

Numerous reported studies have examined the effects of antithyroid therapy on bone metabolism by measuring bone formation and bone resorption markers in patients with hyperthyroidism [4,5]. Bone turnover rapidly decreases after normalization of the thyroid function, and is accompanied by a more rapid and prominent decrease in bone resorption marker [4,5], as was also seen in our study. Bone resorption markers thus seem to be better markers for monitoring bone loss in hyperthyroidism than bone formation markers. However, there are no previous studies on how the bone resorption marker (U.NTx) in GD patients after antithyroid treatment is longitudinally influenced by bisphosphonate, although Lupoli et al. [24] previously showed a significant reduction in a bone formation marker (osteocalcin) resulting from bisphosphonate in patients with Graves' thyrotoxicosis. Our study found that, as expected, RIS in addition to MMI significantly diminished the accelerated bone turnover in our GD patients compared with MMI alone, but not to a level below the normal range. In view of the fact that the bone metabolic markers are good predictors for the change in BMD in response to antiresorptive treatment [33], we speculate that this significant improvement in bone turnover brought about by RIS may account for the significant increase in BMD produced by RIS in our study. Furthermore, some clinical studies have suggested that increased bone turnover independently contributes to an increase in fracture risk [34]. RIS therefore seems all the more beneficial for these patients because a significant improvement in bone turnover by RIS in our GD patients is expected not only to increase their BMD, but also to reduce the risk of future fractures.

Using nasal calcitonin (CT), Jodar et al. [11] evaluated the effects of another antiresorptive therapy on BMD at the LS, the FN, and the whole body in hyperthyroid patients (8 men and 35 women). BMD increased in all the subgroups treated with different doses of CT (0, 800, and 1400 IU/month), but no significant difference was found in the improvement in either BMD or biochemical bone markers among the three subgroups, suggesting that the treatment with nasal CT has no additional effect beyond the attainment of the euthyroid state. Kung and Yeung [35] also found that calcium supplementation prevented bone loss induced by thyroxine suppressive therapy regardless of whether it was with or without the use of nasal CT. Although Akcay et al. [36] recently found that nasal CT siginificantly lowered urinary deoxypyridinoline in 10 patients with hyperthyroidism, they also showed that BMD was similarly increased after treating hyperthyroidism regardless of nasal CT. These results and ours suggest that RIS is superior to CT for improving the reduced BMD in hyperthyroidism.

Although our study demonstrated that RIS significantly increased BMD and improved bone turnover in male patients with thyrotoxic GD, it remains unclear whether RIS can reduce the risk of fractures for these patients. Some clinical studies have reported that changes in BMD may explain only a small portion of fracture risk reduction [37], so it remains a matter of much debate whether changes in BMD are directly linked to changes in fracture risk [38]. However, even if increased BMD does not lead to fracture risk reduction, RIS has been reported to actually decrease the risk of new vertebral fractures by up to 70% within 1 year of treatment for patients with osteoporosis [39], although the specific effect of RIS or bisphosphonates on fracture risk for patients with GD is not known. It also remains unclear whether the added bone mass benefit of RIS is worth the additional cost. As is well known, fractures associated with osteoporosis, especially compressed fracture of the spine or hip fracture, can have a devastating effect on disability and medical costs. However, although the present study suggested some beneficial effects of RIS on the increased risk of fractures in Graves' disease, it was not designed to calculate the cost-effectiveness of RIS. Further studies are therefore needed to clarify these issues.

Fortunately, RIS was well tolerated by our patients, except for some minor febrile reactions that have been reported in the literature, including epigastric discomfort and diarrhea [40]. On the other hand, MMI was also well tolerated whether with or without RIS, except for transient itchiness. Our finding that these adverse events were comparable for patients with or without RIS indicates the adequate safety and tolerability of RIS for patients with GD.

In conclusion, our study has provided new and supportive information to the existing literature. We found that both 6- and 12-month treatment with RIS significantly in-

creased BMD at the LS, FN, and DR, and improved the accelerated bone metabolic markers in male patients with thyrotoxic GD. These potentially beneficial effects of RIS upon the abnormal bone metabolism in GD suggest that osteoporosis/osteopenia in patients with GD should be treated with RS in order to improve their bone metabolism, and consequently to reduce the risk of future fractures.

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Interferon-α improves bone resorption and osteopenia in patients with chronic hepatitis C

Naoshi Nishida [a,b,*], Yasato Komatsu [a], Toshiki Komeda [a,b], Yoshihiro Fukuda [c]

[a] *Department of Medicine and Clinical Science, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan*
[b] *Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan*
[c] *Department of Laboratory Science, School of Health Science, Faculty of Medicine, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan*

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Abstract

Background: Interferon (IFN)-β is known to be involved in the regulation of bone homeostasis. As IFN-α and -β share the same receptor complex and signaling pathway, we speculated that treatment with IFN-α for chronic hepatitis C (CHC) may provide a beneficial effect on bone loss.
Methods: Urinary deoxypyridinoline (uDPD) of 41 patients with CHC who had been receiving IFN-α for 24 weeks was examined during the period of observation. Among them, eight patients showed a bone mineral density (BMD) of less than 0.850 g/cm^2 before IFN therapy and they were examined a BMD again after completion of IFN administration. Relationships between the percentage difference of uDPD after discontinuation of IFN and various factors related to CHC were also examined.
Results: A mean uDPD of 7.1 ± 3.4 nM/mM creatinine before IFN therapy decreased to 4.5 ± 2.4 in the 4th week and 4.2 ± 2.7 in the 24th week of IFN therapy, respectively ($p < 0.0001$). The reduction in uDPD was more prominent in cases with a lower viral load ($p = 0.0266$). The BMD of the eight patients, which was less than 0.850 g/cm^2 before IFN therapy, showed significant increase after the end of therapy ($p = 0.0172$).
Conclusion: IFN-α can improve bone resorption in CHC patients, especially in those with a lower viral load, and increased BMD. These effects are thought to be a result of direct action of IFN on bone homeostasis.
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Keywords: Interferon; Chronic hepatitis C; Bone absorption; Bone mineral density; Deoxypyridinoline; Osteoporosis

1. Introduction

Chronic infection with hepatitis C virus (HCV) is a worldwide problem which can lead to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Rapid advances in interferon (IFN)-based therapy have made the elimination of HCV possible and produced long-term benefits by reducing both liver-related death and overall death, especially in patients with sustained virological responses [1,2]. In addition to the antiviral response induced by IFN-α and -β, IFN has been found to induce several host responses through induction of IFN-stimulated genes and/or crosstalk with other signaling systems [3,4].

Recently, Takayanagi et al. reported that the receptor activator of NF-κB ligand (RANKL) induced the IFN-β gene in osteoclast precursor cells and that IFN-β inhibited differentiation of precursor cells by interfering with RANKL-induced expression of c-fos [5]. Furthermore, mice deficient in IFN-β

* Corresponding author at: Division of Gastroenterology, GI cancer Research Lab., Baylor University Medical Center, 2nd. Floor H.O.B. 3500 Gaston Avenue, Dallas, TX 75246, USA. Tel.: +1 214 820 2692; fax: +1 214 818 9292.
E-mail address: naoshi@kuhp.kyoto-u.ac.jp (N. Nishida).

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signaling exhibit osteopenia with enhanced osteoclast differentiation, and local administration of IFN-β results in the inhibition of osteoclast formation and bone resorption [5]. Because high serum RANKL has been reportedly observed in chronic hepatitis C (CHC) patients, and IFN-α and -β share the same receptor complex and signaling pathways [6,7], we speculated that administration of IFN-α for CHC might also contribute to a reduction in bone resorption and increase the bone mass through an inhibition of excessive osteoclastogenesis. If so, as chronic hepatitis could be a risk of bone loss, the IFN therapy for CHC might have an additional therapeutic benefit, namely, the reversal of bone loss, leading to improved physical activity [8].

In this study, we examined bone resorption markers and bone mineral density (BMD) serially during a therapeutic course for patients with CHC who were being treated with the IFN-α. We report here a novel benefit of IFN-α, the improvement of osteopenia in patients with CHC.

2. Patients and methods

2.1. Patients

Forty-one patients with CHC, including six cases with mild liver cirrhosis who received the IFN-α therapy for 24 weeks at Kyoto University Hospital were enrolled in the study after informed consent had been obtained. We excluded patients who had a history of bone fractures and other diseases that might cause secondary osteoporosis (renal dysfunction, malignancy, hyperthyroidism, hyperparathyroidism, hypercorticoidism, or hypogonadism) or who had a history of taking medications (active Vitamin D3, bisphosphonates, calcitonin injections, estrogens, steroids, thyroid hormone, diuretics, heparin and anticonvulsants) that could influence bone metabolism. Because cirrhosis is sometimes accompanied by diverse derangements of metabolism including that of bone mineral, cirrhosis with serum albumin of less than 3.6 g/dl and/or platelet counts of less than $8 \times 10^4/\mu l$ before treatment was also excluded. All patients were subjected to plain X-ray (anteroposterior and lateral views) of the lumbar spine, and patients with scoliosis, compression fractures or ectopic calcifications that could interfere with bone mineralization studies were excluded. No patients were alcoholics. Their mean age was 56 years (range 36–74). Twenty-four patients were male and 17 were female. Twenty-seven patients received combination therapy for 6 months, consisting of 6 million units of IFN-α2b three times a week plus 600–800 mg of ribavirin per day. Nine patients received 6 million units of natural IFN-α three times a week and five patients received 18 million units of consensus IFN-α three times a week for more than 6 months. Serum viral load of HCV and its genotype of all patients were determined by means of competitive reverse-transcription and reverse transcription-polymerase chain reaction (RT-PCR) assay (Otsuka Pharmaceutical Co., Ltd., Osaka, Japan) before the start of IFN-α therapy. Twenty-nine patients showed evidence of HCV of genotype 1b, eight of 2a and four of 2b. Serum HCV levels were less than 99 KIU/ml in 4 patients, between 100 and 499 KIU/ml in 6 patients and more than 500 KIU/ml in 31 patients. All patients underwent liver biopsy before the IFN therapy and fibrosis grading according to the criteria of Desmet et al. showed 9 patients with F1 (mild fibrosis), 11 with F2 (moderate fibrosis), 15 with F3 (severe fibrosis) and 6 with F4 (cirrhosis) [9].

2.2. Response to the IFN therapy

In 17 patients, HCV RNA could not be detected with a qualitative detection method using RT-PCR (SRL Inc., Tokyo, Japan) for more than 6 months after the end of IFN therapy. These patients were classified as sustained virological responders (SVR). A decrease in serum alanine aminotransferase (ALT) levels to less than 50 IU/ml without the disappearance of HCV for more than 6 months after therapy was seen in 14 patients; these were classified as biochemical responders (BR). Presence of HCV RNA in serum as well as abnormal serum ALT levels at the end of IFN therapy was seen in 10 patients; these were considered non-responders (NR).

2.3. Biochemical markers of bone absorption and bone mineral density

As markers of bone resorption, urinary levels of deoxypyridinoline normalized by creatinine (uDPD) were measured in all patients using the second urine samples in the morning with an enzyme immunoassay (SRL Inc., Tokyo, Japan) before the start of the IFN, in the 4th week and in the 24th week of the IFN therapy, and at 24 weeks after the end of the IFN therapy.

The bone mineral density (BMD) of 17 patients was measured at the lumbar spine (L2–L4), the femoral neck, and the distal radius using dual energy X-ray absorptiometry (DXA) before IFN administration (Hologic QDR 2000, Hologic Inc. Waltham, MA). Statistically, there was no difference between patients who underwent BMD and who did not in terms of their clinical feature, such as age, gender, viral load, genotype of virus, fibrosis grade, response to IFN-α and type of IFN-α used. To eliminate technical variation, the same physician measured all the patients. Values of BMD at the lumbar spine are presented as the mean of those at the L2–L4 level. *T*-scores and *Z*-scores were calculated on the basis of the normal reference values for age- and sex-matched Japanese groups provided by the DXA system manufacturer. Eight of 17 patients showed a BMD of less than $0.850\ g/cm^2$. BMD of these eight patients was examined again within 12 weeks after the end of the IFN-α therapy.

2.4. Statistics

Wilcoxon signed rank test was used to compare the uDPD levels at each time point. Mann–Whitney *U*-test (comparison between two groups) or Kruskal–Wallis test (comparison among three groups) was used to compare the percent

decrease in uDPD and each factor related to CHC. Wilcoxon signed rank test was used to compare the BMD level before and after the IFN-α therapy. The relationship between percentage difference of uDPD and that of BMD was examined by Spearman rank correlation. All of these calculations were performed with StatView-J 4.5 software (Abacus Concepts, Berkeley, CA).

3. Results

3.1. Decrease in uDPD in CHC patients during administration of IFN-α (Figs. 1 and 2)

The mean uDPD in all 41 patients was 7.1 ± 3.4 (mean ± S.D.) nM/mM creatinine before IFN therapy and significantly decreased to 4.5 ± 2.4 in the 4th week and 4.2 ± 2.7 in the 24th week of IFN therapy (Wilcoxon signed rank test; $p < 0.0001$). However, the mean uDPD at 24 weeks after the end of IFN administration was 6.9 ± 3.1 nM/mM creatinine, which was almost identical to that before IFN (Wilcoxon signed rank test; $p = 0.2331$).

A decrease in uDPD during administration of IFN-α was observed for every type of IFN-α regimen examined, such as combination of IFN-α2b plus ribavirin, natural IFN-α and consensus IFN-α (Fig. 1). In addition, this decrease was observed regardless of the final response to IFN-α, as indicated by the elimination of serum HCV and/or a decrease in serum ALT (Fig. 2).

3.2. Decrease of uDPD during administration of IFN-α and clinical background of CHC (Table 1)

The relationships between the percentage difference of uDPD and factors related to CHC (viral load before and during the 2nd week of IFN administration, HCV genotype, fibrosing stage before the treatment, final response of CHC to IFN-α and type of IFN-α used) were examined. The percentage difference in uDPD after the start of IFN was calculated as follows:

$$\left(\frac{\text{uDPD after} - \text{uDPD before}}{\text{uDPD before}}\right),$$

in which "uDPD after" denotes uDPD after the start of IFN therapy and "uDPD before" refers to uDPD before the start of the therapy. In the 4th week of IFN therapy, the group of a viral load of less than 499 KIU/ml before the IFN therapy showed more percentage reduction of uDPD than those with a viral load of more than 500 KIU/ml ($p = 0.0266$ by Mann–Whitney U-test). Similarly, the group of a viral load of less than 0.5 KIU/ml during the 2nd week of therapy showed more reduction of uDPD than those with a viral load of more than 0.5 KIU/ml in the 4th week of therapy ($p = 0.0072$). Other factors, such as HCV genotype, fibrosing stage, final response to IFN and type of the IFN-α used, had no effect on the percentage difference in uDPD during IFN therapy.

3.3. Improvement in BMD after IFN-α therapy (Table 2)

Of the 17 patients whose DXA was examined before IFN therapy, 8 showed a BMD of less than 0.850 g/cm^2. The DXA of these eight patients was examined again within 12 weeks after the end of the IFN, and BMD and T-scores showed significant increase after the end of the IFN therapy compared with basal level ($p = 0.0172$ by Wilcoxon signed rank test). The mean percentage difference between the before and after therapy was 2.57 ± 1.41% (mean ± S.D.). No patients showed a decrease in T-score. Increases in BMD were observed regardless of the type of IFN-α used, the final response of the CHC to IFN, fibrosing stage, genotype or viral load before IFN therapy (Table 2). The percent dif-

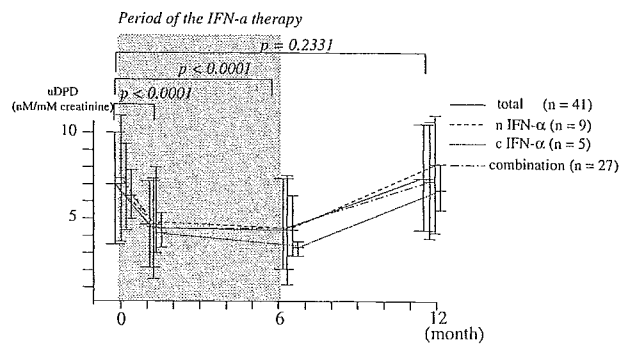

Fig. 1. Changes of mean uDPD before, during and after IFN-α therapy for CHC. The uDPD levels in the 4th week of IFN-α therapy were significantly lower than those before IFN administration with total case ($n = 41$; $p < 0.0001$; Wilcoxon signed rank test). The uDPD levels in the 24th week of therapy were also significantly lower than those before the start of IFN therapy ($p < 0.0001$; Wilcoxon signed rank test), but returned to basal levels at 24 weeks after the end of treatment ($p = 0.2331$). A decrease in uDPD was observed in cases treated with natural IFN-α (n IFN-α; $n = 9$), consensus IFN-α(c IFN-α; $n = 5$) and combination therapy of IFN-α2b plus ribavirin (combination; $n = 27$).

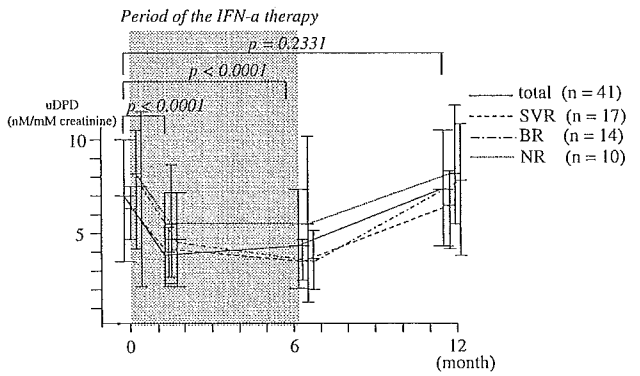

Fig. 2. Changes of mean uDPD before, during and after IFN-α therapy and the final response of CHC to IFN-α therapy. The uDPD levels were significantly lower in the 4th and the 24th week of therapy compared with those before IFN administration ($p < 0.0001$; Wilcoxon signed rank test), but returned to basal levels after the end of treatment ($n = 41$; $p = 0.2331$). A decrease of uDPD was observed in cases showing sustained virological responders (SVR, $n = 17$), biochemical responders (BR, $n = 14$) and non-responders (NR, $n = 10$).

Table 1
Relationships between the percentage difference of uDPD after the start of IFN-α administration and various factors related to CHC

Factors related to CHC (no. of cases)[a]	p Value[b] (4 weeks[c])	p Value[b] (24 weeks[d])
Serum viral load before treatment[e] <499 KIU/ml (10) vs. ≥500 KIU/ml (31)	**0.0266**	0.1143
Serum viral load during the 2nd week of IFN therapy[f] <0.5 KIU/ml (20) vs. ≥0.5 KIU/ml (20)	**0.0072**	0.3958
Genotype 1b (29) vs. 2a (8)	0.8535	0.4383
Fibrosis grade F1 or F2 (20) vs. F3 or F4 (21)	0.6670	0.3150
Final response of CHC to IFN SVR (17) vs. BR (14) vs. NR (10)	0.8537	0.2840
Type of the IFN-α used Combination (27) vs. natural (9) vs. consensus (5)	0.6065	0.4037

[a] Number of cases analyzed in each group.
[b] p Value determined by Mann–Whitney U-test (comparison between two groups) or Kruskal–Wallis test (comparison among three groups). Bold types indicate p value of less than 0.05.
[c] Relationship between percentage difference of uDPD in the 4th week of IFN therapy and various factors related to CHC.
[d] Relationship between the percentage difference of uDPD in the 24th of IFN therapy and various factors related to CHC.
[e] The mean uDPD before IFN, in the 4th and the 24th week of IFN therapy in each subgroup are as follows: the group of HCV titer of less than 499 KIU/ml before therapy; 7.5 ± 3.8 nM/mM (mean ± S.D.) before IFN, 4.4 ± 2.6 in the 4th week and 4.2 ± 3.2 in the 24th week, respectively. The group of more than 500 KIU/ml before therapy; 5.8 ± 1.8 before IFN, 5.0 ± 2.0 in the 4th week and 3.9 ± 0.9 in the 24th week, respectively. The mean percentage difference of uDPD in the 4th week is −39% in the group of less than 499 KIU/ml and −16% in the group of more than 500 KIU/ml. On the other hand, −42% (in the group of less than 499 KIU/ml) vs. −31% (in the group of more than 500 KIU/ml) in the 24th week of IFN therapy.
[f] The mean uDPD with the group of HCV titer of less than 0.5 KIU/ml during the 2nd week; 6.3 ± 1.6 before IFN, 4.8 ± 2.2 in the 4th week and 3.8 ± 1.5 in the 24th week, respectively. The mean uDPD with the group of more than 0.5 KIU/ml; 5.7 ± 3.7 before IFN, 5.0 ± 3.4 in the 4th week and 3.6 ± 1.3 in the 24th week, respectively. The mean percentage difference of uDPD in the 4th week is −42% in the group of less than 0.5 KIU/ml and −6% in the group of more than 0.5 KIU/ml. On the other hand, −40% (in the group of less than 0.5 KIU/ml) vs. −29% (in the group of more than 0.5 KIU/ml) in the 24th week of IFN therapy.

Table 2
Improvement in BMD of eight patients with BMD of less than 0.850 g/cm^2 before the IFN-α therapy

Patient	Age/sex	Genotype	Viral load (KIU/ml)	Fibrosis grade	Responses to IFN[a]	% Δ of uDPD		BMD			Type of IFN used
						At 4 W[b]	At 24 W[c]	Before IFN	After IFN	% Δ[d]	
1	68/F	2b	<50	F4	BR	−43	−31	0.784 (77%)[e]	0.824 (81%)	5.10	Natural
2	63/F	2a	570	F4	BR	−57	−25	0.675 (67%)	0.697 (69%)	3.26	Natural
3	68/M	1b	52	F3	SVR	−46	−39	0.778 (74%)	0.801 (76%)	2.96	Natural
4	70/F	2a	700	F2	NR	−40	−27	0.805 (79%)	0.828 (81%)	2.86	Combination
5	56/F	2a	929	F3	BR	−32	−32	0.713 (71%)	0.732 (72%)	2.66	Natural
6	67/F	2b	>850	F3	SVR	−56	−44	0.620 (61%)	0.634 (63%)	2.26	Combination
7	69/F	1b	>850	F2	NR	−49	−41	0.723 (71%)	0.735 (72%)	1.66	Combination
8	66/F	1b	702	F3	NR	24	−3	0.831 (79%)	0.829 (79%)	−0.24	Combination

[a] Final response of CHC to IFN therapy.
[b] Percentage difference of uDPD between before and in the 4th week of IFN therapy. A minus score indicates a decrease of uDPD in the 4th week compared with base line uDPD.
[c] Percentage difference of uDPD between before and in the 24th week of IFN therapy. A minus score indicates a decrease of uDPD in the 24th week.
[d] Percentage difference of BMD between two DXA measurements.
[e] BMD expressed as T-scores.

ference of BMD between two DXA measurements did not correlate with that of uDPD after the start of IFN ($\rho = 0.310$, $n = 8$, $p = 0.4128$ by Spearman rank test).

4. Discussion

Osteoporosis is a serious problem especially in the elderly because it may cause bone fracture and bone pain resulting in a deterioration of daily activity. Chronic liver disease is one of the risk factors for osteopenia, which is called hepatic osteodystropy [8]. Recent studies have shown that IFN-β inhibits the differentiation of osteoclasts by interfering with RANKL-induced expression of c-fos, which is an essential transcription factor for the formation of the osteoclast, and local administration of IFN-β result in the inhibition of bone resorption [5]. It is known that IFN-α and -β share a common receptor for their signal transduction and high serum

RANKL levels have been detected in CHC patients compared with controls [6,7]. These findings encouraged us to investigate bone resorption during IFN therapy for CHC.

Our serial measurements of uDPD before, during, and after IFN-α therapy indicated that bone resorption was suppressed during IFN therapy regardless of the HCV genotype, fibrosing stage, final response of CHC to IFN, or the type of IFN-α used, although the effect of IFN-α was limited to the time of administration of IFN-α. In addition, among eight patients whose BMD were less than 0.850 g/cm^2 before treatment, seven showed increase of BMD after IFN therapy (Table 2). A decrease in uDPD was observed within the first month and this effect was observed even in NR patients, suggesting that this effect can be attributed to a direct action of IFN-α on bone homeostasis, and was not a consequence of improvements in liver fibrosis and function. Previously, Miki et al. also reported that 10 patients treated with 6 million units of natural IFN-α for 12 weeks showed a reduction of uDPD during the treatment [10]. In the present study, the decrease of uDPD during IFN therapy returned to basal levels after the end of treatment. These evidences also support the fact that IFN-α directly affected the reduction of bone resorption.

A previous study reported that combination therapy consisting of IFN-α2b plus ribavirin resulted in a bone loss after therapy, although they found no evidence for an increase in bone turnover in these cases [11]. On the other hand, our study with serial measurements of uDPD and BMD before, during, and after the treatment did not show any increase in uDPD or decrease in BMD even in the cases of combination therapy. The reason for the discrepancy between the previous study and ours is not clear, but the effect of ribavirin on bone loss reported in the previous study might be suspected because the same cases were not followed serially. In addition, a recent study of recurrent hepatitis C after liver transplantation also showed that bone loss was not detected in the patients treated with combination therapy [12].

In this study, the lower viral loads of less than 499 KIU/ml before the IFN therapy and that of less than 0.5 KIU/ml in the 2nd week of the IFN therapy were significantly associated with the reduction of uDPD in the 4th week of IFN therapy. This association between low viral load and decrease in uDPD became less evident in the 24th week of IFN therapy. It is well known that low viral load is a good predictive factor for effective antiviral response of IFN-α therapy. In this regard, it is conceivable that final response to IFN-α could be associated with alteration of bone resorption and BMD, which we failed to detect in this study. In our cohort of CHC patients, 27 received combination therapy because of high viral load. It is reported that, by using combination therapy, SVR can be achieved even in patients with high viral load and elimination of HCV could be obtained even after early stage of therapy [13]. In fact, in this cohort of 41 patients, 37 out of 41 patients carried viral load of more than 100 KIU/ml and only 4 showed less than 99 KIU/ml, which is a predictive factor of effective INF-α response. Among 41 patients, seventeen showed SVR and 14 showed BR. In addition, 7 out of 10 NR cases showed transient decrease of HCV viral load of less than 0.5 KIU/ml at the 24th week of therapy, although HCV-RNA was still positive. Therefore, considerable effectiveness of IFN-α observed in patients with high viral load in this cohort could obscure a clear relationship between a final response to IFN-α and a decrease of uDPD because both a reduction of HCV viral titer at the end of therapy and a decrease of uDPD were observed in almost every cases. In other words, transient reduction of HCV might be enough for interfering uDPD values but not sufficient for the final response of IFN.

It is reported that HCV was present and could replicate in cells of the monocyte/macrophage lineage [14–18]. As osteoclast precursor cells are known to be monocyte/macrophage origin, it is interesting to speculate that early response of antiviral effect of IFN-α might induce a reduction of uDPD at early phase of IFN therapy. In addition, expression of HCV proteins such as core protein reportedly inhibits IFN-α induced intracellular signaling [19–21]. Therefore, HCV might interfere with the action of IFN in osteoclast precursor cell and thus partially affected the IFN-induced inhibition of osteoclast differentiation, although our study did not provide direct evidence for an inhibitory effect of HCV on the improvement of bone resorption.

Recent in vitro study showed that β subtype of IFN was much more potent than α2 subtype for inhibition of osteoclastogenesis [22]. In this regard, the possibility that indirect effects of IFN-α might induce a decrease of uDPD still linger on because we did not demonstrate the actual intracellular signaling events by IFN-α on osteoclast precursor cells. However, as serum concentration of IFN-α after an intramuscular injection of 3–10 million units usually reaches 50–150 IU/ml, which could, at least partially, inhibit the differentiation of precursor cell to osteoclast in vitro [22]. Therefore it might be possible that high dose of IFN-α induced inhibition of osteoclastogengsis in vivo, although the inhibitory effect of α subtype would be weaker compared with that of β subtype. In this respect, it is interesting to compare the effectiveness of IFN-α and -β to osteoporosis in CHC patients.

Increasing numbers of patients receive the long-term IFN-α therapy, not only for the elimination of HCV, but also for an improvement in inflammation and fibrosis and a reduction in the risk of hepatocellular carcinoma [23]. In addition to preventing the progression of liver damage, we have proposed here that IFN therapy for CHC have an additional benefit, which is an improvement of bone homeostasis and a prevention of bone loss.

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C-Type Natriuretic Peptide, a Novel Antifibrotic and Antihypertrophic Agent, Prevents Cardiac Remodeling After Myocardial Infarction

Takeshi Soeki, MD,* Ichiro Kishimoto, MD,* Hiroyuki Okumura, MD,* Takeshi Tokudome, MD,* Takeshi Horio, MD,† Kenji Mori, PHD,* Kenji Kangawa, PHD*

Suita, Osaka, Japan

OBJECTIVES We assessed the hypothesis that in vivo administration of C-type natriuretic peptide (CNP) might attenuate cardiac remodeling after myocardial infarction (MI) through its antifibrotic and antihypertrophic action.

BACKGROUND Recently, we have shown that CNP has more potent antifibrotic and antihypertrophic effects than atrial natriuretic peptide (ANP) in cultured cardiac fibroblasts and cardiomyocytes.

METHODS Experimental MI was induced by coronary ligation in male Sprague-Dawley rats; CNP at 0.1 μg/kg/min (n = 34) or vehicle (n = 35) was intravenously infused by osmotic mini-pump starting four days after MI. Sham-operated rats (n = 34) served as controls. After two weeks of infusion, the effects of CNP on cardiac remodeling were evaluated by echocardiograpic, hemodynamic, histopathologic, and gene analysis.

RESULTS C-type natriuretic peptide markedly attenuated the left ventricular (LV) enlargement caused by MI (LV end-diastolic dimension, sham: 6.7 ± 0.1 mm; MI+vehicle; 8.3 ± 0.1 mm; MI+CNP: 7.7 ± 0.1 mm, $p < 0.01$) without affecting arterial pressure. Moreover, there was a substantial decrease in LV end-diastolic pressure, and increases in dP/dt_{max}, dP/dt_{min}, and cardiac output in CNP-treated MI rats compared with vehicle-treated MI rats. Importantly, CNP infusion markedly attenuated an increase in morphometrical collagen volume fraction in the noninfarct region (sham: 3.1 ± 0.2%; MI+vehicle: 5.7 ± 0.5%; MI+CNP: 3.9 ± 0.3%, $p < 0.01$). In addition, CNP significantly reduced an increase in cross-sectional area of the cardiomyocytes. These effects of CNP were accompanied by suppression of MI-induced increases in collagen I, collagen III, ANP, and β-myosin heavy chain messenger ribonucleic acid levels in the noninfarct region.

CONCLUSIONS These data suggest that CNP may be useful as a novel antiremodeling agent. (J Am Coll Cardiol 2005;45:608–16) © 2005 by the American College of Cardiology Foundation

The mammalian natriuretic peptide system consists of three structurally homologous peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (1). The actions of the natriuretic peptides are modulated through membrane-bound receptors, two of which are guanylyl cyclase (GC)-coupled receptors (GC-A and GC-B). These receptors are linked to the cyclic guanosine monophosphate (cGMP)-dependent signaling cascade and mediate the biological actions of the peptides (2). Atrial natriuretic peptide and BNP are mainly released from the heart to act as circulating hormones, which bind to their specific receptor, GC-A, in the vascular tissue, kidney, and adrenal gland and induce vasodilation, natriuresis, and diuresis (3). C-type natriuretic peptide, which was originally isolated from porcine brain extracts (4), not only acts in the central nervous system, but also plays a role in the local regulation such as the suppression of neointimal formation after vascular injury (5) through its specific receptor, GC-B. Recently, we have shown that CNP was synthesized in cultured cardiac fibroblasts and that CNP inhibited both deoxyribonucleic acid (DNA) and collagen synthesis of cardiac fibroblasts more potently than ANP and BNP (6). C-type natriuretic peptide also has more potent antihypertrophic effects than ANP in cultured cardiomyocytes (7). These findings might be due to the relative abundance of GC-B over GC-A in cardiac fibroblasts and in cardiomyocytes (6,7). In addition, in a recent clinical study, CNP was produced by the hearts of patients with chronic heart failure, and its level in the coronary sinus correlated with mean pulmonary wedge pressure (8). These basic and clinical results suggest that CNP might represent an important local mediator in the heart.

Left ventricular (LV) remodeling after myocardial infarction (MI) is a major cause of subsequent heart failure and death (9). Postinfarction remodeling has been divided into an early phase (within 72 h), which involves expansion of the infarct zone, and a late phase (after 72 h), which is associated with time-dependent LV dilation, mural hypertrophy, and cardiac fibrosis (10). Given the inhibitory effects of CNP on cardiac fibrosis and hypertrophy in vitro, CNP might act against the progression of cardiac late remodeling after MI. Furthermore, because intravenously administered CNP has been demonstrated to have much less potent

From the *Department of Biochemistry, National Cardiovascular Center Research Institute, Suita, Osaka, Japan; and the †Department of Medicine, National Cardiovascular Center, Suita, Osaka, Japan. This work was supported, in part, by research grants from the Japanese Ministry of Education, Science, and Culture; the Japanese Ministry of Health, Labor and Welfare; the Program for Promotion of Fundamental Studies in Health Sciences of Pharmaceuticals and Medical Devices Agency; the Japan Cardiovascular Research Foundation; and the Kowa Life Science Foundation.

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Abbreviations and Acronyms

ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
CNP = C-type natriuretic peptide
cGMP = cyclic guanosine monophosphate
GC = guanylyl cyclase
LV = left ventricle/ventricular
MHC = myosin heavy chain
MI = myocardial infarction
PKG = cyclic guanosine monophosphate-dependent protein kinase
RV = right ventricle/ventricular
TGF = transforming growth factor

vasorelaxant and natriuretic activities than ANP (4,11), CNP is not expected to perturb systemic hemodynamics after massive MI while ANP or BNP is. However, there has been no in vivo evidence to directly prove these beneficial effects of CNP after MI. Therefore, in the present study, we have assessed the hypothesis that in vivo administration of CNP might attenuate cardiac late remodeling after MI. In addition, to elucidate the mechanism involved in the antifibrotic action of CNP, we investigated the action of cGMP/cGMP-dependent protein kinase (PKG) pathway on collagen synthesis by cardiac fibroblasts in vitro, and to clarify whether CNP is an important local mediator in the heart, we investigated the degree and source of endogenous CNP production in the infarcted heart.

METHODS

Model of MI. All experimental procedures were performed according to the guidelines for animal experimentation of National Cardiovascular Center. Male Sprague-Dawley rats (Nihon SLC, Hamamatsu, Japan) weighing 180 to 220 g were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally). After left thoracotomy, the left coronary artery was ligated 2 to 3 mm from its origin using a 6-0 Prolene suture. The chest was closed, and the rats were allowed to recover. Sham-operated rats underwent the identical surgical procedure as described above without the actual coronary artery ligation.

Administration of CNP. Four days after coronary ligation, the rats with MI were randomly divided into two groups: one to be infused with synthetic CNP (MI+CNP, n = 36) and the other with vehicle (MI+vehicle, n = 42). The CNP group was then fitted with subcutaneous osmotic minipumps (model 2ML2, Alza Corp., Palo Alto, California) filled with synthetic CNP dissolved in a 5% glucose solution and set to release 0.1 μg/kg/min of the peptide for two weeks. The dose of CNP was chosen because our preliminary study revealed that CNP at this dose has no effects on arterial blood pressure and heart rate in rats. Glucose solution was infused in a similar manner in the control group. The pumps were connected to the left jugular vein by a polyethylene catheter. The synthetic CNP was kindly provided by Daiichi Suntory Pharma (Tokyo, Japan).

Noninvasive blood pressure and pulse rate. Systolic blood pressure and pulse rate were measured before MI and one day, one week, and two weeks after MI by the tail-cuff method without use of anesthesia (Softron, Tokyo, Japan).

Echocardiographic and hemodynamic studies. Echocardiographic studies were performed using an echocardiographic system equipped with a 15-MHz phased-array transducer (SONOS 5500, Hewlett Packard, Andover, Massachusetts) under anesthesia with sodium pentobarbital (30 mg/kg, intraperitoneally) 4 and 18 days after the experimental MI or sham operation as described previously (12). Rats with >20% fractional shortening or an early filling wave (E) velocity to atrial filling wave (A) velocity ratio of <3 in the echocardiographic study performed four days after MI were excluded from the study.

Eighteen days after the coronary ligation or sham operation, hemodynamic studies were performed under anesthesia as previously described (12). After completion of hemodynamic measurements, the hearts were arrested by the injection of 30 mM potassium chloride through the carotid artery, excised, and weighed.

Histological examination. After fixation, three cross-sections through the ventricles were obtained and embedded (n = 17 to 19 in each group). Paraffin sections (2 μm) were stained with Masson's trichrome for measurement of infarct size, hematoxylin and eosin for measurement of myocyte size, and Sirius red F3BA for determination of collagen volume fraction. The infarct size was expressed as previously described (13). For the measurement of cardiomyocyte cross-sectional area and diameter in the noninfarcted LV, a total of 30 myocytes sectioned transversely for area and longitudinally for diameter at the level of the nucleus were randomly chosen from each section at ×400 magnification, and traced. To measure collagen volume fraction, 16 fields in the border and remote myocardium of the noninfarcted LV and right ventricle (RV) walls per section were scanned at a magnification of ×200. The interstitial collagen volume fraction was measured while omitting fibrosis of the perivascular, epi-, and endocardial areas from the study. The collagen volume fraction was obtained by calculating the mean ratio of connective tissue to the total tissue area of all the measurements of the section. The collagen-positive areas from all sections were determined by a single investigator who was unaware of the experimental groups.

Northern blot analysis. Total ribonucleic acid (10 μg/lane) was extracted from the RV, noninfarcted LV, and infarcted LV (n = 10 in each group). Hybridization was carried out with cDNA probes for rat α-1 (type I) collagen, rat α-1 (type III) collagen, rat fibronectin, rat transforming growth factor (TGF)-β-1, rat ANP, and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH). We also used synthetic oligonucleotide probes for the α- and β-myosin heavy chain (MHC) messenger ribonucleic

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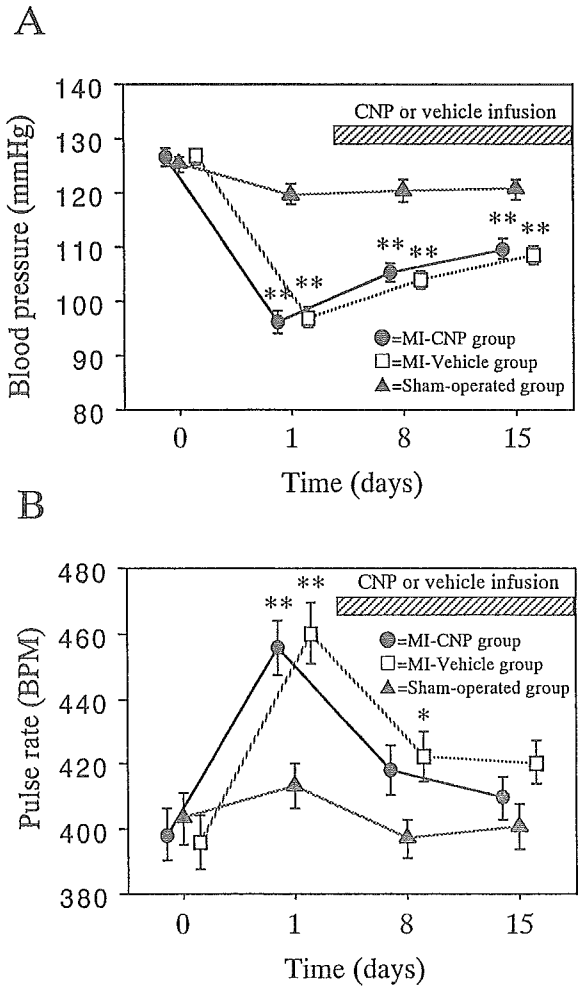

Figure 1. Time course of systolic blood pressure **(A)** and pulse rate **(B)** in sham-operated rats **(closed triangles)** and in rats with myocardial infarction (MI) before and during infusion of 0.1 μg/kg/min C-type natriuretic peptide (CNP) **(closed circles)** or vehicle (5% glucose solution) **(open squares)**. Values are mean ± SEM. A p value for systolic blood pressure by two-way analysis of variance: group <0.001; time course <0.001; group/time course interaction <0.001, and a p value for pulse rate by two-way analysis of variance: group <0.05; time course <0.001; group/time course interaction <0.01. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with the sham-operated group at same stage by Bonferroni multiple-comparison *t* test. BPM = beats/min.

acids (mRNA). The band intensity was estimated by a radioimage analyzer (BAS-5000, Fuji Film, Tokyo, Japan).

Collagen synthesis in vitro. Neonatal cardiac fibroblasts were prepared as described previously (14). The effects of CNP and a cGMP analog on collagen synthesis in cardiac fibroblasts were evaluated on subconfluent cultures by the incorporation of [^{3}H]proline into cells as previously described (6). In brief, after the preconditioning period, CNP with or without Rp-8-pCPT-cGMP (Calbiochem, San Diego, California), or 8-Bromo cGMP (Sigma, St. Louis, Missouri) was added, and 0.5 μCi of [^{3}H]proline was also added. After the cells were incubated for 24 h, the radioactivity of aliquots of the trichloroacetic acid-insoluble material was determined using a liquid scintillation counter.

Table 1. Echocardiographic Parameters

	Sham	MI+Vehicle	MI+CNP
4th day (before treatment)			
AWT diastole, mm	1.2 ± 0.01	1.0 ± 0.01*	1.0 ± 0.01*
PWT diastole, mm	1.3 ± 0.01	1.3 ± 0.01	1.3 ± 0.01
LVDd, mm	6.4 ± 0.1	7.0 ± 0.1*	7.0 ± 0.1*
FS, %	34 ± 1	16 ± 0.3*	15 ± 0.3*
E velocity, cm/s	89 ± 3	102 ± 2*	103 ± 3*
A velocity, cm/s	49 ± 2	18 ± 1*	19 ± 1*
E/A	1.9 ± 0.1	5.8 ± 0.2*	5.6 ± 0.1*
18th day (after treatment)			
AWT diastole, mm	1.2 ± 0.01	0.9 ± 0.02*	0.9 ± 0.01*
PWT diastole, mm	1.3 ± 0.01	1.5 ± 0.02*	1.4 ± 0.02*†
LVDd, mm	6.7 ± 0.1	8.3 ± 0.1*	7.7 ± 0.1*†
FS, %	35 ± 1	16 ± 0.4*	18 ± 0.4*†
E velocity, cm/s	88 ± 2	112 ± 3*	102 ± 3*‡
A velocity, cm/s	51 ± 2	19 ± 1*	26 ± 1*†
E/A	1.8 ± 0.05	6.2 ± 0.2*	4.2 ± 0.2*†

Values are mean ± SEM. *p < 0.01 compared with sham-operated group; †p < 0.01, ‡p < 0.05 compared with MI+vehicle group by analysis of variance and Bonferroni multiple-comparison t test.

A = atrial filling wave; AWT = anterior wall thickness; CNP = C-type natriuretic peptide; E = early filling wave; FS = fractional shortening; LVDd = left ventricular end-diastolic dimensions; MI = myocardial infarction; PWT = posterior wall thickness.

Quantitative reverse transcription-polymerase chain reaction. Endogenous mRNA expressions of ventricular CNP were evaluated in rats killed on day 3, 7, and 18 after MI (without CNP treatment) and on day 3 after sham operation (n = 6 in each group) with quantitative reverse transcription-polymerase chain reaction using a LightCycler system (Roche Applied Science, Penzberg, Germany) according to the manufacturer's instruction.

Immunohistochemical analysis. Immunohistochemical studies were performed to localize endogenous CNP in LV myocardium after MI. The section on day 7 after MI (in rats without CNP treatment) was stained with goat anti-CNP antibody (Santa Cruz Biotechnology, Santa Cruz, California) followed by Alexa-Fluor donkey anti-goat IgG antibody (Molecular Probes, Eugene, Oregon) and stained with rabbit fibronectin antibody (Sigma, St. Louis, Missouri) followed by tetrarhodamine isothiocyanate-conjugated goat anti-rabbit IgG antibody (DakoCytomation, Glostrup, Denmark).

Statistical analysis. All values are expressed as mean ± SEM. Differences among the groups were evaluated by one-way analysis of variance and two-way analysis of variance for repeated measurements, as appropriate. When a statistical difference was detected by analysis of variance, the Bonferroni method of adjusting for multiple pairwise comparisons was used. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The effect of CNP on survival rate and infarct size. Among the MI rats, two of the CNP-infused rats and seven of the vehicle-infused rats died during the two-week infusion period. The survival rate of the MI+CNP group (94%) was higher than that of the MI+vehicle group (83%), but this

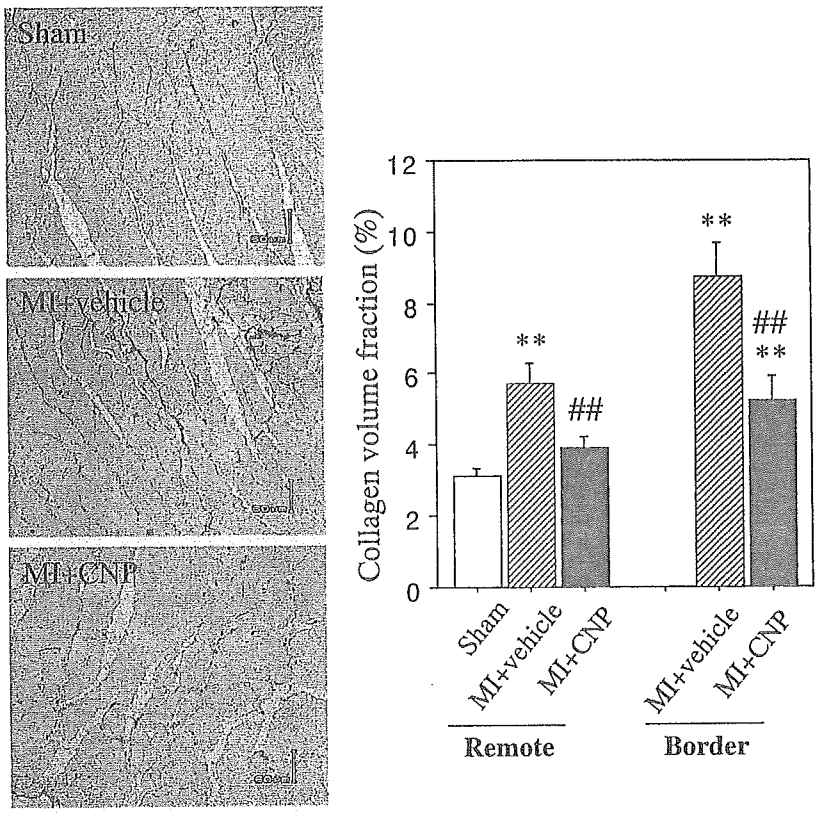

Figure 2. The effect of C-type natriuretic peptide (CNP) infusion on collagen volume fraction in the remote and border noninfarcted left ventricular area after myocardial infarction (MI). Representative photomicrographs of collagen volume stained with Sirius red in the remote noninfarcted LV (×200 magnification) **(left)** and quantitative morphometric analysis **(right)**. Values are mean ± SEM. **p < 0.01 compared with the sham-operated group; ##p < 0.01 compared with the MI+vehicle group by analysis of variance and Bonferroni multiple-comparison *t* test.

difference was not statistically significant by Kaplan-Meier survival analysis (p = 0.13). No rats died in the sham group. Therefore, the total numbers for final analysis were 34 rats in the MI+CNP group, 35 in the MI+vehicle group, and 34 in the sham group. There was no difference in infarct size between the MI+CNP and MI+vehicle groups (45 ± 1% and 46 ± 1%, respectively).

Serial change of noninvasive blood pressure and pulse rate. A significant reduction in the systolic blood pressure was observed in MI+CNP or MI+vehicle rats compared with the sham-operation rats during two weeks after the operation. As shown in Figure 1A, the systolic blood pressure was not perturbed by CNP infusion at any time points. The pulse rate in MI groups significantly increased at day 1 compared with sham animals and decreased gradually. The pulse rate was not significantly affected by CNP treatment at any time points (Fig. 1B).

The effect of CNP on echocardiographic and hemodynamic parameters. Table 1 shows echocardiographic assessments of cardiac geometry and function for the three groups of rats at the 4th and 18th days after MI. At the 4th day (before CNP infusion), when compared with sham, LV enlargement, decreased fractional shortening, and increased ratio of E to A velocities were seen in similar degree in both MI groups. At the 18th day (after two weeks of CNP infusion), hypertrophy of the posterior wall and the LV cavity enlargement caused by MI were significantly attenuated by CNP infusion, although thinning of the anterior wall was not changed; CNP also ameliorated the decrease of fractional shortening. Furthermore, CNP significantly improved LV diastolic filling pattern, resulting in a marked reduction in the ratio of E to A velocities (Table 1).

Table 2 also shows hemodynamic assessments for the three groups of rats at the 18th day after MI. No significant difference was noted in heart rate among the three groups. Mean arterial pressure and LV systolic pressure were lower

Table 2. Hemodynamic Parameters

	Sham	MI+Vehicle	MI+CNP
HR, beats/min	412 ± 5	421 ± 6	410 ± 5
MAP, mm Hg	120 ± 2	99 ± 2*	103 ± 2*
LVSP, mm Hg	139 ± 2	116 ± 2*	118 ± 2*
LVEDP, mm Hg	7 ± 0.4	18 ± 1*	13 ± 1*†
LV dP/dt_{max}, mm Hg/s	7,970 ± 156	5,019 ± 155*	5,743 ± 155*†
LV dP/dt_{min}, mm Hg/s	−6,216 ± 158	−3,791 ± 151*	−4,644 ± 147*†
CO, ml/min	98 ± 2	73 ± 2*	81 ± 2*†

Values are mean ± SEM. *p < 0.01 compared with sham-operated group; †p < 0.01 compared with MI+vehicle group by analysis of variance and Bonferroni multiple-comparison *t* test.

CNP = C-type natriuretic peptide; CO = cardiac output; HR = heart rate; LV dP/dt max or min = peak rate of left ventricular rise or fall; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; MI = myocardial infarction.

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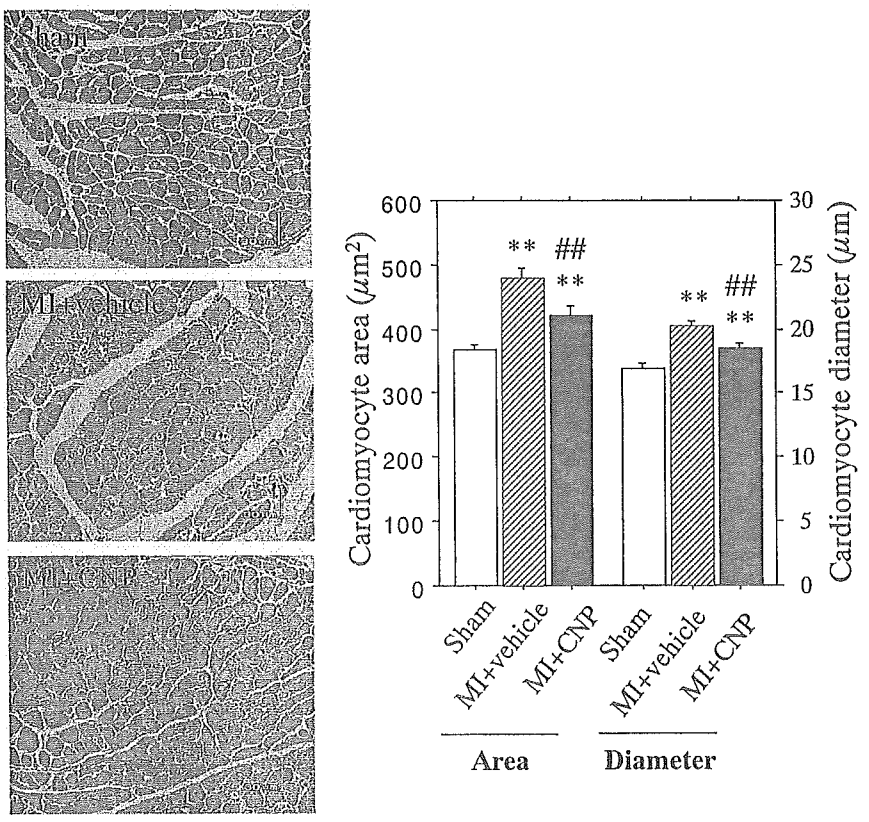

Figure 3. The effect of C-type natriuretic peptide (CNP) infusion on cardiac hypertrophy in the noninfarcted left ventricle. Representative photomicrographs of cardiomyocyte size stained with hematoxylin and eosin (×400 magnification) **(left)** and quantitative morphometric analysis of cardiomyocyte area and diameter **(right)**. Values are mean ± SEM. **p < 0.01 compared with the sham-operated group; ##p < 0.01 compared with the myocardial infarction (MI) + vehicle group by analysis of variance and Bonferroni multiple-comparison *t* test.

in the MI+vehicle and MI+CNP groups than in sham, but there were no differences in these parameters between the two MI groups. Left ventricular end-diastolic pressure was higher, and the peak rate of contraction (dP/dt_{max}), the peak rate of relaxation (dP/dt_{min}), and the cardiac output were lower in MI+vehicle than in sham. As shown in Table 2, the MI-induced systolic and diastolic LV dysfunction was markedly improved by CNP.

The effect of CNP on cardiac collagen volume and hypertrophy. To clarify the mechanism of improved cardiac performance caused by CNP, we examined the effects of CNP treatment on collagen volume and mural hypertrophy in the noninfarcted region; CNP significantly ($p < 0.01$) attenuated an increase in morphometrical collagen volume fraction in the remote LV (Fig. 2) and RV (sham: 3.3 ± 0.3%; MI+vehicle: 5.5 ± 0.5%; MI+CNP: 4.2 ± 0.3%). Furthermore, CNP reduced an increase in collagen volume fraction more effectively in the border region of MI, in which fibrosis was more prominent compared with the remote zone (Fig. 2).

The cross-sectional area and diameter of myocytes in the noninfarcted LV significantly increased in MI+vehicle compared with sham, and hypertrophy of the myocytes was significantly ($p < 0.01$) inhibited by CNP infusion (Fig. 3). In agreement with the above results, the heart-weight-to-body-weight ratio, which was increased in the two MI groups compared with sham, was significantly ($p < 0.01$) lowered by CNP treatment (sham: 3.29 ± 0.03 g/kg; MI+vehicle: 3.96 ± 0.09 g/kg; MI+CNP: 3.69 ± 0.06 g/kg).

The effect of CNP on gene expression. To confirm the effects of CNP on cardiac remodeling, we examined the expression of several mRNAs associated with fibrosis and hypertrophy in the noninfarcted LV and RV after MI (Figs. 4A, representative autoradiograms, and 4B, quantitative analysis, n = 10 in each group). As shown in Figure 4, the increased mRNA expression of collagen type I and collagen type III after MI was significantly suppressed by treatment with CNP. The increased fibronectin mRNA expression tended to be decreased by CNP, but it was not significant. At the 18th day, mRNA expression of TGF-β-1, which is well known to be a fibrotic cytokine and to be upregulated in the acute phase of MI (15), was not different between MI rats with or without CNP infusion; CNP treatment resulted in suppression of the ANP mRNA level, which is a useful marker of cardiac fetal phenotype modulation after MI, and the β-/α-MHC ratio, which is a qualitative marker of cardiac hypertrophy, in the noninfarcted LV (Fig. 4). In the infarcted LV, mRNA levels of collagen type I, collagen type III, fibronectin, TGF-β-1, ANP, and β-/α-MHC were all increased in the MI+vehicle and MI+CNP groups compared with sham, but there was no difference in these