Influence of Thyroid Function on Serum Bone Gla Protein

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Abstract

The serum BGP level was assayed in patients with hyperthyroidism (untreated and remittent cases) and hypothyroidism. The mean serum BGP concentration was 9.7 ± 0.90 ng/ml in 30 patients with untreated hyperthyroidism which was significantly higher than the 2.7 ± 0.38 ng/ml in 15 remittent patients and 1.3 ± 0.31 ng/ml in 13 patients with hypothyroidism ($p < 0.001$, $p < 0.001$). Serum BGP had a significant positive correlation with the concentrations of free triiodothyronine and alkaline phosphatase in the serum, while it had a significant negative correlation with serum PTH. In the patients with hypothyroidism, serum BGP increased significantly in parallel with increases in serum free triiodothyronine with thyroxine therapy. In the patients with hyperthyroidism, serum free triiodothyronine decreased significantly after the first month of methimazole treatment, and fluctuated within the normal range after two months. Serum alkaline phosphatase and BGP did not show significant changes during the first six months of treatment, although they were eventually reduced significantly at the end of one year.

These results suggest that thyroid hormone directly stimulates the synthesis and secretion of BGP in existent osteoblasts and also acts on the bone remodeling cycle, therapy accelerating the rate of bone formation; the latter action may occur over a long period.

Serum bone γ -carboxyglutamic acid (Gla) containing protein (bone gla protein: BGP)

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is thought to be a sensitive and specific marker of bone turnover (Price *et al.*, 1980). The serum concentration level has been studied in patients having various diseases with abnormality of bone metabolism, including primary hyperparathyroidism. The clinical usefulness of this assay has been

reported (Price *et al.*, 1980; Delmas *et al.*, 1983; Yoneda *et al.*, 1986).

Recently, it has been reported that patients with hyperthyroidism show high serum BGP concentrations that reflect abnormal bone metabolism (Orimo *et al.*, 1984; Garrel *et al.*, 1986; Lukert *et al.* 1968). However, the effects of thyroid hormone on serum BGP have not been clarified.

Here we studied the relationship of serum BGP to thyroid hormone and biochemical factors related to bone metabolism in patients at various stages of thyroid function to better understand the effects of thyroid hormone on serum BGP. In addition, we investigated changes in serum BGP following treatment of thyroid disorders.

Based on these data, we have attempted to clarify the effects of thyroid hormone on serum BGP.

Materials and Methods

Our subjects were 30 patients with untreated hyperthyroidism, 15 patients with remittent hyperthyroidism, and 13 patients with untreated hypothyroidism.

The diagnosis of hyperthyroidism was based on classic clinical criteria and confirmed by several thyroid function tests that included free thyroxine (FT₄), free triiodothyronine (FT₃), and elevated thyroid ¹²³I uptake. The patients with remittent hyperthyroidism were those who had maintained a normal thyroid hormone concentration for two years or more with a maintenance dose of the antithyroid drugs, and had a positive T₃ suppression test. Hypothyroidism was diagnosed on the basis of clinical features and serum biochemical data (FT₄, FT₃ and TSH).

Decreased renal function was not found in any patient. Sex difference and age distribution were comparable among the three groups.

All blood samples were collected while the subjects were fasting early in the morning to examine blood chemistry and assay the baseline concentrations of triiodothyronine (T₃), FT₃, FT₄, parathyroid hormone (PTH) and BGP.

BGP was measured with a radioimmunoassay kit (Immuno Nuclear Co., stillwater, Minnesota). The antibody used in this kit was obtained by immunizing rabbits against bovine BGP. The mean $\pm$ S. E. value for serum BGP determined in normal adults by this procedure is 4.5 ± 0.48 ng/ml and the normal range is 2.0–7.0 ng/ml (Yoneda *et al.*, 1986).

PTH was measured by the previously described method of radioimmunoassay (Kawakubo *et al.*, 1986). In this kit, the antibody specific for human PTH 43–68 was used (Middle region assay, Yamasa Shoyu Co., Ltd., Choshi). The normal range is 0.17–0.41 ng/ml for PTH.

T₃, FT₃ and FT₄ were measured with the Dinabot T-3 RIAKIT II (Dainabot Co. Tokyo), Amerlex Free T₃ radioimmunoassay kit and Amerlex Free T₄ radioimmunoassay kit (Amersham International plc, U. K.) respectively. The normal range is 0.9–1.9 ng/ml for T₃, 2.4–6.0 pg/ml for FT₃ and 0.8–1.8 ng/dl for FT₄ in the serum (Mitsuma *et al.*, 1976; Kisamori *et al.*, 1984; Mitsuma *et al.*, 1981). The inter and intra-assay variations were less than 13%.

Serum Ca, P, albumin (Alb) and ALP were measured with an autoanalyzer (Technicon SMAC Jr.). Serum Ca was corrected by Alb according to the formula of Payne (Payne *et al.*, 1973). The normal range is 8.4–10.0 mg/dl for Ca, 2.9–4.5 mg/dl for P and 30–115 IU/l for ALP in the serum.

All data are given as the mean $\pm$ S. E. and were assessed by Student's *t*-test and paired *t*-test for the significance of differences.

Schedule of the clinical study was as following:

1. Effects of methimazole therapy on serum BGP concentration in patients with hyperthyroidism.

Eight patients with hyperthyroidism were given orally 30 mg of methimazole daily. Doses were decreased according to the thyroid function of individual patients. Blood samples were collected before and after 1, 2, 3, 6, and 12 months of treatment.

2. Effects of triiodothyronine (T₃) on serum BGP concentration in patients with remittent hyperthyroidism (T₃ suppression test).

Fifteen patients with remittent hyperthyroidism were given orally 75 μ g of liothyronine sodium daily for 7 days. Blood samples were collected before and on the 7th day of treatment.

3. Effects of thyroxine (T₄) therapy on serum BGP

concentration in patients with hypothyroidism. Nine patients with hypothyroidism were given orally 25 µg of levothyroxine daily. Doses were increased slowly according to the function of individual patients until they reached maintenance doses in all patients after three months of treatment. Blood samples were collected before and after 1, 2, and 3 months of treatment.

Results

Table 1 shows the laboratory data for

Fig. 1. Serum BGP in 30 patients with hyperthyroidism, 15 patients with remittent hyperthyroidism and 13 patients with hypothyroidism. Shaded area represents the value for the normal control (±2SD).

Table 1. Laboratory data for 30 patients with hyperthyroidism and 13 patients with hypothyroidism.

	Sex, Number	Age (yrs)	Serum, Ca (mg/dl)	Serum P (mg/dl)	Serum ALP (IU/L)	Serum FT ₃ (pg/ml)	Serum FT ₄ (ng/dl)	Serum PTH (ng/ml)	M ± SE (Range)
Hyperthyroidism	Male 11	33.7 ± 2.0 (15 ~ 54)	9.5 ± 0.08 (8.7 ~ 10.3)	4.2 ± 0.18 (2.6 ~ 5.8)	170 ± 15 (51 ~ 278)	20.1 ± 1.5 (11.0 ~ 25 <)	11.1 ± 0.99 (3.8 ~ 20 <)	0.33 ± 0.02 (0.05 ~ 0.48)	
	Female 19								
Hypothyroidism	Male 5	42.9 ± 7.3 (23 ~ 80)	8.5 ± 0.12 (7.9 ~ 8.8)	3.4 ± 0.15 (2.7 ~ 3.9)	70 ± 7.1 (49 ~ 104)	0.86 ± 0.15 (0.2 ~ 2.1)	0.21 ± 0.06 (UD ~ 0.8)	0.48 ± 0.04 (0.30 ~ 0.61)	
	Female 8								
Significance level (P)	NS	NS	<0.001	<0.05	<0.005	<0.001	<0.001	<0.001	

Table 2. Correlation between serum BGP and other factors in patients with thyroid disease.

	Ca	P	ALP	FT ₃	FT ₄	PTH
BGP vs	n 33	33	36	58	41	53
	r 0.69	0.37	0.78	0.73	0.62	-0.36
	P< 0.001	0.05	0.001	0.001	0.001	0.05

the patients with hyper- and hypothyroidism. The concentrations of Ca ($p < 0.001$), P ($p < 0.05$) and ALP ($p < 0.005$) in the serum were significantly higher in the patients with hyperthyroidism than in those with hypothyroidism. The serum PTH concentration was significantly lower ($p < 0.001$) in the patients with hyperthyroidism (0.33 ± 0.02 ng/ml) than in the patients with hypothyroidism (0.48 ± 0.04 ng/ml).

As shown in Fig. 1, the serum BGP concentration was significantly higher in the patients with hyperthyroidism (9.7 ± 0.90 ng/ml; range, 2.4–20.4), than in patients with remittent hyperthyroidism (2.7 ± 0.38 ng/ml; range, 1.0–3.9, $p < 0.001$) or those with hypothyroidism (1.3 ± 0.31 ng/mg; range, U. D.–3.4, $p < 0.001$). The serum BGP concentration was significantly lower ($p < 0.01$) in the patients with hypothyroidism than in patients with remittent hyperthyroidism. Abnormally high values were found in 19 of the 30 patients with hyperthyroidism and abnormally low values in 9 of the 13 patients with hypothyroidism.

Table 2 shows the correlation of the serum BGP concentrations with other biochemical factors in the three groups. The serum BGP had a significant positive correlation with Ca ($p < 0.001$), P ($p < 0.05$), ALP ($p < 0.001$), FT_3 ($p < 0.001$) (Fig. 2) and FT_4 ($p < 0.001$) in the serum, and a significant negative correlation with PTH ($p < 0.05$).

Fig. 3 shows the changes in serum BGP and other biochemical parameters with methimazole therapy in the eight patients with hyperthyroidism. Serum FT_3 decreased significantly ($p < 0.01$) from 15.9 ± 1.3 pg/ml before treatment to 8.4 ± 1.9 pg/ml ($p < 0.01$) in one month and to 5.1 ± 1.1 pg/ml ($p < 0.001$) in two months, and an euthyroid state was maintained up to one year thereafter. The serum Ca concentration dropped significantly from 9.3 ± 0.18 mg/dl before treatment to 8.6 ± 0.18 mg/dl three months after treatment was started ($p < 0.05$), 8.7 ± 0.14 mg/dl after six months ($p < 0.05$) and 8.7 ± 0.06 mg/dl after one year ($p < 0.01$). The serum PTH concentration rose significantly ($p < 0.05$) from 0.27 ± 0.05 ng/ml

Fig. 2. The relationship between serum $F-T_3$ and BGP in 30 patients with hyperthyroidism ($\circ$), 15 patients with remittent hyperthyroidism ($\triangle$) and 13 patients with hypothyroidism ($\bullet$).

before treatment to 0.48 ± 0.07 ng/ml three months after treatment was started. Serum ALP increased slightly 2–3 months after treatment was started from 160 ± 22 IU/L before treatment, although the difference was not significant. It had decreased significantly ($p < 0.02$) to 93 ± 8.3 IU/L one year after treatment was started. Serum BGP did not show a significant change up to six months after treatment was started as compared to 8.7 ± 1.1 ng/ml before treatment. It had decreased significantly ($p <$

0.02) to 5.2 ± 0.58 ng/ml one year after treatment was started.

In 15 patients with remittent hyperthyroidism who were given orally $75 \mu\text{g}$ of T_3 daily for a week for T_3 suppression testing, serum T_3 increased significantly ($p < 0.001$) from the pretreatment value of 1.3 ± 0.06 ng/ml to 4.5 ± 0.04 ng/ml at one week, while serum PTH was 0.45 ± 0.04 ng/ml, showing no significant change compared to the pretreatment value of 0.49 ± 0.04 ng/ml. Serum BGP increased significantly

Fig. 3. Changes in serum FT_3 , Ca, ALP, PTH and BGP during methimazole therapy in eight patients with hyperthyroidism. * $P < 0.05$, ** < 0.02 , *** < 0.01 and **** < 0.001 vs the pre-administration value.

Fig. 4. Changes in serum T₃, PTH and BGP after treatment with triiodothyronine (75 µg, daily) for T₃ suppression testing in 15 patients with remittent hyperthyroidism. *P < 0.001 vs pretreatment value.

($p > 0.001$) from the pretreatment value of 2.7 ± 0.38 ng/ml to 3.6 ± 0.38 ng/ml at one week (Fig. 4).

Fig. 5. Changes in serum FT₃, PTH and BGP during levothyroxine (25-150 µg, daily) therapy in nine patients with hypothyroidism. *P < 0.05, ** < 0.005 and *** < 0.001 vs pretreatment value.

Fig. 5 shows the changes in FT₃, PTH, and BGP in the serum accompanying T₄ therapy in nine patients with hypothyroidism. Serum FT₃ increased significantly from the pretreatment value of 0.64 ± 0.10 pg/ml to 1.9 ± 0.31 pg/ml ($p < 0.005$) one month after treatment was started, 2.7 ± 0.37 pg/ml ($p < 0.001$) after two months and 3.4 ± 0.21 pg/ml ($p < 0.001$) after three months. Serum PTH decreased from the pretreatment value of 0.45 ± 0.05 ng/ml to 0.31 ± 0.04 ng/ml one month after treatment was started, but no significant change was observed. Serum

BGP increased significantly, closely paralleling the increases in serum FT_3 from the pretreatment value of 1.3 ± 0.37 ng/ml to 2.8 ± 0.61 ng/ml ($p < 0.05$) two months after treatment was started and 4.8 ± 0.53 ng/ml ($p < 0.001$) after three months.

Discussion

Serum BGP is thought to be a marker of bone turnover, particularly bone formation, in patients with metabolic bone disease such as primary hyperparathyroidism (Price *et al.*, 1980; Delmas *et al.*, 1983; Yoneda *et al.*, 1986).

Recently the serum BGP concentration has been assayed in patients with thyroid malfunction and reported to be high in patients with hyperthyroidism (Orimo *et al.*, 1983; Garrel *et al.*, 1986; Lukert *et al.*, 1986; Johansen *et al.*, 1987). However, there are somewhat different views on the serum BGP concentration in patients with hypothyroidism: Nakai (1984) reported it to be high but Johansen (1987) reported it to be low.

In our study, the serum BGP concentration was abnormally high in many patients with hyperthyroidism, while it was abnormally low in many patients with hypothyroidism. These facts show that thyroid function seriously affects serum BGP. It is known that the synthesis and secretion of BGP in osteoblasts is stimulated by treatment with active vitamin D (Price and Baukol, 1980). In patients with hyperthyroidism, however, parathyroid function is reduced (Bouillon and Moor, 1973; Mosekilde and Christensen, 1977), and serum vitamin D is also decreased (Velentzas *et al.*, 1977; Bouillon *et al.*, 1980).

We had reported that the serum BGP concentration was high in patients with primary hyperparathyroidism and low in those with idiopathic hypoparathyroidism (Yoneda *et al.*, 1985). In the present study,

the parathyroid function was suppressed in the patients with hyperthyroidism, which supports the previous reports. Therefore, we have to seek the other causes for increased serum BGP.

The serum BGP concentration had a positive correlation with serum FT_3 and FT_4 . Moreover, thyroid hormone increased serum BGP in a short time in the patients with remittent hyperthyroidism and in parallel to serum FT_3 in the patients with hypothyroidism.

Rizzoli (1986) reported, using an *in vitro* system, that there are T_3 receptors in the nuclei of rat osteosarcoma cells and that T_3 directly accelerates the synthesis and secretion of BGP. These facts strongly suggest that thyroid hormone directly accelerates the synthesis and secretion of BGP in existent osteoblasts.

In the course of treatment of hyperthyroidism, the serum BGP concentration was normalized conspicuously more slowly than the serum FT_3 . This phenomenon is similar to the slow decrease in the BGP concentration after a parathyroid adenoma was removed from a patient with primary hyperparathyroidism (Delmas *et al.*, 1986). The high serum BGP concentration in the patients with hyperthyroidism cannot be explained by the direct action of thyroid hormone alone. Other factors may also be involved. It is well known that thyroid hormone directly promotes bone resorption (Mundy *et al.*, 1976; Orbai and Gozarium, 1982). The present study suggested that activation of bone resorption by thyroid hormone stimulates the bone remodeling cycle and enhances the number and activity of the osteoblasts. e. g. the activation of bone formation, and that this phenomenon lasts for a long term. However, in order to elucidate the mechanism, further study will be required on various factors involving the bone remodeling cycle.

These results indicated that, *in vivo*, thyroid hormones directly promote the

synthesis and secretion BGP in existent osteoblasts and also stimulates the bone remodeling cycle by accelerating bone resorption: These acute and chronic actions increase serum BGP.

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