IgG4-related disease in pulmonary arterial hypertension on long-term epoprostenol treatment

To the Editor:

IgG4-related disease (IgG4-RD) is a recently described systemic fibro-inflammatory condition characterised by tumefactive lesions and dense lymphoplasmacytic infiltrates rich in IgG4-positive plasma cells [1]. IgG4-RD has been described in a variety of organ systems, including the lacrimal gland, salivary gland, lungs, pancreas and kidneys, and often responds favourably to corticosteroids. The pathophysiological mechanisms for IgG4-RD remain unclear, but type 2 T-helper (Th2) cells are thought to play a critical role [2, 3]. We recently encountered a patient who developed IgG4-RD during the course of idiopathic pulmonary arterial hypertension (PAH). After this experience, we carefully examined PAH patients for IgG4-RD and found four additional cases.

The index case patient was a 19-year-old female who presented with progressive exertional dyspnoea corresponding to World Health Organization functional class (WHO-FC) II in March 2000. She was referred to Keio University Hospital (Tokyo, Japan), where complete pulmonary hypertension (PH) evaluations were conducted. Right heart catheterisation revealed an elevated mean pulmonary arterial pressure (mPAP) of 67 mmHg and a normal pulmonary arterial wedge pressure of 7 mmHg. There was no condition or underlying disease that accounted for her PH, leading to the diagnosis of idiopathic PAH. She was initially treated with warfarin and nifedipine, and an oral prostacyclin analogue, beraprost, was added in January 2002. 1 year later, her dyspnoea had deteriorated (to WHO-FC III), with marked elevation of the mPAP (to 95 mmHg). Beraprost was replaced by epoprostenol, and the dosage was gradually increased to 57 $ng \cdot kg^{-1} \cdot min^{-1}$, resulting in improved symptoms and haemodynamic parameters (mPAP 47 mmHg).

In January 2007, the patient developed chronic sinusitis. Simultaneously, she noticed a gradual enlargement of bilateral lacrimal, parotid and submandibular glands, and of minor salivary glands of the lips, in conjunction with intermittent upper abdominal pain (fig. 1a–g). A computed tomography scan revealed diffuse enlargement of bilateral lacrimal and salivary glands and pancreas. Her serum IgG4 level was remarkably elevated (to 824 $mg \cdot dL^{-1}$ from a normal range of <135 $mg \cdot dL^{-1}$). Histopathological analysis of specimens obtained from labial glands showed a dense lymphoplasmacytic infiltrate around the ducts and acini. The inflammatory infiltrate was mainly composed of $CD20^+$ B-cells and $CD138^+$ plasma cells. The infiltrating plasma cells were predominantly IgG4-bearing cells, which exceeded 50% of the total plasma cells. The diagnosis was IgG4-related dacryoadenitis, sialadenitis and pancreatitis. The patient was treated with 35 mg prednisolone daily and showed prompt improvement of the tumefactive lesions. However, 4 months later, she died of uncontrolled right heart failure due to exacerbation of PAH.

After we encountered the index case in 2007, we examined PAH patients who were being followed at the specialised PH clinic of Keio University Hospital for IgG4-RD. Between 2008 and 2012, we found four additional cases of idiopathic/heritable PAH with complicating IgG4-RD during the disease course. To examine the incidence rate, clinical characteristics and predisposing factors for IgG4-RD in patients with PAH, we retrospectively obtained clinical information for 75 consecutive patients with idiopathic/heritable PAH and 40 consecutive patients with PAH associated with connective tissue disease (CTD), who first visited the specialised PH clinic of Keio University Hospital between 2000 and 2009. IgG4-RD was diagnosed based on the characteristic organ involvement with elevated serum IgG4 level and/or typical histopathological findings [4]. All the clinical information was collected in June 2012. The study was approved by the Keio University Institutional Review Board.

IgG4-RD was observed in five (6.7%) out of the 75 patients with idiopathic/heritable PAH, but in none of the 40 patients with PAH-CTD. None of the patients without IgG4-RD showed enlarged lacrimal or salivary glands upon careful physical examination, or an increase in serum IgG4. The incidence rate of IgG4-RD in patients with idiopathic/heritable PAH was 14.3 per 1000 patient-years. Since the estimated prevalence of IgG4-RD in Japan was 0.28–1.08 per 100 000 in a community-based study [5], patients with idiopathic/heritable PAH are at extremely high risk for developing IgG4-RD.

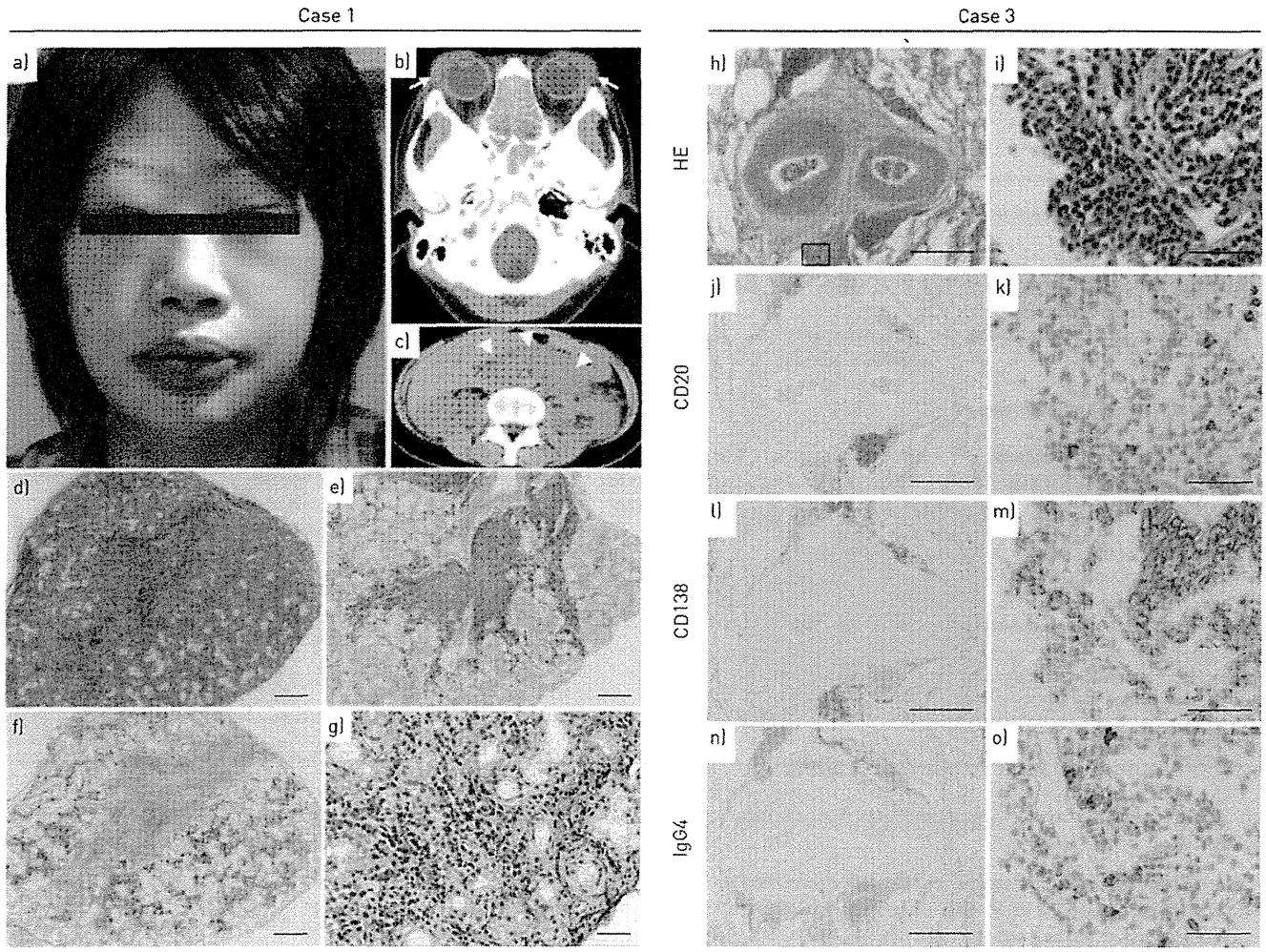

FIGURE 1 a–g) Clinical, radiological and histopathological findings of the index case (case 1) and h–o) histopathological findings of the consecutive lung sections from case 3. a) Bilateral enlargement of the lacrimal, parotid and submandibular glands. Lips of the mouth were markedly swollen because of beaded enlargement of the labial glands. b) Computed tomography (CT) scan showed diffusely enlarged bilateral lacrimal glands (arrows). c) CT scan showed a diffusely enlarged pancreas (arrowheads). d) Haematoxylin and eosin (HE) staining of a labial gland specimen obtained from lip biopsy. A dense lymphoplasmacytic infiltrate was found in the interstitium around the ducts and acini. e) CD20 immunostaining of the labial gland specimen showed B-cell accumulation predominantly around the duct. f) CD138 immunostaining of the labial gland specimen showed diffuse infiltrates of plasma cells in the peri-acinal area. g) IgG4 immunostaining of the labial gland specimen showed a predominance of IgG4-bearing plasma cells. h, i) HE staining showed a focal and dense lymphoplasmacytic infiltrate around the muscular pulmonary arteries with medial and adventitial thickening. h) Box indicates part of image that is shown at higher magnification in i, k, m and o. j, k) CD20 immunostaining showed focal B-cell accumulation predominantly around the narrowed pulmonary arteries. l, m) CD138 immunostaining showed focal infiltrates of plasma cells in the peri-acinal area. n, o) IgG4 immunostaining showed an excess of IgG4-bearing cells. Scale bars: d–f) 250 μm; g, i, k, m, o) 50 μm; h, j, l, n) 500 μm.

Table 1 summarises clinical characteristics of the five patients with idiopathic/heritable PAH who developed IgG4-RD. Interestingly, IgG4-RD occurred during the course of PAH, between 65 and 125 months after diagnosis of PAH. The lacrimal and salivary glands were symmetrically affected in all five cases, consistent with a previously recognised condition called Mikulicz disease, while none of them had sicca syndrome or positive anti-SSA or anti-SSB antibodies, which are typical features of Sjögren's syndrome. Pancreas involvement was also detected in two patients. Concomitant onset of allergic diseases was common.

As treatment for the IgG4-RD, four patients received corticosteroids (prednisolone >0.5 mg·kg^{-1} daily), and the remaining patient (case 3) was not treated because of an upcoming lung transplant. Histopathological analysis of lung specimens obtained at transplantation showed focal and dense lymphoplasmacytic infiltrates around the muscular pulmonary arteries with medial and adventitial thickening. The infiltrate consisted mainly of CD20$^+$ B-cells and CD138$^+$ plasma cells with an excess of IgG4-bearing cells (fig. 1h–o). Favourable responses, including resolution of tumorous swellings and decrease in serum IgG4, were observed in all patients who received corticosteroids, but none of them

TABLE 1 Clinical characteristics of patients with idiopathic/heritable pulmonary arterial hypertension (PAH) who developed IgG4-related disease (IgG4-RD) during the course of the disease

	Case				
	1#	2	3	4	5
Age at IgG4-RD diagnosis years	26	36	54	70	48
Sex	Female	Female	Female	Female	Female
Classification of PAH	Idiopathic	Idiopathic	Idiopathic	Heritable	Idiopathic
Interval between diagnoses of PAH and IgG4-RD months	78	69	65	125	82
Organ involvement of IgG4-RD	Lacrimal and salivary glands, pancreas	Lacrimal and salivary glands	Lacrimal and salivary glands	Lacrimal and salivary glands	Lacrimal and salivary glands, pancreas
Serum IgG4 level at diagnosis of IgG4-RD mg·dL^{-1}	824	1190	896	2220	92
Concomitant allergic disease	*Chronic sinusitis*	*Bronchial asthma*	None	*Chronic sinusitis*	*Chronic sinusitis*
PAH drugs at IgG4-RD diagnosis	Epoprostenol	Epoprostenol, bosentan	Epoprostenol, sildenafil	Epoprostenol, bosentan, sildenafil	Epoprostenol, bosentan, sildenafil
Duration of epoprostenol treatment at IgG4-RD diagnosis months	61	60	65	54	79
Maximum dose of epoprostenol ng·kg^{-1}·min^{-1}	85	33	57	53	93
Accumulated lifetime dose of epoprostenol mg·kg^{-1}	73	71	78	64	96

#: index case.

showed improvement in PAH. None experienced recurrence of IgG4-RD, and one patient died of exacerbation of the PAH.

Comparisons of the demographic and clinical characteristics of 75 patients with idiopathic/heritable PAH stratified by the presence or absence of IgG4-RD revealed no significant difference in age, sex, WHO-FC or haemodynamic parameters at the time of PAH or IgG4-RD diagnosis. Regarding the drugs used to treat the PAH, all five patients were on epoprostenol when IgG4-RD was diagnosed, while 46 out of 70 patients without IgG4-RD were treated with epoprostenol ($p=0.16$, Fisher's exact test). Notably, long-term treatment with a high concentration of epoprostenol appeared to be a risk factor for development of IgG4-RD. Specifically, the relative risk for developing IgG4-RD was >14.4 in patients who had received epoprostenol treatment for >4 years ($p=0.002$), and was >10.2 in patients with accumulated lifetime dose of epoprostenol >50 mg·kg^{-1} ($p=0.006$).

This is the first report describing a potential link between PAH and IgG4-RD. Since PAH is observed in association with various inflammatory conditions, such as CTD, one could speculate that PAH is one of the manifestations of IgG4-RD. In this regard, lung specimens obtained from one of the cases showed histological features compatible with IgG4-RD, but it remains unclear how lymphoplasmacytic infiltration present outside the vasculature contributes to remodelling of pulmonary arteries. IgG4-RD occurred >5 years after diagnosis of PAH, when the WHO-FC and haemodynamics were stable. In addition, corticosteroid treatment dramatically improved IgG4-RD but was ineffective for PAH, although rituximab, a potential agent effective for refractory IgG4-RD [6], was not tried for any of our patients. Conversely, long-term treatment with a high epoprostenol dose was suggested as a risk for IgG4-RD. Epoprostenol, a synthetic prostacyclin analogue, exerts its function mainly through a prostaglandin I_2 (IP) receptor, but also through other prostaglandin receptors such as DP1 and EP2 [7]. These receptors are reported to modulate development and function of T-helper cells [8], such as enhancement of Th2 expansion [9, 10]. It is intriguing to speculate that long-term exposure to a high concentration of epoprostenol contributes to IgG4-RD development by shifting the T-helper cell balance to Th2. However, further studies accumulating similar cases are necessary to clarify a causal relationship between these two conditions.

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IgG4-related disease may be associated with patients with PAH, especially those treated with long-term epoprostenol http://ow.ly/rFwlO

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Point-of-care urine test for assessing adherence to isoniazid treatment for tuberculosis

To the Editor:

Good adherence to treatment for tuberculosis (TB) is essential, both to cure disease and prevent development of drug resistance. Adherence to chemoprophylaxis (preventive therapy) for latent TB infection (LTBI) is particularly poor [1].

Methods for measuring adherence to TB medication include detecting urine colour change due to the presence of rifampicin. However, this effect is short lived, peaking at 2–6 h and is only seen in <50% of patients [2]. Directly observed therapy (DOT) will ensure adherence to antituberculous treatment, but this can be unacceptable and many patients do not tolerate a three times a week regimen. DOT is costly in terms of personnel and is seldom employed in chemoprophylaxis patients [1]. The highly reliable Arkansas method for detecting isoniazid metabolites in urine relies on a laboratory colorimetric assay, involving adding drops of prepared solutions of reagents, including potassium cyanide, to a urine sample [3]. There are obvious risks involved in handling and storing the reagents.

IsoScreen (GFC Diagnostics, Bicester, UK) uses the reagents of the Arkansas Method but in an enclosed plastic testing device (SafeTube; GFC Diagnostics), allowing safe and rapid point-of-care testing in clinics and patients' homes [2]. The urine colour change is apparent within a few seconds but 5 min is allowed to ensure stable colour development. Purple or blue samples are deemed positive, green intermediate and

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ORIGINAL ARTICLE

Lifestyle and other related factors for the development of mixed connective tissue disease among Japanese females in comparison with systemic lupus erythematosus

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Abstract

Objective. The etiology of mixed connective tissue disease (MCTD) has not been elucidated in detail. Case control studies of MCTD and systemic lupus erythematosus (SLE) were conducted in order to compare factors related to these two diseases.

Methods. We selected 48 MCTD and 54 SLE female patients throughout Japan from 2009 to 2010. Controls were 182 female patients who visited the clinics of general internal medicine during the study periods.

Results. Smoking and walking a longer time showed an increased age-adjusted risk for MCTD as well as SLE. On the other hand, frequent intake of bread increased the risk of MCTD and high intake of green tea decreased the risk of MCTD. Even after an additional adjustment of smoking and drinking, frequent intake of bread increased the risk of MCTD, while walking increased the risk of SLE.

Conclusion. The present study suggests that Westernization of dietary habits (i.e. frequent intake of bread and low intake of green tea) may increase the risk of MCTD, while walking may increase the risk of SLE (probably due to exposure to the sunlight) among Japanese females. Further studies are needed to confirm the result of the present study.

Keywords

A case control study, Japan, Mixed connective tissue disease, Risk factor, Systemic lupus erythematosus

History

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Introduction

Mixed connective tissue disease (MCTD), which was first introduced as a distinct clinical entity by Sharp and co-workers in 1972 [1], is an autoimmune disease with combined clinical features of systemic lupus erythematosus (SLE), scleroderma and polymyositis/dermatomyositis, as well as with high titers of antibodies to U1 small nuclear ribonucleoprotein [1–3]. The common primary clinical features of MCTD are Raynaud's phenomenon, swollen fingers or hands and arthralgia with or without arthritis, while pulmonary involvement is a serious complication of MCTD and pulmonary arterial hypertension is one of the most common diseases related to causes of death in MCTD [2].

The Japanese Ministry of Health, Labour and Welfare designated MCTD and SLE as intractable diseases because there is no established way to cure or prevent them [4]. Based on this definition, the Ministry has financially supported scientific research to cure or prevent them [4]. However, risk or preventive factors for MCTD have not been clearly shown in these studies [4] although several case control studies revealed some factors related to the development of SLE in learned journals [5–10].

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In case control studies of SLE [5–10], information before the diagnosis of SLE was collected in cases, while information before the interview day was collected in controls so that they could find what factor may be related to the development of SLE.

Like SLE [11], MCTD affects females more frequently than males [2,3]. Therefore, in the present study, a case control study of MCTD as well as a case control study of SLE was conducted so that we could compare factors related to these two rheumatic diseases in Japanese women.

Methods

Profile of Japan MCTD study

The Japan MCTD study was a case control study to evaluate risk factors for MCTD as well as SLE among Japanese females. From 2009 to 2010, cases (i.e. MCTD patients and SLE patients) were recruited from rheumatic outpatients in our collaborative hospitals from all over the Japan, except Shikoku Island, while controls were recruited from outpatients who visited the clinics of general internal medicine in our collaborative hospitals.

MCTD patients were diagnosed according to the criteria proposed by the Ministry of Health, Labour and Welfare in Japan [3], while all SLE patients fulfilled the American College of Rheumatology criteria [12].

In the Japan MCTD study, we used the same questionnaire that was used in the Kyushu Sapporo SLE Study [7,8,10]. An

anonymous self-administered questionnaire was obtained from MCTD patients, SLE patients and control patients, along with informed consent for co-operation in this study. All questions referred to the subject's status before the diagnosis of MCTD or SLE in cases and before the interview day for controls. The present study was approved by the institutional review boards of St. Mary's College, Kyoto University, and each of other institutions involved.

In the present study, 91 female MCTD patients and 91 female SLE patients (i.e. one SLE patient for every one MCTD patient) were recruited for cases, while 182 female patients (i.e. two controls for every pair of one MCTD patient and one SLE patient) who visited the clinics of general internal medicine were recruited for controls during the study periods. However, we excluded the patients who had been treated for MCTD or for SLE for 10 years or longer in order to obtain accurate information. Thus, we used 48 MCTD patients and 54 SLE patients as cases in the present study.

MCTD patents were older than the controls at the time of the survey (43.0 ± 14.3 years old vs. 36.5 ± 15.0 years old, $p < 0.01$), while the age at the diagnosis of MCTD did not differ from the controls (38.3 ± 14.6 years old vs. 36.5 ± 15.0 years old, $p = 0.47$) (not shown in the table). On the other hand, SLE patients and the controls did not differ in age at the time of the survey (37.5 ± 11.8 years old vs. 36.5 ± 15.0 years old, $p = 0.54$), while SLE patients at the time of diagnosis tended to be younger than the controls (32.9 ± 36.5 years old vs. 36.5 ± 15.0 years old, $p = 0.06$) (not shown in the table).

Statistical analysis

Unconditional logistic regression was used to evaluate the odds ratio (ORs) and their 95% confidence intervals. Age was treated as a continuous variable, and indicator variables were used for smoking and drinking. We treated current and former smokers as smoking positive, while those who drank 1 day/week or more were defined as having drinking habit. Ages at the time of the survey were used for controls, while the ages at diagnosis were used for MCTD patients and SLE patients. All statistical analyses were conducted by use of a statistical analysis system package (SAS Institute, Cary, NC, USA). All P values were two-sided, with those less than 0.05 considered statistically significant.

Results

Table 1 shows life style and dietary habits and the age-adjusted ORs of MCTD and SLE. In the case control study of MCTD, smoking and eating bread (once/day or more vs. less than once/day) increased the risk of MCTD, and walking (30 min/day or more vs. less than 30 min/day) tended to increase the risk. On the other hand, green tea (7–9cups/day or more vs. 4–6 cups/day or less) tended to decrease the risk. In the case control study of SLE, smoking, drinking and walking (30 min/day or more vs. less than 30 min/day) increased the risk of SLE.

Age, smoking and drinking-adjusted ORs are shown in Table 2. In the case control study of MCTD, either drinking or smoking did not show any meaningful association with the development of MCTD after additional adjustment, while frequent bread intake (once/day or more vs. less than once/day) remained as a significant risk factor even after additional adjustment for smoking and drinking. In the case control study of SLE, smoking and drinking failed to be a significant risk factor after additional adjustment, while walking (30 min/day or more vs. less than 30 min/day) increased the risk even after additional adjustment of smoking and drinking.

Discussion

Smoking has been demonstrated to increase the risk of SLE in several pieces of research for intractable diseases supported by the Japanese Ministry [5–8,10]. Cigarette smoke contains many

Table 1. Lifestyle and dietary habits and the risk of MCTD and the risk of SLE.

	MCTD ($n = 48$) number (%)	Control ($n = 182$) number (%)	Age-adjusted OR (95% CI)	p value
Lifestyle factors				
Smoking (former and current smokers vs. non-smokers)	16 (33.3)	35 (19.2)	2.20 (1.08–4.48)	<0.05
Drinking (1–3 times/week or more vs. less than once/week)	9 (18.8)	39 (21.4)	0.80 (0.35–1.80)	0.59
Walking (30 min/day or more vs. less than 30 min/day)	34 (70.8)	104 (57.1)	1.83 (0.92–3.65)	0.09
Sports activity (3–4 times/week or more vs. 1–2/week or less)	12 (25.0)	37 (20.3)	1.28 (0.60–2.71)	0.52
Sleeping (7–8 h/day or more vs. 5–6 h/day or less)	41 (85.4)	157 (86.3)	0.91 (0.37–2.27)	0.84
Felt psychological stress (yes vs. no)	22 (45.8)	72 (39.6)	1.37 (0.71–2.62)	0.35
Dietary habits				
Green tea (7–9 cups/day or more vs. 4–6 cups/day or less)	2 (4.2)	24 (13.2)	0.26 (0.06–1.15)	0.08
Black tea (4–6 cups/day or more vs. 2–3 cups/day or less)	1 (2.1)	2 (1.0)	2.13 (0.19–24.45)	0.55
Coffee (4–6 cups/day or more vs. 2–3 cups/day or less)	4 (8.3)	15 (8.2)	1.00 (0.31–3.16)	1.00
Rice (2–3 times/day or more vs. once/day or less)	19 (39.6)	92 (50.6)	0.60 (0.31–1.16)	0.13
Bread (once/day or more vs. less than once/day)	21 (43.8)	53 (29.1)	1.93 (1.00–3.72)	<0.05
	SLE ($n = 54$) number (%)	Control ($n = 182$) number (%)	Age adjusted (95% CI) OR	p value
Lifestyle factors				
Smoking (former and current smokers vs. non-smokers)	18 (33.3)	35 (19.2)	2.13 (1.08–4.19)	<0.05
Drinking (1–3/week or more vs. less than once/week)	18 (33.3)	39 (21.4)	2.03 (1.02–4.02)	<0.05
Walking (30 min/day or more vs. less than 30 min/day)	40 (74.1)	104 (57.1)	2.15 (1.09–4.24)	<0.05
Sports activity (3–4 times/week or more vs. 1–2/week or less)	16 (29.6)	37 (20.3)	1.68 (0.84–3.35)	0.14
Sleeping (7–8 h/day or more vs. 5–6 h/day or less)	51 (94.4)	157 (86.3)	2.83 (0.82–9.83)	0.10
Felt psychological stress (yes vs. no)	25 (46.3)	72 (39.6)	1.29 (0.70–2.39)	0.41
Dietary habits				
Green tea (7–9 cups/day or more vs. 4–6 cups/day or less)	4 (7.4)	24 (13.2)	0.57 (0.19–1.75)	0.30
Black tea (4–6 cups/day or more vs. 2–3 cups/day or less)	1 (1.9)	2 (1.0)	1.76 (0.15–20.07)	0.65
Coffee (4–6 cups/day or more vs. 2–3 cups/day or less)	8 (14.8)	15 (8.2)	2.12 (0.84–5.38)	0.11
Rice (2–3 times/day or more vs. once/day or less)	27 (50.0)	92 (50.6)	1.01 (0.55–1.89)	0.98
Bread (once/day or more vs. less than once/day)	17 (31.5)	53 (29.1)	1.10 (0.57–2.13)	0.78

OR, odds ratio; 95% CI, 95% confidence intervals.

Table 2. The risk of MCTD and the risk of SLE after adjusting age, smoking and drinking.

Factors	MCTD ($n=48$) number (%)	Control ($n=182$) number (%)	Smoking, drinking and age-adjusted OR (95% CI)	p value
Smoking (former and current smokers vs. non-smokers)	16 (33.3)	35 (19.2)	1.90 (0.67–5.44)	0.23
Drinking (1–3/week or more vs. less than once/week)	9 (18.8)	39 (21.4)	0.68 (0.21–2.22)	0.52
Walking (30 min/day or more vs. less than 30 min/day)	40 (74.1)	104 (57.1)	1.95 (0.71–5.30)	0.19
Green tea (7–9 cups/day or more vs. 4–6 cups/day or less)	4 (7.4)	24 (13.2)	0.25 (0.03–2.05)	0.20
Bread (once/day or more vs. less than once/day)	21 (43.8)	53 (29.1)	3.47 (1.36–8.87)	<0.01
Factors	SLE ($n=54$) number (%)	Control ($n=182$) number (%)	Smoking, drinking and age-adjusted OR (95% CI)	p value
Smoking (former and current smokers vs. never smokers)	18 (33.3)	35 (19.2)	1.37 (0.76–2.50)	0.29
Drinking (1–3/week or more vs. less than once/week)	18 (33.3)	39 (21.4)	1.16 (0.89–1.52)	0.28
Walking (30 min/day or more vs. less than 30 min/day)	40 (74.1)	104 (57.1)	2.74 (1.23–6.10)	<0.05
Green tea (7–9 cups/day or more vs. 4–6 cups/day or less)	4 (7.4)	24 (13.2)	0.82 (0.26–2.58)	0.73
Bread (once/day or more vs. less than once/day)	17 (31.5)	53 (29.1)	1.42 (0.70–2.88)	0.33

OR, odds ratio; 95% CI, 95% confidence intervals.

chemical compounds including aromatic amines, which may play a role in the development of SLE because hydrazine, a drug containing aromatic amines, is an inducer of SLE [13]. Cigarette smoking may increase the risk of SLE through the autoimmune response promoted by the exposure to reactive oxygen species [14]. Costenbader et al. [15] demonstrated that smoking was significantly associated with an increased risk of SLE in a meta-analysis based on nine studies. The result of this meta-analysis supports the results of Japanese case control studies [5–8], which suggest that smoking contributes to an increased risk of the development of SLE in a Japanese population.

Like SLE, rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that causes immune-medicated destruction of tissue and organs. Although the etiology of these autoimmune diseases has not yet been elucidated in detail, some of their environmental determinants may be considered similar. Like the meta-analysis of studies on SLE [14], Sugiyama et al. [16] demonstrated that smoking was a significant risk factor for the development of RA in a meta-analysis based on 16 studies. The results of these two studies suggest that smoking may be a common risk factor for rheumatic diseases such as SLE and RA. In the present study, smoking showed an increased age-adjusted OR of MCTD as well as SLE. These findings suggest that smoking may be a common risk factor for both SLE and MCTD. However, in the present study, smoking failed to remain as a significant risk factor for either SLE or MCTD after controlling drinking habit. These findings may be partly explained by the small number of cases because smoking is a significant risk factor for SLE in the previous studies in Japan [5–8,10], as well as in the meta-analysis based on studies on SLE [15].

Alcohol consumption is suggested to decrease the risk of SLE in some studies in Japan [5,8] as well as in Western countries [17–19]. Recently, Wang et al. [20] demonstrated that moderate alcohol consumption decreased the risk of SLE in a meta-analysis based on five studies. Similarly, in Japan, Kiyohara et al. [8] found that light/moderate alcohol consumption was inversely associated with SLE risk among Japanese females, irrespective of the type of alcoholic beverage.

In the present study, there was no meaningful association between drinking alcohol and the risk of MCTD, while drinking showed an increased age-adjusted OR for SLE. However, after additional control of smoking, there was no meaningful association between drinking and the risk of SLE. It may be partly explained by the following way. First, smokers were more likely to drink than their counterparts [21]. Second, drinkers may include heavy drinkers or binge drinkers. Heavy drinkers and binge drinkers may have felt emotional stress, which is a risk factor for SLE [10,22]. Thus, we could not find any meaningful association between drinking alcohol and the risk of MCTD or SLE in the present study after additional adjustment of smoking.

Physical activity (e.g. exercise) is reported to reduce the risk of cancer [23] and coronary heart disease [24]. However, there is little information about leisure-time physical activity and the risk of SLE. Nagai et al. [6] reported that neither outdoor sports nor physical active behavioral characteristics had any meaningful association with the risk of SLE, while skin sensitivity to sunlight increased the risk of SLE [6]. Washio et al. [7] also failed to show any meaningful association between leisure-time physical activity and the risk of SLE. However, they reported that walking (30 min/day or more vs. less than 30 min/day) increased the risk of SLE not in northern Japan (i.e. Hokkaido) but in southern Japan (i.e. Kyushu) [7]. Walking in southern Japan may be a surrogate of staying outdoors under sunlight because outdoor work is reported to increase the risk of SLE [25]. In the present study, there was no meaningful association between sports activity and MCTD or SLE, while walking (30 min/day or more vs. less than 30 min/day) showed a tendency of increased age-adjusted OR for MCTD and a significantly increased OR for SLE. However, after additional control for smoking and drinking, the ORs showed higher than the unity but failed to show the statistical significance.

Dietary factors are thought to affect the course of SLE as well as the development of SLE [26]. Minami et al. [26] reported that the frequent intakes of meat increased the risk of SLE, while Oumi et al. [9] demonstrated that total calorie intake, protein intake and carbohydrate intake were positively associated with the risk of SLE.

Compared with controls, SLE patients preferred beef or pork to chicken [26]. In addition, milk (once/day or more vs. less than once/day), bread (once/day or more vs. less than once/day) and canned tuna (once/week or more vs. less than once/day) increased the risk of SLE [9]. Since the frequent intakes of meat, milk, bread and canned tuna may be indicators of Westernized dietary habits, these findings suggest that Westernized dietary habits may increase the risk of SLE.

In the present study, there was no meaningful association between dietary factors and the risk of SLE, which may be explained by the small number of SLE patients in the present study. On the other hand, frequent intake of bread (once/day or more vs. less than once/day) showed a significantly increased age-adjusted OR for MCTD, while frequent intake of green tea (7–9 cups/day or more vs. 4–6 cups/day or less) may reduce the age-adjusted risk of MCTD. Green tea drinking is reported to be positively correlated with the traditional Japanese diets (rice, soy paste soup and pickles), and negatively correlated with eating bread for breakfast and coffee consumption [27]. Therefore, green tea is an indicator of a

traditional Japanese dietary habit, while bread may be an indicator for a Westernized dietary habit. Further studies should be recommended to confirm the hypothesis that a Westernized dietary habit increase the risk of MCTD.

Although green tea is thought to be an indicator of a traditional Japanese dietary habit, green tea is demonstrated to have anti-inflammatory [28], anti-apoptotic [28], radical scavenging [28,29] and anti-oxidative effects [29]. Further studies are needed to answer whether green tea per se may prevent the development of MCTD or if a traditional Japanese dietary habit may reduce the risk of MCTD.

In the present study, we conducted two case control studies (i.e. a case control study of MCTD and a case control study of SLE), and compared the risk factors for MCTD and the risk factors for SLE. Despite the limitations of the case control study, the case control study remains the most proper study design of epidemiological studies for rare diseases such as MCTD or SLE because case control studies need smaller participants than cohort studies [30,31]. Case control studies represent a high achievement of modern epidemiology, and if conducted well, they can reach the highest standards of validity [30,31]. However, we must confess the limitations of the present study. First, the number of cases was small because we recruited 91 MCTD patients and 91 SLE patients but half of them were excluded for the analyses in order to obtain accurate information. Second, since MCTD was a rare disease, the small number of MCTD patients obliged us to use prevalent cases in the present study. Third, controls were recruited not from a general population but from outpatients who visited the clinics of general internal medicine in our collaborative hospitals. Therefore, the present study was not free from recall bias or selection bias.

However, our study has strengths as well. As far as we know, this is the first case control study to evaluate the risk factors for MCTD in the world. Second, the validity of the study for MCTD risk was similar to the validity of the study for SLE risk because the two studies were conducted at the same time in the same way. In addition, the same controls were used in these two case control studies. So, we can compare the risk factors for MCTD and the risk factors for SLE.

In conclusion, the present study suggests that smoking may be a common risk factor for both MCTD and SLE among Japanese females. On the other hand, a Westernized dietary habit (i.e. frequent intake of bread and low intake of green tea) was suggested to increase the risk of MCTD, while walking a long time every day was proposed as a risk factor for SLE. However, further studies are required to confirm the results of the present study.

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Conflict of interest

None.

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Appendix

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ORIGINAL ARTICLE

Effects of the endothelin receptor antagonist bosentan on hemodynamics and exercise capacity in Japanese patients with mildly symptomatic pulmonary arterial hypertension

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Abstract Pulmonary arterial hypertension (PAH) trial has mostly enrolled patients with World Health Organization functional class (WHO FC) III or IV. However, PAH is rapidly progressive in nature even in patients with less severe forms at diagnosis. Following the recent studies in Western population, here we assessed the efficacy of bosentan in Japanese patients with WHO FCII PAH. In this open-label trial, bosentan 125 mg twice daily was administered for 12 weeks in 16 patients, and a hemodynamic evaluation was performed. Treatment was continued for a further 12 weeks, where the effect on exercise capacity was assessed in 13 patients. In 16 patients, mean pulmonary arterial pressure decreased from 40.4 ± 10.4 to 35.6 ± 12.6 mmHg ($p = 0.018$) and cardiac index increased from 2.54 ± 0.73 to 2.96 ± 0.82 L/min/m^2 ($p = 0.023$). Thus, pulmonary vascular resistance decreased from 792 ± 565 to 598 ± 558 dyn·sec/cm^5 ($p = 0.006$). In 13 patients followed up for 24 weeks, 6-min walking distance increased from baseline at Week 12 ($p = 0.003$) and Week 24 ($p = 0.011$). All patients were mildly symptomatic at baseline with dyspnea index (Borg scale) of 2.50 ± 1.58 and the specific activity scale (SAS) of 5.0 ± 1.4 METs. These values remained unchanged throughout the study. These results suggest that bosentan treatment was beneficial for Japanese patients

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with WHO FC II PAH and treatment should be started in the early stage of the disease.

Keywords Bosentan · Endothelin receptor antagonist · Pulmonary arterial hypertension · Hemodynamics · World Health Organization functional class

Introduction

Enormous strides have been made in understanding the basic mechanisms of pulmonary arterial hypertension (PAH) and we have witnessed a number of innovative strategies to combat this devastating disorder in the past decades. Nevertheless, the long-term prognosis for patients with PAH is ultimately poor as the disease often leads to right ventricular failure and death. World Health Organization functional class (WHO FC) has been identified as an important factor in predicting the outcome of the affected patients. The NIH cohort study that enrolled 194 patients with idiopathic PAH (IPAH) documented that the risk of death was higher among patients in WHO FC III or IV than among those in WHO FC I or II, the median survival time being 2.5 years for patients in WHO FC III and 6 months for patients in WHO FC IV while nearly 6 years among WHO FC I or II patients [1]. Not only in untreated patients with IPAH but also in those receiving therapeutic intervention, mortality rate is increased associated with higher WHO FC.

Since the discovery that endothelin 1 plays an important role in the pathogenesis of PAH, inhibition of ET receptors by a dual endothelin receptor antagonist, bosentan, has been proved to have significant clinical benefits.

Under these circumstances, results of the Endothelin Antagonist Trial in Mildly Symptomatic Pulmonary Arterial Hypertension Patients (EARLY) study, which pioneered early therapeutic management trial, were presented in 2008 [2]. This study was designed to assess the effect of bosentan exclusively in mildly symptomatic WHO FC II PAH patients. Following the studies in the Western populations, we also assessed the efficacy of bosentan in Japanese patients with PAH to bridge the Japanese status to the large-scale evidence for the expansion of bosentan indication. As the preceding studies had mostly enrolled patients with advanced disease, the patient population of the first Japanese study of bosentan also involved those exclusively in WHO FC III and IV. According to the growing body of evidence for the benefits of early detection and early therapeutic intervention in patients with PAH, this study was designed to assess the effects of bosentan in Japanese patients with WHO FC II PAH.

Materials and methods

This prospective open-label study was performed at 11 centers in Japan from November 2009 to December 2011. The protocol was reviewed and approved by the appropriate Institutional Review Board before study commencement. All subjects gave written informed consent prior to any screening procedures. The study consisted of a 12-week efficacy evaluation period followed by a 24-week safety evaluation period. Patients were treated with bosentan 62.5 mg twice daily for the first 4 weeks as an initial dose, and with bosentan 125 mg twice daily from Week 5 onwards as the target dose. If the drug was not well tolerated, down-titration to, or maintenance at, the 62.5 mg twice daily dose was available at any time, with possible subsequent up-titration to the target dose. At Week 12, all endpoints were assessed and, at Week 24, all endpoints except hemodynamics were assessed.

Japanese patients eligible for the study were aged 18–74 years; had PAH, either idiopathic/heritable or secondary to connective tissue disease (CTD) or congenital heart disease (CHD, atrial septal defect of less than 2 cm in a diameter, ventricular septal defect of less than 1 cm in diameter, patent ductus arteriosus); were in WHO FC II; and were PAH-specific treatment naive or treated with beraprost sodium for more than 3 months. Patients were also required to meet the following hemodynamic criteria: mean pulmonary arterial pressure (PAP) $\geq$25 mmHg at rest, pulmonary arterial wedge pressure (PAWP) $\leq$15 mmHg, and pulmonary vascular resistance (PVR) $\geq$240 dyn·sec/cm^5. Patients were excluded if they had any of the following: PAH associated with conditions other than those mentioned above, severe obstructive lung disease (forced expiratory volume [FEV]$_1$/forced vital capacity [FVC] <0.5), low total lung capacity (<70 %), acute or chronic impairment limiting the ability to comply with study requirements, hypotension (systolic blood pressure <85 mmHg), low hemoglobin concentration (<75 % of the lower limit of the normal range), moderate-to-severe hepatic impairment (aspartate aminotransferase [AST] and/or alanine aminotransferase [ALT] >1.5 times the upper limit of the normal range [ULN]). All other PAH-specific treatments, excluding beraprost, anticoagulation and diuretics, were prohibited as were calcineurin inhibitors, glibenclamide, fluconazole, and other investigational drugs. Because bosentan treatment has been associated with the development of reversible, dose-related abnormalities in liver aminotransferases, frequent liver function tests were repeated every 2 weeks throughout the study period and strict guidelines for treatment and monitoring were provided. In case of associated clinical symptom of liver injury and/or increases in total bilirubin >2× ULN, study treatment was to be stopped. Hemodynamics via right heart catheterization (RHC) (cardiac output,

mean right arterial pressure [RAP], systolic PAP, diastolic PAP, PAWP, and mixed venous oxygen saturation [SvO_2]), height, and body weight were assessed immediately before the start of treatment and at Week 12. Cardiac output was measured by the thermodilution method. Based on these measurements, PVR, total pulmonary resistance (TPR), and cardiac index (CI) were calculated. Treatment efficacy was also assessed by 6-min walk distance (6MWD), specific activity scale (SAS), and WHO FC. The 6-min walk test and Borg dyspnea index score were performed immediately before the start of treatment and at Weeks 12 and 24. WHO FC and SAS were assessed immediately before the start of treatment and at Weeks 4, 8, 12, and 24. Safety was assessed on the basis of recorded adverse events (AEs), clinical laboratory parameters, vital signs, and electrocardiography.

The primary objective of this study was to demonstrate that bosentan improves PVR in Japanese patients with WHO FC II PAH. The secondary objectives were firstly to demonstrate that bosentan is safe and well tolerated in Japanese patients with WHO FC II PAH, and secondly that bosentan improves cardiopulmonary hemodynamics (mean RAP, mean PAP, TPR, CI, SvO_2) and exercise capacity (6MWD) in Japanese patients with WHO FC II PAH.

Statistical analysis

Statistical hypothesis testing was not defined and a confirmatory analysis was not performed. However, a supplementary analysis was performed. Changes in PVR, which was the primary efficacy parameter, and other hemodynamic parameters from baseline to Week 12 were analyzed using a paired Wilcoxon test. Changes in 6MWD from baseline to Weeks 12 or 24 were analyzed using the same test. A significant change was defined as $p < 0.05$ (two-tailed).

To minimize bias, missing data at the Week 12 assessment were derived from predefined replacement rules. Discontinuation of study medication due to clinical worsening was analyzed based on the patient's assessment at the time of premature withdrawal. If no assessment was recorded, these patients were assigned the worst rank values—0 m for 6 MWD, a score of 10 for Borg dyspnea index, class IV for WHO FC, and the highest PVR recorded in the same population. All data analysis was performed with SAS version 9.2 (SAS statistical Software, version 9.2, 2009; SAS Institute Inc., Cary, NC, USA).

Results

Patient demographics and baseline characteristics

Twenty-nine Japanese patients with PAH were initially screened at 11 centers but 10 patients did not meet inclusion (mPAP less than 25 mmHg in 6 patients/exclusion (unstable clinical condition in 2 patients, total lung capacity less than 70 % in patient and PAH with reverse shunt in 1 patient) criteria. Nineteen patients with WHO FC II PAH were enrolled in the study. Four patients discontinued the study prematurely: 3 due to an elevation of liver aminotransferases without assessment of hemodynamics and one patient discontinued the study due to worsening of right heart failure with assessment of hemodynamics at the time of discontinuation. Underlying disease of this patient was systemic sclerosis which was characterized by rapid progression and poor prognosis. Therefore, 16 patients (5 males, 11 females, including 6 with idiopathic/heritable PAH, 7 with CTD, and 3 with CHD) were finally assessed for efficacy and 19 patients for safety. The mean age was 49.8 ± 15.9 (range 18–73) years. Patient demographics and baseline characteristics are shown in Table 1.

Efficacy

Bosentan was discontinued in one patient because of worsening of right heart failure and decrease in PVR was not apparent in this case. However, treatment efficacy was evaluated by treating the worst rank value as the observation from the last visit. Overall, at Week 12 mean PAP decreased from 40.4 ± 10.4 to 35.6 ± 12.6 mmHg ($p = 0.018$) and Cardiac Index increased from 2.54 ± 0.73 to 2.96 ± 0.82 L/min/m^2 ($p = 0.023$; Table 2). Therefore, calculated PVR was reduced from 792 ± 565 to 598 ± 558 dyn·sec/cm^5 ($p = 0.006$; Fig. 1).

Primarily 6 MWD was analyzed in the 16 patients at Week 12, thereby mean 6 MWD increased from 424.0 ± 116.1 to 442.7 ± 154.7 m ($p = 0.016$). In the 13 patients in whom additional measurement was available, 6MWD increased from a baseline value of 436.3 ± 125.0

Table 1 Patient demographics and baseline characteristics

	$n = 19$
Sex	
Male	6 (31.6 %)
Female	13 (68.4 %)
Age (years)	49.8 (15.9)
Weight (kg)	53.57 (9.50)
Time from diagnosis (years)	4.81 (8.96)
Etiology	
Idiopathic/heritable pulmonary arterial hypertension	8 (42.1 %)
Connective tissue disease	8 (42.1 %)
Congenital heart disease	3 (15.8 %)

Data are n (%) or mean (standard deviation)

Table 2 Changes in hemodynamic parameters after a 12-week treatment program with bosentan

Hemodynamic parameter	Patients	Baseline	Week 12	*p* value
PVR (dyn·s/cm^5)	16	792 ± 565	598 ± 558	0.006
mPAP (mmHg)	16	40.4 ± 10.4	35.6 ± 12.6	0.018
mRAP (mmHg)	16	5.5 ± 3.0	5.1 ± 3.9	0.687
CI (L/min/m^2)	16	2.54 ± 0.73	2.96 ± 0.82	0.023
TPR (dyn·s/cm^5)	16	989.1 ± 611.6	741.3 ± 623.8	0.006
SvO_2 (%)[a]	15	70.70 ± 5.86	71.53 ± 7.18	0.208

Mean ± standard deviation; paired Wilcoxon test

PVR pulmonary vascular resistance, *mPAP* mean pulmonary arterial pressure, *mRAP* mean right atrial pressure, *CI* cardiac index, *TPR* total pulmonary resistance, *SvO_2* mixed venous oxygen saturation

[a] Data in 1 patient were excluded due to missing baseline values

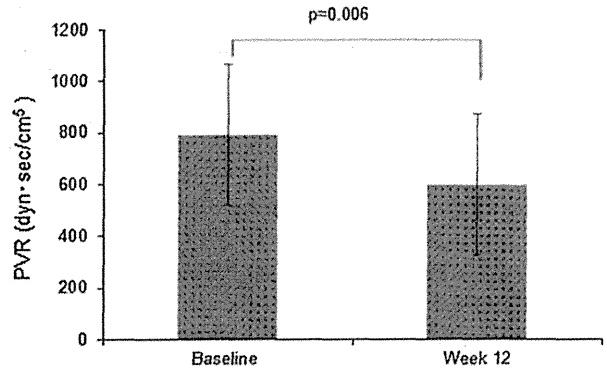

Fig. 1 Effect of bosentan on pulmonary vascular resistance from baseline to Week 12. *Error bars* are 95 % confidence intervals. $n = 16$, paired Wilcoxon test

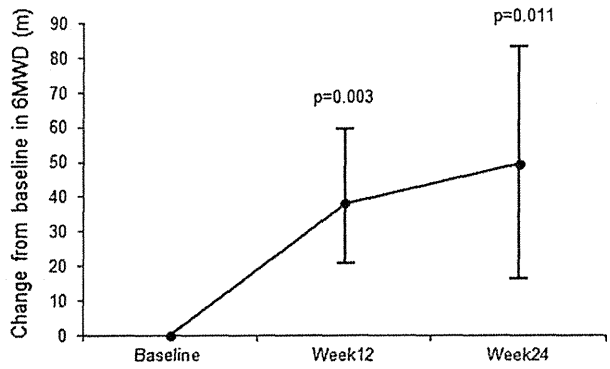

Fig. 2 Mean change in 6-min walk distance from baseline through Week 24. *Error bars* are 95 % confidence intervals. $n = 13$, paired Wilcoxon test

to 474.5 ± 111.4 m at Week 12 ($p = 0.003$) and to 485.8 ± 113.0 m at Week 24 ($p = 0.011$; Fig. 2).

The Borg dyspnea index was 2.50 ± 1.58 at baseline and remained the same, being 2.47 ± 2.91 at Week 12 ($p = 0.526$). The SAS value was substantially higher at baseline averaging 5.0 ± 1.4 METs and remained unchanged throughout the study period. At baseline, all the 16 patients were in WHO FC II that remained unchanged throughout the study period of 24 weeks in all patients who completed the study.

Eight patients (50 %) were pretreated with beraprost. There was no significant difference in the reduction of PVR among patients treated with or without beraprost (18 ± 723 vs −405 ± 660 dyn·sec/cm^5, $p = 0.713$, Wilcoxon rank sum test).

Safety

During the 24-week study period, adverse events (AEs) were observed in 18 of the 19 patients (94.7 %). The most commonly reported adverse events were abnormal liver function tests (36.8 %) and increase in aminotransferases of more than three times ULN in 4 patients (21.1 %). This result was due to the small number of patients. In fact, in post-marketing surveillance of more than 4000 patients, the incidence of aminotransferase elevation was 15.8 % and more than three times elevation was only 3.6 % [3]. These increases returned towards baseline values for all patients, either with discontinuation of bosentan or dose reduction. The incidence of nasopharyngitis was 21.1 %, and other AEs that occurred in more than 2 patients included constipation, headache and insomnia. Peripheral edema developed only in 1 patient, which was not considered by the investigators to be related to bosentan treatment.

Serious adverse events (SAEs) were observed in 4 of the 19 patients (21.1 %), although the investigators considered that all of SAEs were not related to the study drug. Four of the 19 patients (21.1 %) discontinued the study due to AEs (3 patients; an elevation of liver aminotransferases and 1 patient; worsening of right heart failure). No patients died during the study period.

Discussion

Since the NIH registry conducted in the mid 1980s, the prognosis for patients with PAH has indisputably improved along with the introduction of targeted therapy. Effects of modern management are unsatisfactory and PAH still remains as a progressive and fatal disease with an estimated survival at 1, 2, and 3 years of 85.7, 69.6, and 54.9 %, respectively [4]. The most commonly reported predictors of survival are shown to be 6 MWD, WHO FC, and pulmonary hemodynamics [5].

Since the dual endothelin receptor antagonist bosentan was developed as the first oral therapy for PAH, it has been shown that this agent is effective at improving the exercise capacity, right ventricular function, functional capacity and clinical outcome of patients suffering from this devastating disease. However, clinical studies performed so far have mostly enrolled patients in WHO FC III and IV.

Bosentan was approved for treatment of PAH in Japan in 2005 based on our first clinical trial in which patients were eligible if they had PAH in WHO FC III or IV [6]. This study demonstrated that 12 weeks of treatment with bosentan at a dose of 125 mg twice daily resulted in significant improvement in symptoms as measured by Borg dyspnea index, exercise capacity as assessed by 6 MWD and hemodynamic parameters together with an improvement in the SAS which was used to quantitatively expresses exercise capacity in terms of energy cost of physical activities and known to linearly correlate with peak oxygen consumption. Ever since, bosentan has been approved only for WHO FC III and IV PAH patients in Japan.

Hachulla and coworkers [7] reported that 3-year survival of PAH patients in WHO FC II was 80 % and significantly better than those in WHO FC III (72 %) and IV (30 %). Benza and coworkers [5] also analyzed the surrogate markers predictive of long-term survival using data from a total of 811 patients with WHO FC II to IV PAH in a large cohort study. In this study, symptom severity, as assessed by functional class, was shown to be a strong prognostic indicator of survival. In this study, it was demonstrated that patients in WHO FC II had a lower risk of death, with a 3-year survival rate of 80 %, compared with patients in WHO FC III and IV, who had survival rates of 72 and 53 %, respectively. McLaughlin and coworkers assessed the long-term effects of epoprostenol on survival in 162 consecutive patients with IPAH and reported the observed 3-year survival rate to be 89 % for patients who were in WHO FC I or II, 62 % for those in WHO FC III and 0 % for those in WHO FC IV [8]. The French Network on Pulmonary Hypertension registry prospectively enrolled 354 patients with PAH during 1 year from October 2002 and followed up for 3 years to analyze survival rate. Thereby, PAH patients in WHO FC I or II were shown to have significantly better long-term survival rates compared with those in WHO FC III or IV [4]. In China, 72 patients with IPAH and FPAH were enrolled from 1999 to 2004 and followed up for a mean duration of 40 months [9]. In this study, the survival rate was 39 % at 3 years but there was significant difference between WHO FC I/II and WHO FC III/IV.

Despite a growing body of evidence of better survival rates of WHO FC I or II patients than those in WHO FC III or IV, patient with PAH is commonly recognized at an advanced stage when irreversible pathologic changes associated with the substantial delay between symptom onset and diagnosis may have occurred. In the Registry to Evaluate Early and Long-term Pulmonary Arterial Hypertension Disease Management (REVEAL) which enrolled nearly 3,000 patients, 20 % of patients complained of symptoms for more than 2 years prior to the diagnosis [10]. The median time from onset of the symptoms until the diagnosis of PAH was 1.3 years in the NIH registry [11], and 1.1 years in the REVEAL Registry [12]. In the Japanese registry study conducted from 2004 to 2009, 88 % of the 65 consecutive IPAH patients enrolled were diagnosed as WHO FC III and 12 % were diagnosed as WHO FC I or II at baseline [13]. Since PAH is a rapidly progressing disease even in the less advanced stages, current guidelines recommend early diagnosis and early initiation of therapeutic intervention to maximize the opportunities to improve survival [14].

Nevertheless, PAH is rapidly progressive and even patients in WHO FC II at diagnosis will deteriorate to WHO FC III or IV if left untreated, mildly symptomatic PAH can progressively deteriorate both clinically and in terms of hemodynamics, despite the maintenance of exercise capacity. The fact that WHO FC II deteriorated to WHO FC III or IV within 5-year period [7] suggests need for more aggressive management during the early stage. Hoeper and coworkers demonstrated that IPAH is now frequently diagnosed in elderly patients who are mostly in WHO FC I or II and have better exercise capacity and less likely to meet current treatment targets [15]. Nevertheless, they respond less well to medical therapy and have a higher age-adjusted mortality as compared to younger patients. Therefore, early initiation of treatment is also inevitable in these patient groups.

The primary analysis of EARLY study demonstrated that deterioration was still seen in the hemodynamic and clinical variables in the placebo-treated patients after 6 months. At 6 months, geometric mean PVR was 83.2 % of the baseline value in the bosentan group and 107.5 % of the baseline value in the placebo group, and the treatment effect was statistically significant in favor of bosentan. Mean 6 MWD increased from baseline in the bosentan

group and decreased in the placebo group with a mean treatment effect of 19.1 m though the treatment effect on the change from baseline was not statistically significant at month 6. Though the sample size was limited in the present study, we could also demonstrate definitively that PVR was reduced together with an increase in cardiac index at 3 months. Moreover, 6MWD significantly increased from baseline by 38.2 m at 3 months and 49.5 m at 6 months.

In EARLY study, time to clinical worsening was significantly delayed in the bosentan compared with placebo. Considering the similarity in change in the hemodynamic and 6MWD values in both the EARLY and present study, the prognosis for patients in the present study may be similar to the favorable results seen in EARLY study.

In the present study, the SAS values as a measure of Quality of Life remained were 5.0 METs at baseline and remained the same throughout the study period. This is in contrast to the previous Japanese PAH study involving patients in WHO FC III and IV, in which the SAS value was averaged 2.9 METs at baseline but increased continuously and significantly, reaching 4.6 METs at the final assessment at 3 months. This difference may be related to a mild exercise capacity in WHO FC II patients. Actually, 5 METs are moderately intense in activity, to be strived for at least 30–45 min on most days of the week that includes walking at a very brisk pace (1.6 km in 15 min), doubles tennis, dancing (quite rapidly), or using certain exercise apparatuses [16]. These high physical activities remained unchanged by bosentan for 24 weeks.

The safety profile seen in this study was consistent with that seen in the first Japanese PAH study and the rate of aminotransferase elevations was almost similar, being 38.1 and 31.6 %, respectively. Any elevation of liver enzyme was regarded as adverse event. However, globally only more than 3 times ULN has been considered significant. According to data from the results of Japanese post-marketing surveillance of bosentan, elevation in liver transaminases occurred during the early stages of treatment and the probability of developing lever dysfunction is greatly reduced after 1 year [3].

These observations support the role of an early diagnosis in PAH and early therapeutic intervention to improve long-term outcomes. The REVEAL registry reinforced that many patients suffer from symptoms of PAH prior to the recognition of the disease [12]. The main reason for this delay is the mild and non-specific nature of the symptoms [17]. To implement the early diagnosis, effective screening programs are crucial. In this regard, the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) expert consensus recommended periodic screening of patients at risk with a doppler echocardiographic evaluation of a peak velocity of tricuspid regurgitation [18] or acute pharmacological vasodilator testing to assess the presence of pulmonary vasoreactivity, with final confirmation by RHC [19]. RHC is less likely to be performed at an early stage; however, RHC is the gold standard to establish PAH diagnosis. In a French Nationwide Prospective Multicenter Study, out of 599 patients with SSc, PAH was suspected in 33 patients of whom 18 were confirmed to have PAH based on RHC [20]. It has also been recommended that a focus should be placed on younger, symptomatic patients and those suspected as having obstructive lung disease and sleep apnea when their symptoms are out of proportion to their underlying disease or they are not responding to therapy [10]. The limitations of this study are described below. At first, the patient population was small and study design was non-randomized, non-blinded and open labeled. Nevertheless, results were the same as those of the EARLY study. Second, etiology of PH was different in comparison with the EARLY study. However, differences in the pathogenesis of PH did not make any differences in the results of these two studies. In, conclusion, the present study confirmed the results of the EARLY study in Japanese population. Thus, treatment with bosentan may have similar benefit to Japanese patients with WHO FC II PAH and treatment should be started in the early stage of the disease. Strict control of liver enzymes is warranted.

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Conflict of interest Dr. Sasayama has received consultant fees from Actelion Pharmaceuticals Japan Ltd. Drs. Hatano, Yamada, Fukuda, Kuwana, Nakanishi and Saji have received lecture fees from Actelion Pharmaceuticals Japan Ltd. Other investigators have no conflict of interest.

Ethical standards All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients included in the study.

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Elevated Pentraxin 3 in Systemic Sclerosis: Associations with Vascular Manifestations and Defective Vasculogenesis

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Running head: PTX3 AND SSc VASCULOPATHY

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ABSTRACT

Objective. To clarify the role of pentraxin 3 (PTX3), a multi-functional pattern recognition protein that can suppress fibroblast growth factor-2 (FGF2), in systemic sclerosis (SSc)-related vasculopathy.

Methods. This study assessed 171 SSc patients and 19 age- and sex-matched healthy control subjects. Circulating PTX3 and FGF2 levels were measured by enzyme immunoassay, and $CD34^+CD133^+CD309^+$ endothelial progenitor cells (EPCs) were counted by flow cytometry. Correlations between PTX3 and FGF2 and the presence or future development of vascular manifestations, including digital ulcers (DU) and pulmonary arterial hypertension (PAH), were identified by univariate and multivariate analysis. The effect of PTX3 on EPC differentiation was evaluated in pro-angiogenic cultures of mouse bone marrow cells in combination with colony formation assay.

Results. Circulating PTX3 and FGF2 levels were significantly higher in SSc patients than in healthy control subjects. PTX3 was elevated in SSc patients who had DU or PAH, while FGF2 was reduced in SSc patients with PAH. Multivariate analysis identified elevated PTX3 as an independent parameter associated with the presence of DU and PAH, and PTX3 was a useful predictor of future occurrences of DU. Reduced FGF2 was independently associated with the presence of PAH. EPC counts were significantly lower in patients with DU or PAH, and correlated negatively with circulating PTX3 concentrations. Finally, PTX3 inhibited EPC differentiation *in vitro*.

Conclusion. In SSc patients, exposure to high PTX3 concentrations may suppress EPC-mediated vasculogenesis and promote vascular manifestations such as DU and PAH.

Systemic sclerosis (SSc) is a multi-system disease characterized by microvascular abnormalities and excessive fibrosis [1]. It has been suggested that the pathogenic process of SSc is triggered by endothelial damage and the subsequent activation of immune cells and fibroblasts, resulting in the excessive accumulation of extracellular matrix [2]. Although several soluble mediators, including growth factors, cytokines, chemokines, and pro-angiogenic and anti-angiogenic factors, are known to play critical roles in the pathogenesis of SSc, the mechanisms regulated by the interactions of these mediators are not clearly understood [2].

Pentraxin 3 (PTX3) is a pattern recognition protein belonging to the pentraxin superfamily [3]. C-reactive protein (CRP), a short pentraxin, is primarily produced in the liver in response to interleukin (IL)-6. In contrast, PTX3 is produced locally at the inflammation site by macrophages, dendritic cells, endothelial cells, smooth muscle cells, and fibroblasts [4], and it is induced by Toll-like receptor agonists or pro-inflammatory cytokines such as IL-1β and tumor necrosis factor (TNF)-α, but not IL-6. Recently, several lines of evidence have shown that PTX3 has non-redundant roles in antimicrobial innate immunity, inflammation, extracellular matrix deposition, and neovascularization [4]. Specifically, PTX3 binds to apoptotic cells and selected pathogens, and it activates and modulates the classical complement pathway by binding to C1q. PTX3 is also as a component of the extracellular matrix, and contributes to fibrosis in this role. Finally, PTX3 acts as an anti-angiogenic factor by binding to fibroblast growth factor-2 (FGF2) with high affinity and specificity, and inhibiting FGF2-dependent endothelial cell proliferation and neovascularization [5].

PTX3's pleiotropic effects on inflammation and fibrosis, along with its inhibition of

neovascularization, suggest PTX3 as an intriguing candidate for mediator in the pathogenesis of SSc. In fact, circulating PTX3 levels are elevated in SSc patients [6], and PTX3 is upregulated in endothelial cells and fibroblasts in affected skin [6-8]. In addition, cultured fibroblasts derived from SSc skin constitutively expressed PTX3 in the absence of agonistic stimulation [6, 7]. The silencing of PTX3 gene expression by small interfering RNA restored the impaired ability of cultured SSc microvascular endothelial cells to form capillary-like tubes and promote vascularization [9]. These findings suggest that PTX3, constitutively produced at the affected site, is involved in the pathogenesis of SSc. To test this hypothesis, we examined PTX3's roles in SSc pathogenesis by evaluating potential correlations between SSc manifestations and the circulating levels of PTX3 and FGF2, and by investigating the mechanisms underlying these correlations.