Bartter's Syndrome Associated with Graves' Disease and Myasthenia Gravis: Report of a Case

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Synopsis

Described here is a 21-year-old man, who had several episodes of generalized muscle weakness, blepharoptosis and a diffuse goiter. The results of thyroid function tests and histological findings of specimen obtained by thyroid biopsy was compatible with mild Graves' disease. Blepharoptosis was moderately improved immediately after edrophonium injection, thus established the diagnosis of ocular type of myasthenia gravis. In addition, the patient had markedly diminished serum potassium level, in association with markedly elevated plasma renin activity (PRA). Plasma aldosterone concentration (PAC) and urinary aldosterone excretion (UAE) were also moderately increased. The insensitivity to pressor effect of infused synthetic angiotensin II and hyperplasia of the juxtaglomerular apparatus of the kidney in histological examination substantiated the diagnosis of Bartter's syndrome complicated with Graves' disease and myasthenia gravis.

In order to investigate the effect of indomethacin, an inhibitor of prostaglandin biosynthesis, on the biochemical abnormalities of Bartter's syndrome, indomethacin (75 mg/day) was given with spironolactone and potassium treatment. However, the biochemical abnormalities were not corrected and, moreover, muscle weakness and further decrease in potassium level were observed during the treatment with indomethacin alone. The findings suggest that the abnormalities observed in this patient could not be explained by the overproduction of renal prostaglandin.

The present case with Bartter's syndrome was characterized by complicating of Graves' disease and myasthenia gravis, both of which have been regarded as autoimmune diseases. Immunological studies, including subpopulation of lymphocyte determined by surface immunoglobulin bearing cells, rosette formation and blastoid formation, revealed the suppression of B lymphocyte population in the present case. Although these immunological abnormalities were corrected after administration of azathioprine, an immunosuppressive agent, serum potassium level was not ameliorated. The result suggest that immunological mechanism is not related to the etiology of Bartter's syndrome in the present case.

Since hyperaldosteronism associated with hypokalemic alkalosis, normal blood pressure and juxtaglomerular cell hyperplasia was described by Bartter *et al.* (Bartter *et al.*, 1962), a number of patients with similar syndrome, which is now called Bartter's syndrome, have been reported. Although

the controversy regarding the etiology of Bartter's syndrome has continued, several investigators have shown that indomethacin, an inhibitor of prostaglandin synthesis, could correct all the abnormalities of this disorder, and they suggested a possible important role of renal prostaglandin overproduction in the pathogenesis of this syndrome (Verberckmoes *et al.*, 1976; Fichman

et al., 1976; Gill *et al.*, 1976; Halushka *et al.*, 1977).

In the present paper, we report a patient with Bartter's syndrome associated with Graves' disease and myasthenia gravis, both of which have been regarded as autoimmune diseases. Endocrine studies, including the investigation on the effect of indomethacin, and immunological studies before and after the administration of azathioprine, an immunosuppressive agent, were made in the present case.

Case Report

A 21-year-old man was admitted to Tenri Hospital on October 31, 1975, for evaluation of blepharoptosis and episodes of generalized muscle weakness. The patient had been in good health until March, 1970, when he became aware of palpitation, increasing fatigue and perspiration. He was pointed out a diffuse goiter and diagnosed as having Graves' disease. Partial thyroidectomy was performed in a hospital, Okinawa, Japan, in May, 1970.

In December, 1972, he began to note blepharoptosis and, therefore, he had been treated with eye drop of anti-cholinesterase. The first episode of generalized muscle weakness occurred in the summer, 1973, when he woke in the morning to find himself unable to stand up or to move his extremities. There was no convulsion, incontinence or respiratory and swallowing difficulties. He had cleared spontaneously following bed rest for 2 days. Thereafter, he had several episodes of muscle weakness. Three months before admission, he complained of palpitation and perspiration. He was pointed out a diffuse goiter, with slightly elevated serum thyroxine (T_4) and tri-iodothyronine (T_3) levels in outpatient clinic of Tenri Hospital. He received an antithyroid drug for about one month, which induced euthyroid state lasting without

maintenance dose of the drug. He denied both the habitual usage of laxatives and diuretics and the frequent occurrence of nausea, vomiting and diarrhea.

Past history was non-contributory. Family history was negative for muscle weakness, although one of his cousins had Graves' disease.

Physical examination on admission revealed a well-nourished man, 153 cm in height and 41 kg in weight. The blood pressure was 100/50 mmHg in the supine position, and the pulse was 70/min in rate and regular. Exophthalmos was not present, but ptosis of the right eyelid was noted and movement of his eyes were slightly limited in all directions. Although there was an operation scar in the neck, the diffuse goiter was still noted. It was elastic soft and not tender with a smooth surface. Chvostek' sign and Trousseau's phenomenon could not be elicited. There was no pitting edema in the extremities. Neurological examinations were non-contributory.

Following the intravenous administration of 2 mg of edrophonium chloride, moderate improvement in blepharoptosis was noted (Fig. 1). Thus, the diagnosis of ocular type of myasthenia gravis was made.

Roentgenogram of the chest was interpreted as normal, but an electrocardiogram showed flat T wave and prominent U wave. ^{67}Ga scintigraphy showed no evidence of mediastinal tumor.

Urinary volume varied 1000 to 3300 ml/24 hr and a routine urinalysis revealed a specific gravity of 1008 with no protein nor sugar. Complete blood count showed no anemia, but slight leucocytosis was noted. Serum transaminases and alkaline phosphatase were almost within normal limits. Circulatory blood volume and plasma volume determined by the dilution method using iodinated serum albumin were 78.6 ml/kg body weight and 46.0 ml/kg body weight, respectively. Endogenous creatinine clearance was 100 ml/min. Serum sodium varied

135 to 140 mEq/l and chloride 89 to 96 mEq/l, while serum potassium was markedly low (1.6–2.3 mEq/l). Moreover, renal potassium clearance rate on 80 ml $\text{Na}_2\text{S}_2\text{O}_3$ loading was markedly elevated (52 ml/min *vs* 10–30 ml/min in the normals). Arterial blood gases revealed metabolic alkalosis with an oxygen tension of 77 mmHg, carbon dioxide tension of 36.5 mmHg, base excess of +5.2 mEq/l and pH of 7.493. Plasma renin activity (PRA) in supine position, determined by angiotensin I radioimmunoassay kit (Schwarz / Mann, New York, USA), was markedly increased to 20.6 ng/ml/hr on an ordinary diet, compared with normal values below 1.0 ng/ml/hr. On the same ordinary diet, plasma aldosterone concentration (PAC) and urinary aldosterone excretion (UAE) measured by radioimmunoassay, using commercial kit (CEA-IRESORIN, GIF-SUR-YVETTE, France), were

19.3 ng/100 ml and 26 $\mu\text{g/day}$, respectively, which were above the normal range (PAC: 5 to 15 ng/100 ml and UAE: 5 to 20 $\mu\text{g/day}$).

Methods

The patient was hospitalized in the Endocrine Section of Tenri Hospital. Urine was consecutively collected for 24-hr period, except days of infusion studies. Plasma samples were obtained from the patient in the supine position after a bed rest for at least 30 min. Serum T_4 and T_3 were measured by radioimmunoassay kit provided by the Dainabot Radioisotope Laboratories, Tokyo, Japan and thyroid stimulating hormone (TSH) was measured by radioimmunoassay kit purchased from Daiichi Radioisotope Laboratories, Tokyo, Japan.

Blood pressure responses to the intravenous infusion of synthetic angiotensin II or noradrenalin were assessed in the following way. The patient was kept supine for several hours before and during the test period. Blood pressure was determined with a sphygmomanometer every two or three min after the infusion was started. Angiotensin II-amide (Hypertensin®, Ciba) was infused intravenously for five consecutive 15 min periods at rates of 5, 10, 20, 50 and 100 ng/kg body weight/min and noradrenaline for four consecutive 13-min periods with infusion rates of 40, 100, 200, and 500 ng/kg body weight/min. Moreover, changes of blood pressure, PRA and PAC were observed during intravenous infusion of 600 ng/kg body weight/min of 1-sarcosine, 8-isoleucine angiotensin II (Daiichi Pharmaceutical Company, Tokyo, Japan) for 30 min. The influence of the alterations of dietary sodium (9 to 175 mEq/day) on serum potassium, PRA and PAC were also studied.

Circulatory thyroid antibodies were examined by the thyroglobulin-coated and the microsome-coated tanned red cell hemagglutination tests prepared by Fuji Zoki Co., Tokyo, Japan. Another circulating antibodies, including anti-nuclear, anti-parietal cell, anti-smooth muscle, anti-striated muscle and anti-mitochondrion antibodies, were examined by the fluorescent antibody technique (Paronetto *et al.*, 1967). Proportion of lymphocytes bearing surface immunoglobulin was also determined by the fluorescent antibody method (Ault *et al.*, 1973). Percentages of blastoid forming lymphocytes by either phytohemagglutinin (PHA) or poke weed mitogen (PWM) and those of EAC and E rosette forming lymphocytes in peripheral blood were determined by the methods reported previously (Mendes *et al.*, 1973; Janossy and Greaves, 1971).

Delayed type cell-mediated immunity was studied by means of Mantoux reaction and dinitro-

Fig. 1. Effect of intravenous injection of 2 mg edrophonium on blepharoptosis. Top: Before the injection. Bottom: After the injection.

chlorobenzene (DNCB) skin reaction. Sensitization of DNCB was performed by applying 0.1 ml of 2% DNCB in acetone to the forearm. Testing was carried out with 0.1% DNCB in acetone 14 days after the sensitization.

Results

Thyroid function tests

T_4 and T_3 concentrations in serum about 3 months before admission were $15.3 \mu\text{g}/100 \text{ ml}$ and $195 \text{ ng}/100 \text{ ml}$, respectively (T_4 : 5 to $13 \mu\text{g}/100 \text{ ml}$ and T_3 : 85 to $180 \text{ ng}/100 \text{ ml}$ in the normals). Twenty-four hour ^{131}I thyroid uptake was 56.7%, which was not suppressed after the administration of $75 \mu\text{g}/\text{day}$ of T_3 for 8 days. Serum TSH concentration was under the limit of determination and did not increase significantly after the intravenous injection of $500 \mu\text{g}$ of thyrotropin releasing hormone (TRH). Histologic examination of the specimen obtained by thyroid needle biopsy showed an increase in the height of the lining epithelial cells, pale pink colloid and the accumulation of lymphoid cells in some

area, all of which were compatible with the diagnosis of Graves' disease. After anti-thyroid treatment of one month duration, T_4 and T_3 concentrations in serum were almost within normal limit even without medication.

Angiotensin II and noradrenalin infusion tests (Fig. 2)

The blood pressure response to the infusion of synthetic angiotensin II was studied when the patient was taking 175 mEq/day of sodium. As shown in Fig. 2, the infusion rate of greater than $20 \text{ ng}/\text{kg}/\text{min}$ was required to obtain an increase over 20 mmHg in diastolic pressure, and systolic pressure only slightly responded to infused angiotensin II with an infusion rate of as great as $100 \text{ ng}/\text{kg}/\text{min}$. As the increase over 20 mmHg in systolic and diastolic pressure was obtained in infusion rate of $20 \text{ ng}/\text{kg}/\text{min}$ of angiotensin II in normal subject, marked resistance to pressor action of exogenous angiotensin II was evident in the present patient. Furthermore, the patient showed grossly diminished pressor sensitivity

Fig. 2. Blood pressure response to the infusion of angiotensin II and of noradrenalin.

to noradrenalin (Fig. 2). The increment of systolic pressure was only 10 mmHg or less at an infusion rate of as great as 500 ng/kg/min, whereas the significant increase of systolic pressure was observed in infusion rate under 100 ng/kg/min of noradrenalin in normal subjects.

Effect of 1-sarcosine, 8-isoleucine angiotensin II on blood pressure, PRA and PAC (Fig. 3)

After the infusion of 600 ng/kg/min of 1-sarcosine, 8-isoleucine angiotensin II for 30 min under the condition of daily sodium intake of 175 mEq, blood pressure fell from 94/62 mmHg to 88/30 mmHg. PRA increased from 13.5 to 85.0 ng/ml/hr, while PAC decreased from 19.3 to 12.0 ng/100 ml (Fig. 3).

Fig. 3. Blood pressure response to the infusion of angiotensin II antagonist (1-sarcosine, 8-isoleucine angiotensin II).

PRA: Plasma renin activity.

PAC: Plasma aldosterone concentration.

Effect of alterations in dietary sodium on serum potassium concentration, PRA and PAC (Fig. 4)

PRA and PAC were markedly increased (51.5 ng/ml/hr and 30.7 ng/100 ml, respectively) in this patient on a daily sodium intake of about 9 mEq for 4 days. When the patient's sodium intake was increased from 90 to 175 mEq/day, PRA decreased from 32.5 to 22.7 ng/ml/hr, associated with a slight decrease in PAC from 21.0 to 19.1 ng/100 ml. However, serum potassium concentration was further decreased, despite the diminution of PAC (Fig. 4).

Results of histological studies

A specimen of the right kidney obtained by percutaneous needle biopsy was fixed in Zenker's solution and stained with hematoxylin and eosin, periodic acid-Schiff (PAS) and Bowie's stains. In hematoxylin and eosin stained sections, slightly congested

Fig. 4. Effect of various sodium intake on serum potassium level, PRA and PAC.

PRA: Plasma renin activity.

PAC: Plasma aldosterone concentration.

glomeruli were seen with extreme hyperplasia of juxtaglomerular (JG) cells. No remarkable changes were seen in the tubules, blood vessels and interstitium. In Bowie's stained sections JG cells contained little granulations and a PAS-positive membrane between macula densa cells and glomerular cells was not seen.

Results of immunological studies (Table 1)

Blood sedimentation rate was 38 mm/hr and serum concentrations of immunoglobulin G and A were slightly elevated.

The anti-microsome antibody of circulatory thyroid antibody was positive ($\times 10^5$), but another autoantibodies, including anti-smooth muscle, anti-parietal cell, anti-striated muscle, anti-nuclear and anti-mitochondrion antibodies, were not detectable. Markedly diminished proportion of surface immunoglobulin-bearing cells in peripheral lymphocytes was evident (14 % *vs* 34 ± 7 % in the normals). Moreover, rosette-forming lymphocytes to sheep red cell incubated with complement (EAC rosette) and of blastoid-forming lymphocytes to PWM were also markedly reduced in percentage (EAC rosette: 0.8 % *vs* 19 ± 13 % in the normals and blastoid formation to PWM: 3 % *vs* 18 ± 6 % in the normals), suggesting the suppression of B-lymphocyte population. In contrast, rosette-forming lymphocytes to sheep red cell (E rosette) was almost within the normal range (61 % *vs* 55 ± 12 % in the normals) and blastoid forming lymphocytes to PHA was slightly elevated (82 % *vs* 55 ± 21 % in the normals). Moreover, both Manteau and DNCB skin tests were positive in the present case. The results suggested almost the normal cellular immunity in the present patient.

Effect of azathioprine treatment on immunological findings and serum potassium levels (Table 1)

In order to determine or not the abnormalities in immunological findings might

Table 1. Immunological findings and effect of Azathioprine therapy.

	before	after
Blood sedimentation rate (mm/hr)	38	17
Serum gamma-globulin (g/100ml)	1.2	1.1
Serum immunoglobulin		
Ig G (mg/100 ml)	1860	1160
Ig A	400	300
Ig M	60	35
Surface immunoglobulin-bearing cell		
Ig G (%)	12	30
Ig A	1	14
Ig M	1	1
Total	14	45
EAC rosette formation (%)	0.8	25
Blastoid formation (PWM added) (%)	3	38
E rosette formation (%)	61	36
Blastoid formation (PHA added) (%)	82	30
Serum K (mEq/l)	2.1	2.1

play a role in pathogenesis of Bartter's syndrome, he was treated with 50 to 100 mg/day of azathioprine for 3 months and thereafter the immunological studies were repeated. Blood sedimentation rate and subpopulation of lymphocytes determined by surface immunoglobulin-bearing cells, rosette formation and blastoid formation were all improved by the treatment with azathioprine, while serum potassium level was not increased at all.

Effects of indomethacin on serum potassium and PRA

Although slightly elevation of serum potassium concentration was observed after the administration of potassium and spironolactone, it was still below the normal range and the reduction of PRA was not evident.

During the treatment with 75 mg/day of indomethacin combined by potassium and spironolactone for 4 weeks, serum potassium level was not increased and PRA remained high. Furthermore, during the treatment with 150 mg/day of indomethacin alone for 2 days, serum potassium level was rather decreased and the patient complained of muscle weakness.

Discussion

Described herein is a patient who had several episodes of generalized muscle weakness, blepharoptosis and a diffuse goiter. Blepharoptosis was moderately improved immediately after the injection of edrophonium, thus established the diagnosis of ocular type of myasthenia gravis. Results of thyroid function tests and histological findings of the thyroid are compatible with mild Graves' disease.

In addition to Graves' disease and myasthenia gravis, it was noticeable that the patient had several episodes of generalized muscle weakness associated with markedly diminished serum potassium level. This muscle weakness was not ascribed to thyrotoxicosis, because serum potassium level was consistently low even after thyroid function returned to normal by the administration of an anti-thyroid drug. Moreover, he had markedly elevated PRA in association with increased PAC and UAE. Furthermore, the insensitivity to the pressor effect of infused synthetic angiotensin II and the hyperplasia of the juxtaglomerular apparatus in histological examination substantiated the diagnosis of Bartter's syndrome complicated with Graves' disease and myasthenia gravis.

Despite numerous studies (Gardner *et al.*, 1972; White, 1972; Tomko *et al.*, 1976; Fujita *et al.*, 1977), the pathogenesis of Bartter's syndrome remains unknown. Bartter *et al.* had postulated the vascular insensitivity to angiotensin II as a basic defect of Bartter's syndrome (Bartter *et al.*, 1962). However, recent studies (Kono *et al.*, 1976; Sasaki *et al.*, 1976) have demonstrated the hypotensive response to 1-sarcosine, 8-isoleucine angiotensin II, a specific competitive antagonist of angiotensin II, in Bartter's syndrome, suggesting that endogenous angiotensin II might exert its effect on arteriolar smooth muscle in patients with Bartter's

syndrome.

In the present case, the infusion of the angiotensin II antagonist also produced significant hypotensive reaction, increased PRA and decreased PAC. The results were consistent with those in the previous studies. Furthermore, both PRA and PAC were decreased with an increase of sodium intake. The findings suggest that renin-angiotensin-aldosterone system is still operative in this syndrome. On the other hand, serum potassium concentration was not increased in this period, suggesting that hypokalemia observed in this patient might be due to another factor than hyperaldosteronism. However, the possibility that the increase of exchangeable sodium in tubulus might play a role in hypokalemia by augmenting the renal loss of potassium could not be excluded.

Recently, it has been postulated that there is an overproduction of renal prostaglandins in Bartter's syndrome, based on the observation that biochemical abnormalities of the syndrome were corrected, at least in the short term, by indomethacin, an inhibitor of prostaglandin biosynthesis (Verberckmoes *et al.*, 1976; Fichman *et al.*, 1976; Gill *et al.*, 1976; Halushka *et al.*, 1977). Although concentrations of prostaglandins in serum or in urine were not determined in the present case, addition of indomethacin to spironolactone and potassium treatment for 4 weeks did not correct the biochemical abnormalities. Moreover, muscle weakness and further decrease in serum potassium level were observed during the treatment with indomethacin alone. These findings suggest that the abnormalities observed in this patient could not be explained by the overproduction of renal prostaglandins.

The association of Graves' disease and myasthenia gravis has been frequently noted (Namba and Grob, 1971). To our knowledge, however, no case report of Bartter's syndrome associated with Graves' disease

and myasthenia gravis has appeared in literatures. Since both Graves' disease and myasthenia gravis are considered to be based on autoimmune mechanism, of special interest is the interrelation between Bartter's syndrome and autoimmune diseases. It has been frequently observed that the subpopulation of B lymphocyte is decreased in patients with myasthenia gravis (Nakata *et al.*, 1974). Therefore, the suppression of B lymphocyte population, observed in the present case, is not ascribed to Bartter's syndrome. Despite the fact that the immunological abnormalities were corrected after administration of azathioprine, an immunosuppressive agent, serum potassium level was not ameliorated. This suggests that immunological mechanism is not related to the etiology of Bartter's syndrome in the present case. Further studies should clarify whether Bartter's syndrome and Graves' disease and myasthenia gravis occurred independently or interrelated each other.

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