549 patients in France and Italy, while the other involved 897 patients in the United States (US trial[11]). The primary outcome measure in these two trials was walking distance as evaluated by the treadmill test. In addition, vascular events were assessed as a secondary outcome measure.

2. General protocol

Both trials consisted of patients who met the inclusion criteria after a single-blind placebo run-in-phase and who were randomly assigned to receive either beraprost (40 μg t.i.d.) or a placebo (t.i.d.) for six months.

3. Outcome assessments

The present analysis used vascular events as outcome measures. Since the US trial[11] and the BERCI-2 trial[10] were conducted according to similar protocols, these two trials had the same definition of cardiovascular events including : death of cardiovascular origin (confirmed or sudden death), nonfatal myocardial infarction, unstable angina, stroke or transient ischemic attack, critical leg ischemia (rest pain necessitating an urgent medical intervention or a surgical procedure to avoid amputation), subacute critical ischemia (continuous rest pain for>2 weeks requiring analgesics), peripheral angioplasty, peripheral bypass surgery, and amputation at any level.

To avoid any potential bias by the investigator in event evaluation, in the US trial[11], all vascular events were adjudicated by an independent Critical Cardiovascular Events Committee, while, in the BERCI-2 trial[10], every potential vascular event was fully documented and evaluated blindly by three experienced cardiologists.

4. Study patients

Both trials had similar inclusion criteria with the exception of patient age (BERCI-2[10], 35-75 years ; US trial[11], 40-80 years) and concomitant medication (aspirin, clopidogrel, and ticlopidine were allowed in the US trial[11] but not in the BERCI-2 trial[10]).

5. Endpoints

The primary endpoint was defined as all vascular events for this meta-analysis. These events include lower limb deterioration and cardio/cerebrovascular events, which were assessed separately. Lower limb deterioration was regarded as a measure of PAD progression, while cardio/cerebrovascular events were evaluated to focus on ischemic heart disease and ischemic stroke.

6. Statistical analysis

Statistical analysis was performed according to the intention-to-treat population for the primary studies. P-values were computed using the Mantel-Haenszel chi-square test based on a 2×2 contingency table. A fixed effects model was used to estimate the pooled risk ratio based on a 2×2 table and its 95% confidence interval (CI) according to Mantel-Haenszel method. Heterogeneity between the trials was examined using the Cochran's Q-test[12]. A two-sided p-value of less than 0.05 was considered to be statistically significant. Statistical analyses were performed by using Comprehensive Meta-Analysis® software version 1.0.23.

Results

1. Baseline characteristics

At randomization after the run-in-period, the BERCI-2 trial consisted of 209 patients in the beraprost group and 213 patients in the placebo group, while the US trial had 385 and 377 in the beraprost and placebo groups, respectively. Baseline characteristics are shown in Table 1. As compared with the patients in the BERCI-2 trial, those in the US trial had slightly lower ankle-brachial indices (ABIs) and shorter maximum walking distances (MWDs). In addition, they were more likely to have hypertension, diabetes mellitus, or dyslipidemia. In the US trial, 65%, 6.3%, and 0.5% of the patients concomitantly used aspirin, clopidogrel, and ticlopidine, respectively.

2. Incidence of vascular events

Vascular events occurred in 29 patients (6.9%) in the BERCI-2 trial and 71 patients (9.3%) in the US trial. Cardio/cerebrovascular events other than lower limb events were documented in 7 patients (1.7%) in the BERCI-

Table 1 Baseline characteristics by treatment group for US trial and BERCI-2 trial

	US trial		BERCI-2	
	Beraprost	Placebo	Beraprost	Placebo
Number	385	377	209	213
Mean age (yrs)	65.9	65.7	63.3	61.5
Male	79%	74%	85%	84%
Mean duration of claudication (yrs)	6.4	6.6	6.4	5.3
Previous surgical treatment for PAD	23%	24%	28%	26%
Hypretension	73%	75%	41%	43%
Dyslipidemia	70%	71%	43%	46%
Diabetes	29%	29%	18%	18%
Smoking status				
Current smoker	33%	34%	34%	40%
Previous smoker	61%	58%	58%	51%
Non-smoker	6.2%	8.2%	8.6%	9.4%
Mean ABI	0.64	0.65	0.73	0.71
Mean MWD (m)	164	171	275	271
Mean PFWD (m)	85	90	130	134

ABI : ankle-brachial index, MWD : maximum walking distance,
PFWD : pain-free walking distance

2 trial and 45 patients (5.9%) in the US trial. Overall, the incidence was higher in the US trial.

Comparison between beraprost and the placebo revealed that beraprost was associated with a reduced incidence of vascular events in both trials ; events occurred in 10 beraprost-treated patients (4.8%) and 19 placebo-treated patients (8.9%) in the BERCI-2 trial while 28 beraprost-treated patients (7.3%) and 43 placebo-treated patients (11.4%) were reported to have had events in the US trial (Table 2). Both trials showed similar risk reductions for vascular events with 46.4% in the BERCI-2 trial and 36.2% in the US trial. The number needed to treat was also quite similar, 24 for the US trial and 25 for the BERCI-2 trial.

3. Meta-analysis

Figure 2 shows the results of the meta-analysis of the two trials examining vascular events. The pooled risk ratio was 0.608, indicating a significant risk reduction of beraprost on all vascular events (95%CI : 0.41 to 0.90, p=0.012). The pooled risk ratio for lower limb deterioration was 0.598 (95%CI : 0.34 to 1.06, p=0.079) and the pooled risk ratio for cardio/cerebrovascular events was 0.619 (95%CI : 0.36 to 1.09, p=0.085) ; these were statistically insignificant but similar to that for all vascular events. Heterogeneity among the two studies was not found in the risk ratio for any of these endpoints.

Discussion

1. Risk of vascular events in patients with PAD

The incidence of cardio/cerebrovascular events was 1.7% in the BERCI-2 trial[10] and 5.9% in the US trial[11], a finding that highlights an increased risk of cardio/cerebrovascular events in patients with PAD. The incidence of nonfatal cardiovascular events in patients with IC has been reported to be 2-4% annually[13]. The value for BERCI-2 was similar to the previously reported one ; however, the US trial gave a higher incidence only for six months.

In the Cardiovascular Health Study[14], ABIs was closely correlated to the number of patients with myocardial infarction, angina, and congestive heart disease. ABIs, smoking, diabetes, hypertension, white cell count,

Table 2 Summary of the vascular events in intention-to-treat population

	US trial		BERCI-2	
	Beraprost	Placebo	Beraprost	Placebo
Cardio/cerebrovascular event	**18**	**27**	**2**	**5**
Cardiovascular death	1	4		
Myocardial infarction	0	5		
Unstable angina	5	7		
Cardiovascular revascularization	7	7		
Cerebrovascular accident	5	4		
Limb deterioration	**10**	**16**	**8**	**14**
Worsening limb ischemia	6	8		
Limb revascularization	4	8		
Limb amputation	0	0		
Total	28/385 (7.3%)	43/377 (11.4%)	10/209 (4.8%)	19/213 (8.9%)
Risk reduction	36.2%		46.4%	
Number needed to treat	24		25	

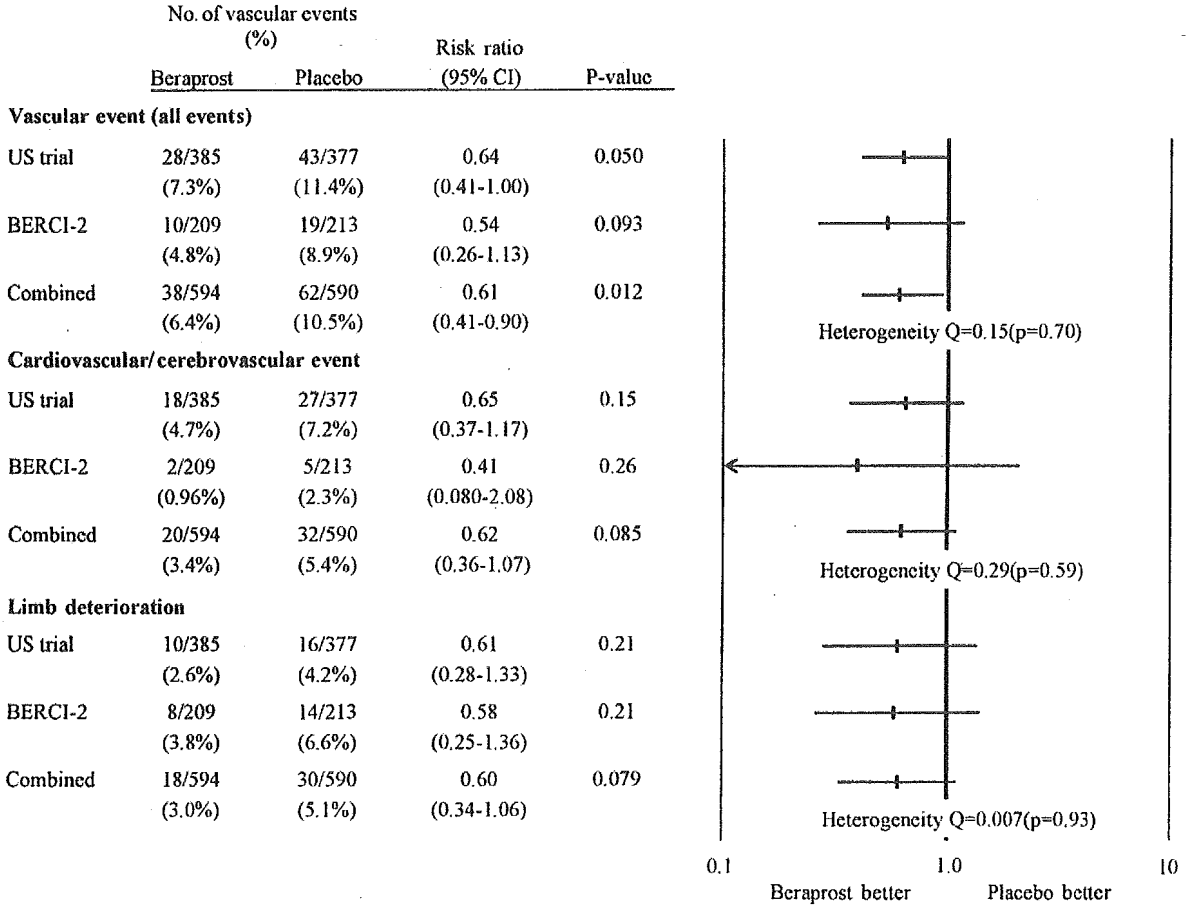

Fig. 2 Meta-analysis of two randomized trials of beraprost sodium therapy for vascular events

asymptomatic carotid disease and fibrinogen have all been reported as predictors of mortality[13].

The US trial included patients with lower ABIs and a high number of patients with diabetes mellitus, hypertension, or dyslipidemia. These factors may have affected the difference between the two trials.

2. Risk reduction of beraprost on cardio/cerebrovascular events and limb deterioraion

In both trials, the total number of vascular events was not statistically significant, but it was relatively low in the beraprost group. In the US trial, there was a significant reduction in the combination of cardiovascular death and myocardial infarction in the beraprost group. As expected, the meta-analysis of two trials has demonstrated the significant risk reduction of beraprost in vascular events. Analyses showed similar risk reductions of 39% ($p=0.012$) for overall events, 40% ($p=0.079$) for lower limb events, and 38% ($p=0.085$) for cardio/cerebrovascular events. Since stratification reduced the number of events and statistical power, these figures failed to reach significant levels. Taken together, the results suggest that beraprost may prevent the progression of arteriosclerosis not only in peripheral arterial disease but also in "systemic arterial disease".

A report by Antithrombotic Trialists' Collaboration[15] described 26 trials of antiplatelet agents in patients with IC due to PAD, estimating a 23% odds reduction for antiplatelet therapy. The present analysis with beraprost also gave a similar result. The goal of treatment in patients with intermittent claudication is to extend walking distance, and the prevention of the progression of lower limb disease is a therapeutic goal of PAD medications. The present meta-analysis showed promising effects of beraprost in preventing the progression of lower limb arteriosclerosis.

3. Relevance of these findings to PAD treatment in Japan

Ojiro and Yamazumi reported an epidemiological study of nursing homes for the elderly in Amami Island, Japan[16]. The three-year survival rate was 66.3% for patients with arteriosclerosis obliterans (ASO) and 74.3% for non-ASO individuals (p=NS). ASO was frequently associated with cardiovascular deaths, with the most common cause of death being acute myocardial infarction ($p<0.05$). As in other countries, ASO is a disease with a poor life prognosis in Japan.

For the life prognosis of patients with ASO, Miyazaki et al. reported a retrospective study of pharmacologic interventions[17]. In patients with ASO receiving various antiplatelet agents after undergoing femoral-peripheral artery bypass graft, a multiple logistic regression analysis including potential prognostic factors revealed that only beraprost significantly improved lifelong prognosis among antiplatelet agents such as aspirin and ticlopidine. This report suggests that beraprost also reduces vascular events in Japanese PAD patients.

These promising effects should be evaluated prospectively in future trials of beraprost with vascular events as the primary outcome. Six months is a widely accepted period for evaluating treadmill walking distance as a primary outcome. However, periods of a year or longer are suggested in such prevention trials to obtain clinical relevance.

4. Methodological limitation

The two selected studies[10,11] have utilized the log-rank test for comparison between groups. They also presented the p-value obtained by a log-rank test. However, they did not show a hazard ratio and have just shown the number of events per total number of patients. Thus, the information obtained from articles was nothing but several 2×2 contingency tables. The present meta-analysis should combine the hazard ratio across the studies since the time to event was a primary outcome. However, their detailed data was not presented in the articles. So that, there was only a way to combine the risk ratios computed by 2×2 contingency tables. The bias caused by using a risk ratio rather than a hazard ratio is considered to be

quite small since the two studies had a common 6-month follow-up and the hazard is considered to be constant during this period.

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Effects of the Endothelin Receptor Antagonist Bosentan on Hemodynamics, Symptoms and Functional Capacity in Japanese Patients With Severe Pulmonary Hypertension

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Background Endothelin (ET)-1 has a pathogenic role in pulmonary arterial hypertension (PAH). Recent clinical studies carried out in Western populations showed that blockade of the ET receptors by bosentan improves pulmonary hemodynamics and exercise capacity. In the present study, the efficacy of bosentan was assessed in Japanese patients with PAH.

Method and Results Because the pharmacokinetics of bosentan and its metabolites are similar in Japanese and Caucasian subjects, the same dose of bosentan, 125 mg twice daily, was administered in the Japanese open-label clinical trial. In 18 patients, mean pulmonary arterial pressure decreased from 52.4±13.8 to 46.8±13.8 mmHg (p=0.003) and cardiac index increased from 2.20±0.74 to 2.61±0.72 $L \cdot min^{-1} \cdot m^{-2}$ (p=0.002). The 6-min walking distance increased from 410±89.5 to 494±86.0 m (p<0.0001). The dyspnea index (Borg scale) decreased from 3.2±2.4 to 2.2±1.7 (p=0.02). The specific activity scale (SAS) gradually increased throughout the study period from 2.9±0.8 to 4.6±1.9 METs (p=0.0005). WHO Class improved in 10 patients.

Conclusion Bosentan was well tolerated and improved the hemodynamics, symptoms, exercise capacity, and quality of life of Japanese patients with PAH. Thus, bosentan can be a valuable therapeutic option in Japanese patients. (*Circ J* 2005; **69:** 131–137)

Key Words: Bosentan; Endothelin receptor antagonist; Pulmonary arterial hypertension; Quality of life

Pulmonary arterial hypertension (PAH) is a rare and debilitating disease, characterized by an increase in pulmonary vascular resistance that ultimately leads to right heart failure and death[1] When a definite cause can not be demonstrated, the condition is termed primary pulmonary hypertension (PPH), which predominantly affects women most commonly in their third decade of life[2] No ethnic predisposition is apparent in the National Institutes of Health registry, and the proportions by ethnic group parallel those in the general population[2] Similar pulmonary vascular lesions are produced by many illnesses such as scleroderma, human immunodeficiency virus infection, liver disease or the use of certain anorectic drugs, and these are now classified as types of PAH[3] A limited number of innovative strategies for the treatment of PAH have been developed over the past decades, but their effectiveness is largely limited by their nonselectivity for the pulmonary vasculature and significant drawbacks have been reported[5]

Recently, it was shown that PAH is associated with increased concentrations of endothelin (ET)-1, a potent vasoconstrictor, in plasma and the lungs[6,7] suggesting that inhibition of ET receptors is a potential therapeutic alternative for this life-threatening disorder. In fact, studies with Caucasian PAH patients have demonstrated significant clinical benefits of bosentan, a dual ET receptor antagonist[8–10] In the present study, the effects of bosentan on cardiopulmonary hemodynamics, symptoms and functional capacity were assessed, as well as the 6-min walk test and the specific activity scale (SAS), in Japanese patients with PAH.

The pharmacokinetics of bosentan are dose-proportional up to a dose of 500 mg and in Caucasians, the absolute bioavailability of bosentan is 50%, being mainly excreted via the bile in the form of metabolites[11,12] However, ethnic differences in the pharmacokinetics of many drugs have been demonstrated[13] Therefore, prior to the start of the clinical trial, the multiple-dose pharmacokinetics of bosentan were compared in Caucasian and Japanese subjects.

Methods

Comparative Study of the Ethnic Differences in the Pharmacokinetics of Bosentan

This part of the study was performed at FOCUS GmbH

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(Neuss, Germany). The protocol was approved by the independent Ethics Committee of the "Aerztekammer Nordrhein" (Düsseldorf, Germany). All subjects gave written informed consent before any screening procedures were performed. Six male and 7 female healthy Caucasians (age 23–49 years) and 6 male and 7 female Japanese subjects (age 21–45 years) were assigned to treatment with 125 mg b.i.d. of bosentan for 7.5 days. Although the pharmacokinetics of bosentan are not influenced by food[11,12] the meals were standardized and Japanese subjects received typical Japanese food and European food was served to the Caucasian subjects throughout the study period.

Blood samples of 4 ml were obtained immediately before drug administration in the morning of days 2–8 and at several time points (every hour for the first 6 h, every 2 h for the subsequent 10 h and finally after 24 h) after drug administration on day 8. Plasma was separated and stored at –20°C pending analysis. The concentration of bosentan and its active metabolite, Ro 48-5033 were determined by a liquid chromatography method with tandem mass spectrometry detection[14] The limit of quantification was 1.0 ng/ml for bosentan and 2.0 ng/l for Ro48-5033.

The pharmacokinetic evaluation for bosentan and Ro48-5033 used model independent methods[15] The peak plasma concentration (C_{max}) and the time to C_{max} (t_{max}) were read directly from the concentration–time data. The area under the plasma concentration–time curve (AUC) was estimated by the linear trapezoidal rule and log-linear regression analysis of the terminal phase. Pharmacokinetic parameters were analyzed descriptively, calculating the geometric mean and 95% confidence intervals or for t_{max}, the median and range.

The study was powered to detect with 90% power a difference of 50% in AUC_τ between the 2 ethnic groups. Differences between Caucasian and Japanese subjects for the bosentan and metabolite pharmacokinetic parameters were explored using the 2-sample t-test on logarithmically transformed C_{max}, AUC_τ, and $t_{1/2}$ values, and the 2-sample Wilcoxon test for t_{max}.

Clinical Study of the Effects of Bosentan in Japanese Patients With PAH

Japanese patients aged over 20 years were eligible for enrollment in the study if they (1) had symptomatic, severe PPH or PAH because of connective-tissue disease (scleroderma or systemic lupus erythematosus (SLE)), (2) were in functional classes III–IV according to the 1998 World Health Organization (WHO) classification despite conventional therapy, (3) met the following hemodynamic criteria within 2 months of enrollment: mean pulmonary arterial pressure (mPAP) >25 mmHg at rest, pulmonary capillary wedge pressure (PCWP) <15 mmHg, and pulmonary vascular resistance (PVR) >240 dyn·s/cm^5, (4) had a baseline 6-min walk test between 150 and 500 m. Patients were excluded if they were pregnant, had hypotension (systolic blood pressure <100 mmHg), hypokalemia or other significant systemic disease. The institutional ethics review committees approved the protocol and written informed consent was obtained from all patients.

At baseline, within 2 months prior to the start of treatment, hemodynamic measurements were performed with a Swan-Ganz catheter while patients were recumbent. Cardiac output (CO) was obtained by the thermodilution method using the mean of 3 measurements. The cardiac index (CI) was derived by normalization of CO with the body surface area (BSA) (CI=CO/BSA). PVR was calculated from the transpulmonary gradient and CO (PVR=[mPAP–PCWP]/CO). The patients' symptoms were evaluated by the Borg dyspnea index (a measure of perceived breathlessness on a scale of 0–10, with higher values indicating more severe dyspnea)[16] and the WHO functional class for pulmonary hypertension. Efficacy of treatment was also assessed by the 6-min walk test and the specific activity scale (SAS)[17] To determine the SAS, patients were asked to specify the extent of physical activity they could perform without symptomatic limitation. Summarizing these data, the patient was categorized by the metabolic costs expended with the most strenuous possible activity.

After the baseline assessments, bosentan (Tracleer, Actelion, Allschwil, Switzerland) was started at a dose of 62.5 mg once daily for the first week, then 62.5 mg twice daily for the next 3 weeks, and finally 125 mg twice daily for the subsequent 8 weeks. Hemodynamic measurements were performed after the 12 weeks of treatment. Symptoms, physical examinations, electrocardiogram, 6-min walk test, WHO classification, and SAS were assessed every 4 weeks. Safety was assessed on the basis of recorded adverse events, clinical laboratory parameters, vital signs, and electrocardiography.

Statistical Analysis

The PVR as the primary efficacy parameter, and other hemodynamic values at week 12 were compared with the baseline on a per protocol population basis by using the signed-ranks test as primary analysis. A significant change was defined as $p<0.05$ (two-tailed). In a patient in whom bosentan treatment was terminated because of worsening of the disease, the hemodynamic data obtained at the last observation were used for analysis. If data were not available, the imputation rule of using the worst data (pre-treatment value in this case) was used. If the data at 12 weeks were not available because of termination of the treatment for other reasons, the last data between 8 and 12 weeks were adopted for analysis. The missing values for other measurements were excluded from the analysis. To confirm the robustness of the results, sensitive analysis based on the ITT (intention to treat) was used.

Results

Comparative Study of the Ethnic Differences in the Pharmacokinetics of Bosentan

Twenty-six subjects participated in the study and 24 completed the entire study in accordance with the protocol. Two subjects prematurely withdrew because of adverse events: myalgia of moderate intensity in 1 female Caucasian and a first-degree atrioventricular block in 1 Japanese female subject. Therefore, 26 subjects were evaluated for safety and 24 for pharmacokinetics.

Steady-state concentrations of bosentan were attained after 5–6 days of administration in both ethnic groups (data not shown). The mean plasma concentration–time curves and pharmacokinetic parameters of bosentan and its metabolite, Ro48-5033, are presented in Fig 1 and Table 1. The 2-sample t-test did not yield any statistically significant differences between the 2 ethnic groups.

Of the 47 adverse events that occurred during the study, 19 were reported by Caucasian and 28 by Japanese subjects. Headache of mild to moderate intensity was the most frequently reported adverse event in both ethnic

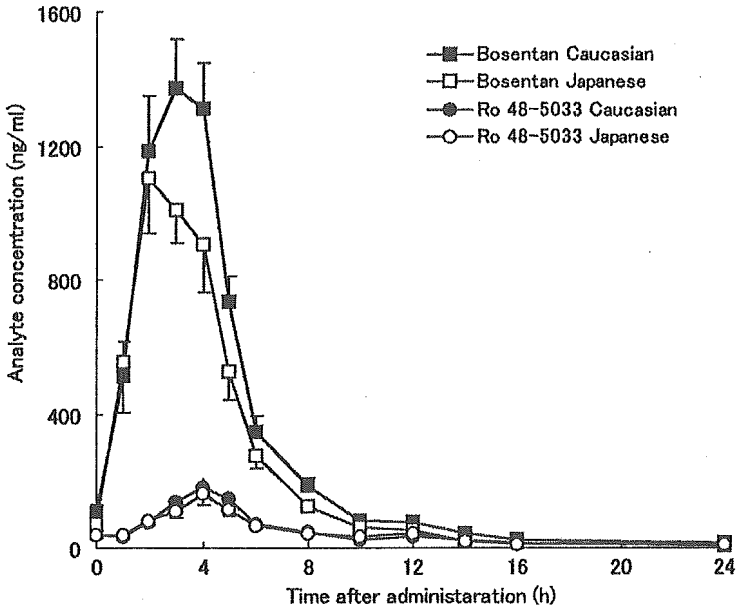

Fig 1. Mean plasma concentration–time curves of bosentan and its metabolite, Ro48-5033 in 12 healthy Caucasian and Japanese subjects after administration of 125 mg of bosentan. Data are presented as arithmetric means±SEM.

Table 1 Pharmacokinetic Parameters of Bosentan and Its Metabolite in Caucasian and Japanese Subjects After Administration of 125 mg of Bosentan

Group	*C_{max} (ng/ml)*	*t_{max} (h)*	*AUC (ng·h/ml)*	*$t_{1/2}$*
Bosentan				
Caucasian	*1,434*	*3.5*	*6,046*	*7*
	(1,137, 1,808)	*(2.0, 4.0)*	*–49,997,311*	*(5.3, 9.3)*
Japanese	*1,212*	*3*	*4,640*	*5.6*
	(940, 1,564)	*(1.0, 4.0)*	*(3,641, 5,914)*	*(4.6, 6.9)*
Ro 48-5033				
Caucasian	*175*	*4*	*859*	*10.6*
	(138, 221)	*(3.0, 5.0)*	*(3,641, 5,914)*	*(4.6, 6.9)*
Japanese	*136*	*4*	*721*	*9.6*
	(92, 201)	*(3.9, 5.0)*	*(532, 977)*	*(7.7, 11.8)*

Data are expressed as geometric mean (95% confidence limits).
AUC, area under curve; C_{max}, peak plasma concentration; t_{max}, time to C_{max}.

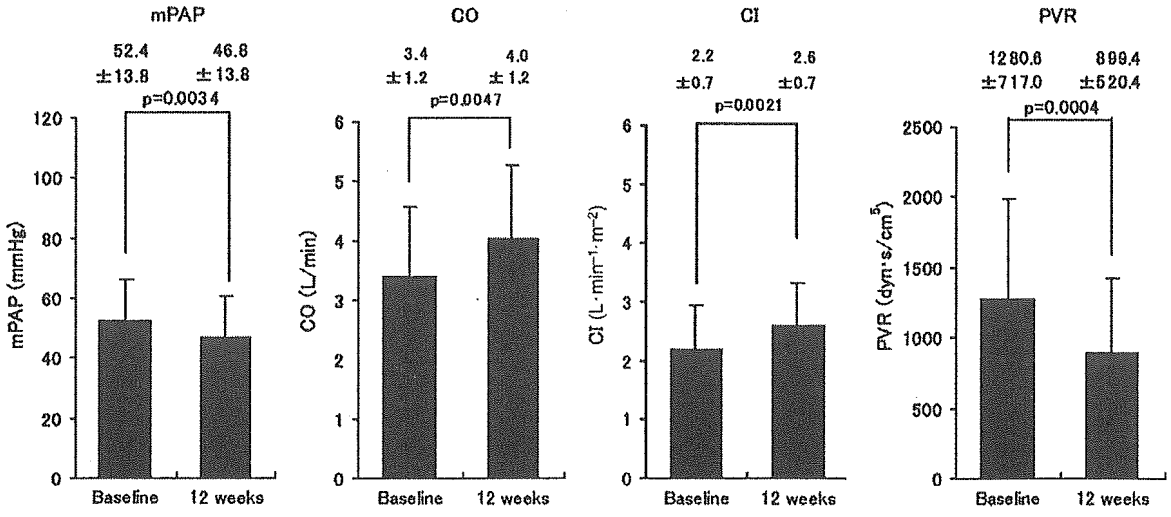

Fig 2. Effect of bosentan on hemodynamic parameters from baseline to week 12 (mean±SEM). mPAP, mean pulmonary arterial pressure; CO, cardiac output; CI, cardiac index; PVR, pulmonary vascular resistance.

Table 2 Changes in the Hemodynamic Parameters After 12-Week Treatment Program With Bosentan 125 mg b.i.d. in 18 Patients With Severe Pulmonary Hypertension

Hemodynamic parameters	*Baseline*	*Week 12*	*p value*	*ITT**
Systolic pulmonary arterial pressure (mmHg)	*85.9±23.6*	*76.7±23.7*	*0.0106*	*0.0074*
Diastolic pulmonary arterial pressure (mmHg)	*33.1±8.6*	*28.3±9.1*	*0.0147*	*0.0182*
Mean pulmonary arterial pressure (mmHg)	*52.4±13.8*	*46.8±13.8*	*0.0034*	*0.0030*
Pulmonary capillary wedge pressure (mmHg)	*6.3±2.7*	*7.8±3.4*	*0.0309*	*0.0297*
Cardiac output (L/min)	*3.39±1.19*	*4.02±1.22*	*0.0047*	*0.0192*
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	*2.20±0.74*	*2.61±0.72*	*0.0021*	*0.0135*
Pulmonary vascular resistance ($dyn \cdot s/cm^5$)	*1,281±717*	*899±520*	*0.0004*	*0.0003*
Right arterial pressure (mmHg)	*4.9±4.0*	*5.4±3.7*	*0.3134*	*0.3510*

**Sensitive analysis by intension-to-treat.*

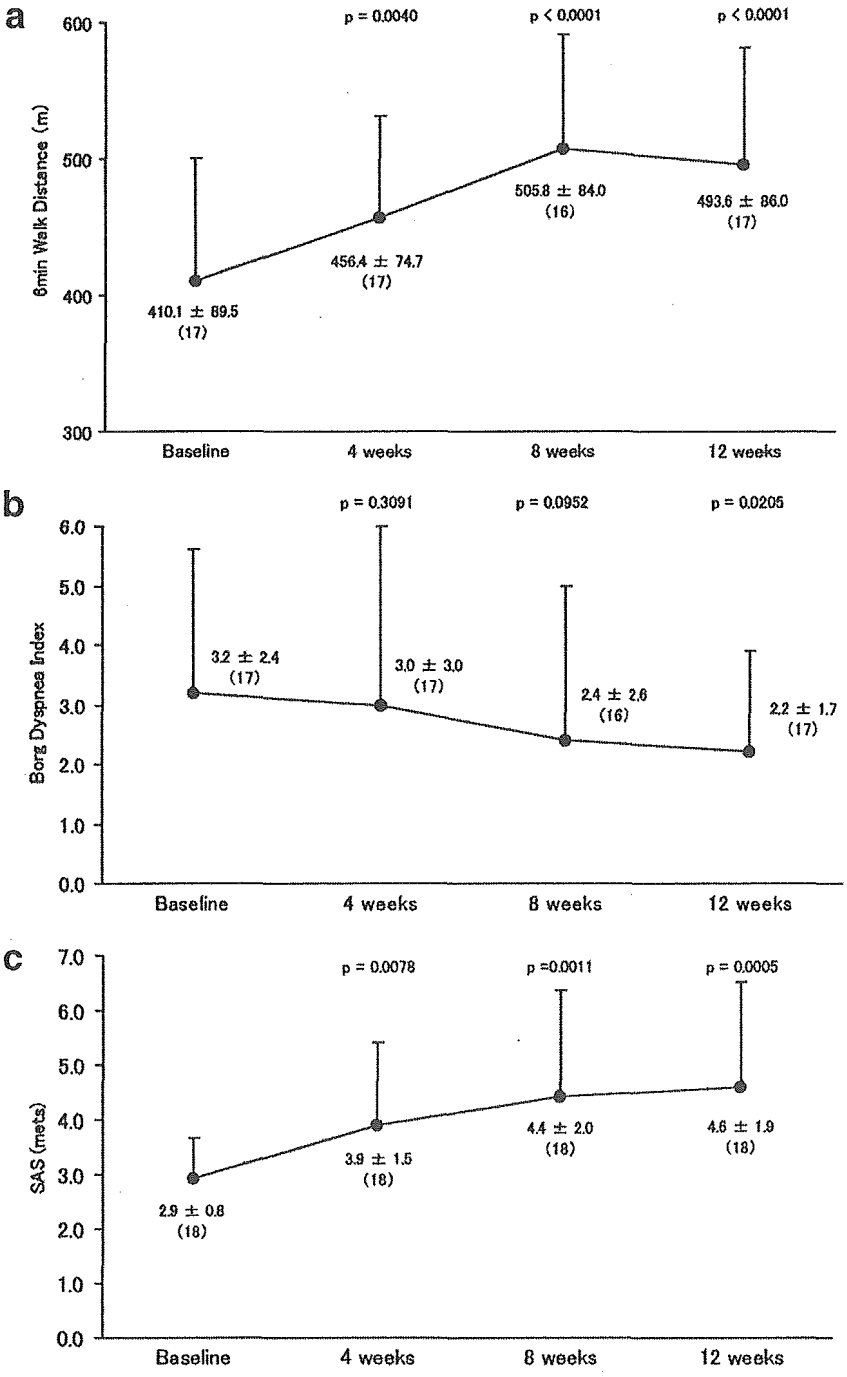

Fig 3. (a) Change in the 6-min walking distance from baseline to week 12. (b) Change in the Borg dyspnea scale from baseline to week 12. (c) Change in the specific activity scale (SAS) from baseline to week 12. Data are expressed as mean± SEM and numbers in parentheses indicate the number of patients assessed.

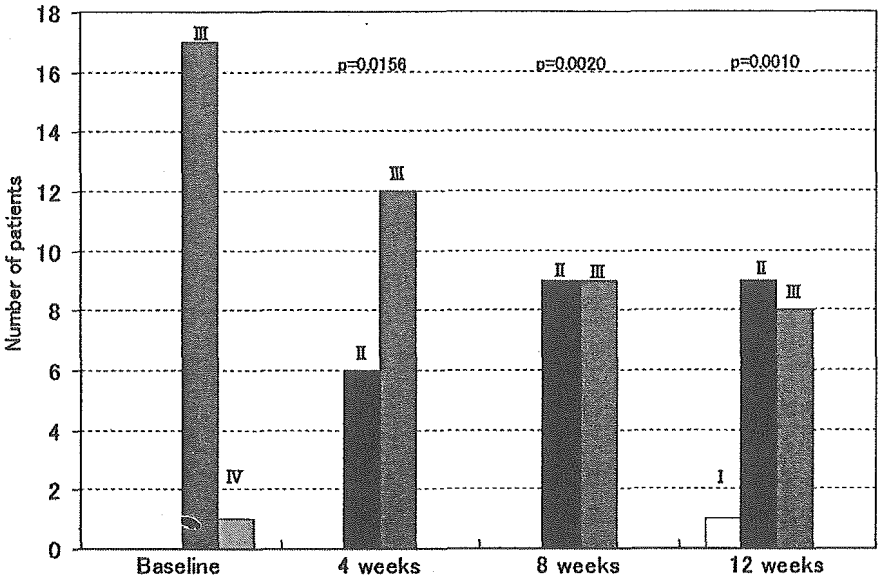

Fig4. Change in World Health Organization (WHO) functional class from baseline to week 12.

groups. Following administration of bosentan, there were slight and transient decreases in systolic and diastolic blood pressure (5–7 mmHg) but these changes did not appear to be clinically significant. At the end-of-study examination, 4 of 13 Japanese subjects and 2 of 13 Caucasian subjects had alanine aminotransferase (ALAT) values above the upper limit of normal, defined as 23 and 19 U/L in male and female subjects, respectively. The absolute values of ALAT did not exceed 67 U/L in any subject and the increases were not considered clinically significant.

Clinical Study of the Effects of Bosentan in Japanese Patients With PAH

Twenty-one patients were recruited from 11 centers. One patient was excluded from the analysis of efficacy because hemodynamic data at week 12 were not available and another 2 patients were excluded because of technical problems that precluded an accurate measurement of hemodynamic parameters. Therefore, 18 patients (2 males, 16 females), 13 with PPH and 5 with PAH (4 secondary to SLE and 1 secondary to mixed connective tissue disease) were finally included in the analysis of efficacy and 21 were assessed for safety. The mean age was 36±10 years (range, 21–60 years).

After 12 weeks of treatment with bosentan, PVR decreased from 1,281±717 to 899±520 dyn·s/cm^5 ($p<0.0004$). Improvements in other hemodynamic parameters were also observed; for example, mPAP reduced from 52.4±13.8 to 46.8±13.8 mmHg ($p<0.0034$), and CI increased from 2.20±0.74 to 2.61±0.72 L·min^{-1}·m^{-2} ($p<0.0021$) (Fig 2, Table 2). Systolic blood pressure was reduced from 113.0±13.3 to 106.6±9.7 mmHg and diastolic blood pressure from 72.7±11.6 to 66.2±5.2 mmHg, but neither of these changes reached statistical significance. No cases of clinically significant hypotension were observed during the study.

After 12 weeks of treatment, the distance walked in 6 min increased by 83.5±64.1 m ($p<0.0001$) (Fig 3a) and the changes in the Borg dyspnea index paralleled the improvements observed in the 6-min walk test; that is, the index decreased gradually from 3.2±2.4 to 2.2±1.7 throughout the study period, but the changes reached statistical significance only at week 12 ($p=0.0205$) (Fig 3b). The SAS values averaged 2.9±0.8 METs at baseline and increased continuously and significantly, reaching 4.6±1.9 METs at the final assessment ($p=0.0005$) (Fig 3–3). At the beginning of the study, 17 patients were in WHO Class III and 1 in Class IV, but by the end of the study 10 patients had improved to Class I or II ($p=0.0010$) (Fig 4), leaving 8 patients in Class III.

Bosentan, at a dose of 125 mg twice daily, was well tolerated. Adverse drug reactions (excluding unrelated) were observed in 14 of 21 patients (66.7%), including headache (38.1%), dizziness (19.0%), and myalgia (14.3%). Abnormal values of laboratory tests were noted in 10 patients. Bosentan treatment was associated with an increase in aspartate aminotransferase and ALAT (38.1%), an increase in bilirubin (14.3%), a decrease in hemoglobin (14.3%) and a decrease in leukocytes (14.3%). Of 8 patients who had increases in liver aminotransferase concentrations, 3 had concentrations more than 3-fold the upper limit of normal, necessitating discontinuation of the study medication in 1 case. In the other 2 cases, the aminotransferase concentrations returned to normal without discontinuation of treatment, continuing either at the same dose or at a reduced dose of 62.5 mg twice daily. In the other 5 cases, aminotransferase concentrations did not increase more than twice the upper limit of the normal range and returned to the normal range by the end of the study in 4 cases without dose adaptation.

Discussion

Pulmonary arterial hypertension is rapidly progressive, leading to right heart failure and death in a median of 2.8 years from diagnosis. For the majority of cases, the treatments so far developed have been only palliative and the limited oral treatment options include long-term anticoagulant therapy and therapy with calcium-channel blockers, prostacyclin analogues, or phosphodiesterase inhibitors[19,20] The introduction of intravenous epoprostenol in 1990s greatly improved survival, but this treatment is expensive, the dosage required to sustain these effects increases with time, adverse effects are frequent because of pump malfunction, catheter-related infections and thrombosis, or the

drug induces significant side effects. The efficacy of epoprostenol analogues that can be inhaled (iloprost) or administered orally (beraprost) remains to be confirmed.[21]

It has been recently suggested that local production of ET-1 plays a pathogenic role in PAH, as evidenced by its high plasma concentrations in patients with PPH or PAH,[7,22] the increased expression of ET-1 in the lungs of patients with pulmonary hypertension[6] or idiopathic pulmonary fibrosis.[23] Endothelin-1 has 2 receptors, A and B. Activation of ETA receptors produces vasoconstriction and smooth muscle growth, whereas activation of ETB receptors induces nitric oxide production and vasodilation. Therefore, development of an ET-receptor blocker specific for ETA appears to be desirable. On the other hand, because the ETB receptor mediates release of aldosterone from the adrenal cortex,[24] nonselective blockade of both ETA and ETB receptors may have additional benefits by inhibiting collagen synthesis. Bosentan is an orally effective, nonselective antagonist of ETA and ETB receptors and recent clinical trials have documented promising results in patients with severe pulmonary hypertension[9,10,18] although its effects are yet to be well characterized in Japanese subjects.

Numerous clinical studies have shown that ethnic groups may differ in their responsiveness to drugs,[25–27] and it has also been suggested that racial differences in the catalytic activity of cytochrome P450 (CYP) isozyme may be responsible for the differences in drug kinetics.[28] The International Conference on Harmonization guideline (ICHE5) document "Ethnic Factors in the Acceptability of Foreign Data" recommends the measurement of pharmacokinetic/pharmacodynamic parameters to permit the clinical effects obtained in one population to be extrapolated to a different population.[13] Ethnic differences in the drug pharmacokinetics depend on gut metabolism/transport and most commonly on hepatic first pass metabolism, but the ethnic differences in hepatic metabolism are known to be unpredictable by race and specific enzyme.[13] The present study showed that the pharmacokinetics of bosentan at the dose of 125 mg are similar in Caucasian and Japanese subjects. Bosentan is metabolized by CYP2C9 and CYP3A4 to 3 metabolites and excreted in bile.[29] A study that used healthy volunteers from broadly defined ethnic groups to assess the adenine to guanine transition in the 5' promoter region of the CYP3A4 gene in a sequence motif known as the nifedipine-specific element, indicated considerable racial differences in the frequency of this polymorphism between Caucasian and Japanese subjects, but there was no ethnic difference in the rate of CYP3A4-dependent drug metabolism and this promoter region polymorphism was considered not to play a major role in determining constitutive CYP3A4 expression.[30] When differences in CYP3A activity between Caucasian and Japanese subjects were assessed using midazolam as an in vivo probe, no statistically significant or clinically important interracial/ethnic difference was observed.[31] Therefore, we assumed that no dose adjustment was necessary when bosentan was used to treat Japanese patients and conducted the first open-label clinical trial of bosentan at the same dose as used in the previous studies carried out in Western populations.

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 the 6-min walk test and the SAS, together with an improvement in hemodynamic parameters. The changes in the 6-min walking distance and Borg dyspnea index indicated that patients were able to walk further with less dyspnea; however, the standard deviation of both parameters was greater than the absolute differences from the baseline values to those at the conclusion of the study at 12 weeks, leading to difficulty in interpreting the efficacy of the treatment.[32]

Because patients with cardiopulmonary disorders are usually more symptomatic during exertion, the most direct approach to an evaluation of functional capacity is to inquire about symptoms at rest and during exertion. The majority of exercise tests are designed to evaluate exercise performance at maximal workloads, but daily activities do not generally require energy expenditure in the maximal range. The SAS that we used in the present study quantitatively expresses exercise capacity in terms of energy cost of physical activities and this scale has been shown to linearly correlate with peak oxygen consumption. The reproducibility of measurement was substantial with a mean difference of 0.4±0.5 METs in interobserver variability,[17] prompting us to consider changes greater than 1 MET as clinically relevant. In the present study, SAS increased continuously and significantly throughout the study period, the mean change of 1.7±1.4 indicating a significant treatment effect in favor of bosentan.

In the placebo-controlled studies reported in the literature, treatment with 125 mg of bosentan twice daily was not associated with significant adverse events when compared with placebo.[9,10] However, increased doses led to a frequent elevation of aminotransferase concentrations in accord with the known incidence of abnormal hepatic function.[10] In the present study, 3 patients had increases in aminotransferase with bosentan at a dose of 125 mg twice daily and another 4 patients and 1 patients had increases at doses of 62.5 mg twice daily and 62.5 mg once daily, respectively. In those cases, the abnormal hepatic function progressively improved during bosentan therapy continued at either the same dose or at a reduced dose, except for one case in whom drug withdrawal was necessary. Liver injury induced by bosentan and its metabolites is thought to be mediated through inhibition of the canalicular bile salt export pump (BSEP), as evidenced by a dose-dependent increase in serum bile salts and alkaline phosphatase concentrations in a significant percentage of bosentan-treated patients, the increased cholestatic potency of bosentan with concomitant administration of a known BSEP inhibitor, the reproduction of similar effects in the experimental setting, or in vitro observation of inhibition of BSEP-mediated taurocholate transport by bosentan and metabolites.[33] Recently, it has also been reported that individual differences in susceptibility to the development of intrahepatic cholestasis observed during pregnancy are related to genetic variability in the gene encoding the BSEP.[34] Therefore, if detection of the responsible BSEP and other transporter polymorphisms becomes possible in future, individual susceptibility to drug-induced hepatotoxicity may be predicted.

In conclusion, there are no ethnic differences in the pharmacokinetics of bosentan, and dose adjustment is not necessary for Japanese patients. Japanese patients with severe pulmonary hypertension showed a significant improvement in cardiopulmonary hemodynamics, symptoms, and functional capacity over a 12-week treatment regimen of bosentan 125 mg twice daily. Aminotransferase concentrations were elevated in some cases but mostly returned to normal without discontinuation of therapy. Therefore, bosentan 125 mg twice daily is considered the clinically

preferable dose and is a valuable treatment option for Japanese patients with pulmonary hypertension, though close monitoring of liver function is necessary.

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Appendix 1

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Electrocardiographic events and cholesterol reduction with pravastatin in patients with hypercholesterolemia: The Hokuriku Lipid Coronary Heart Disease Study-Pravastatin Atherosclerosis Trial

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Abstract

Background: Cholesterol lowering therapy may offset the development of coronary atherosclerosis, and the resulting reduction in coronary ischemia may be observed in the electrocardiogram (ECG). *Methods:* A total of 2039 Japanese adults with hypercholesterolemia were divided into two groups (receiving 10–20 mg pravastatin daily or a normal diet) and were followed up for 5 years. ECG studies were performed at entry and every year during the follow-up period. The occurrence of myocardial infarction and the appearance or worsening of ischemic ST changes were assessed in terms of effects on the ECG. *Results:* Of the 2039 patients registered, 827 were excluded from the study for various reasons. Consequently, a total of 1212 patients were analyzed. There was a lower degree of worsening in the pravastatin group ($n=757$) than in the normal diet group ($n=455$) in the primary prevention cohort [11 (1.8%) vs. 16 (4.3%), respectively, $P=0.031$]. On the other hand, there was no difference in the frequency of worsening between the two groups in the secondary prevention cohort [7 (4.4%) in the pravastatin group vs. 4 (4.9%) in the diet group, $P=0.25$]. Event-free survival was better in the pravastatin group than in the normal diet group in the primary prevention cohort ($P=0.011$), but there was no difference between the two groups in the secondary prevention cohort. *Conclusions:* These results suggest that pravastatin may reduce the incidence of coronary heart disease and that this effect may be predominantly observed in patients with early atheromatous lesions.

Keywords: Hypercholesterolemia; Cholesterol reduction; Pravastatin; Coronary artery disease

1. Introduction

Hypercholesterolemia is an independent risk factor for coronary heart disease and death [1]. For this serious condition, the usefulness of cholesterol reduction therapy, especially using statins, has been reported in Western countries [2–7] and Japan [8–12]. End points of these many reports were coronary events, such as the occurrence of acute myocardial infarction, angina pectoris, or sudden cardiac death. However, development of coronary atherosclerosis may initially appear as changes in the electrocardiogram (ECG) without other symptoms. In addition, angina pectoris may be misdiagnosed when it is predominantly based on the patient's symptoms. In such cases, there are very few reports of patient evaluation based on objective data, such as ECG changes [8,13,14].

The Hokuriku Lipid Coronary Heart Disease Study-Pravastatin Atherosclerosis Trial (HOLICOS-PAT) [15] is a prospective cohort study in Japan investigating the relationship between lipid-lowering effects of pravastatin and the incidence of coronary heart disease. In the present study, we examined the ability of pravastatin to prevent coronary heart disease based on ECG evidence.

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2. Materials and methods

2.1. Study design

The design of the HOLICOS-PAT has been described previously [15]. In brief, this study enrolled 2232 patients, aged 40–70 years, with a serum total cholesterol concentration ≥220 mg/dl. If the patients had been already treated with an antilipidemic agent, they were screened for eligibility after the drug had been withdrawn for at least 4 weeks. Exclusion criteria included familial hypercholesterolemia, secondary hypercholesterolemia, severe liver disease, and severe renal disease. A total of 193 patients were excluded from the study for reasons such as a breach of inclusion criteria, and consequently 2039 patients were analyzed in the HOLICOS-PAT. Patients were classified into two groups, not randomly but based on the decision of the personal physician; one group received pravastatin (10–20 mg/day) and the other was followed with a normal diet. Patients were followed up for 5 years and lipid concentrations, coronary heart disease-related events, other adverse events, and ECGs were monitored. Patients without any history of angina pectoris, previous myocardial infarction, or coronary intervention at entry were assigned to the primary prevention cohort. Those with some of these histories were assigned to the secondary prevention cohort.

2.2. Electrocardiographic study

Exercise ECG studies were performed at entry and each year during the follow-up period. The form of exercise was Master's two-step test. In addition, the treadmill test, ergometer test, and Master's one-step test were also employed, depending on the patient's condition. Exercise methods during the follow-up period were the

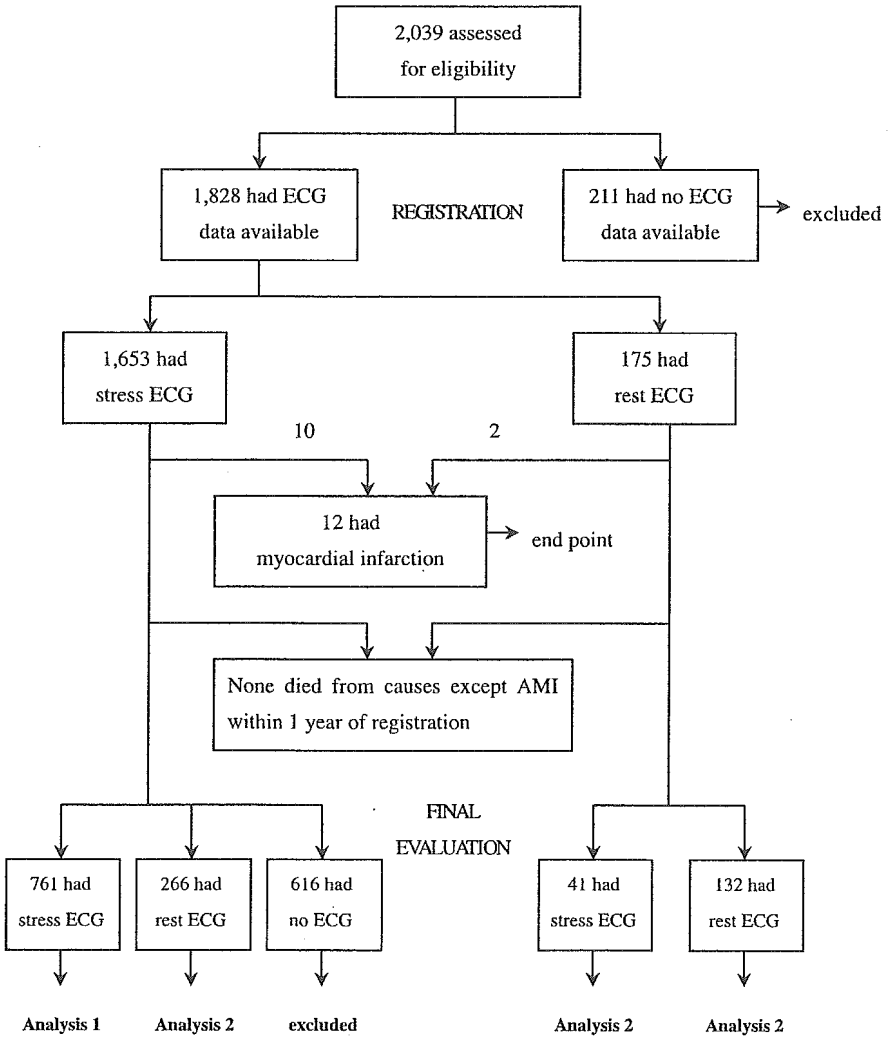

Fig. 1. Trial profile. Analyses were performed using stress (Analysis 1) or rest (Analysis 2) electrocardiograms recorded at registration and final evaluation.

same as those at entry. Patients not performing the ECG at entry and/or during follow-up were excluded from the study. If patients could not undergo exercise for a particular reason or if the methods of exercise at entry and follow-up were different, the analysis was performed using rest ECGs at entry and follow-up (Fig. 1).

2.3. Evaluation and diagnostic criteria

In this study, (i) the occurrence of myocardial infarction and (ii) the appearance or worsening of ischemic ST changes were assessed as ECG events. All ECGs, except those relating to acute myocardial infarction, were reviewed and judged by cardiology experts (Subcommittee for ECG Judgment) who were unaware of the patient's clinical data, including the groups to which they had been assigned. Acute myocardial infarction ECGs were identified by expert cardiologists and/or the personal physicians. The ECG criteria for acute myocardial infarction were ST-segment elevation, or depression of more than 0.1 mV, with elevation of creatine kinase (CK) more than twice the upper limit of the normal range, elevation of CK–MB isoenzyme more than 3% of the total CK, and/or coronary artery occlusion on coronary arteriograms. The appearance of Q-waves >0.04 s in duration and/or 1/4 of the ensuring R-wave in depth were also judged to indicate the presence of myocardial infarction. Myocardial ischemia was diagnosed as follows: (i) ST-segment depression >0.1 mV at 0.08 s after the J-point, (ii) negative U-wave in precordial leads after exercise. If ST-segment depression existed in the ECG at rest, then ST-segment depression on effort >0.1 mV more than in the ECG at rest was judged positive for myocardial ischemia. When resting ECGs at entry and during the follow-up period were compared, ST-segment depression >0.1 mV more than in the ECG at entry was also judged positive for myocardial ischemia. If the patient underwent coronary angioplasty, coronary bypass graft intervention, or non-cardiac death, the last ECG recorded before these events was defined as the final ECG.

2.4. Statistical analysis

Values are expressed as the mean ± S.D. Differences between groups were analyzed by the Wilcoxon rank sum test. Categorical data were compared using the chi-square test. The relative risk of administering pravastatin and 95% confidence interval (CI) were obtained using the Cox proportional hazards model. Kaplan–Meier product-limit survival curves were constructed using the information of the occurrence of ECG events as time variables. Event-free survival curves for both groups were compared using the log-rank test. Statistical analyses were performed using SAS version 8.2 (SAS Institute, Carry, NC). A two-tailed P value <0.05 was considered statistically significant.

3. Results

Of 2039 patients, a total of 827 patients were excluded from the present study; 211 patients had no ECG recording at entry and 616 had no ECG recording at the end of the study (Fig. 1). Consequently, 1212 patients were analyzed, and 973 of these were eligible for inclusion in the primary prevention cohort, and the remaining 239 patients were eligible for the secondary prevention cohort.

3.1. Baseline characteristics of patients

Baseline characteristics of patients in both groups are summarized in Table 1. The proportion of men was significantly lower, and the mean age at entry was significantly higher in the pravastatin group than in the normal diet group. The proportion of patients who were male, diabetic and who were smokers was significantly lower in the pravastatin group than in the normal diet group.

3.2. Changes in lipid levels

Changes in lipid levels of patients in both groups are summarized in Table 2. Total cholesterol and low-density lipoprotein (LDL)-cholesterol levels at entry were significantly higher in the pravastatin group than in the normal diet group. After treatment, levels of both of these lipids were significantly decreased in both groups. Furthermore, at the end of the study, these levels were significantly lower in the pravastatin group than in the normal diet group. High-density lipoprotein (HDL)-cholesterol levels in both groups were increased after treatment, but there was no significant difference in levels between groups at the end of the study.

3.3. ECG changes

Schematic presentation of ECG changes is shown in Fig. 2. Of the 1212 patients, 38 were judged to have worsened. Worsening on ECG was observed in 20 patients (4.4%) in the normal diet group compared to 18 patients (2.4%) in the

Table 1
Baseline characteristics of study patients

	Pravastatin ($n=757$)	Diet ($n=455$)	P-value
Male	250 (33.0%)	198 (43.5%)	<0.001
Age (year)	58.8 ± 8.2	55.9 ± 9.0	<0.001
Angina pectoris	122 (16.1%)	63 (13.9%)	0.29
Previous MI	42 (5.6%)	15 (3.3%)	0.073
Hypertension	312 (41.2%)	182 (40.0%)	0.68
Diabetes mellitus	100 (13.2%)	96 (21.1%)	<0.001
Smoking	168 (22.2%)	126 (27.7%)	0.031
SBP (mm Hg)	135 ± 20	133 ± 21	0.095
DBP (mm Hg)	81 ± 12	79 ± 13	0.003
HR (bpm)	67.5 ± 12.1	66.4 ± 10.9	0.19

MI, myocardial infarction; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

Table 2
Changes in total cholesterol, triglyceride, LDL-cholesterol, and HDL-cholesterol at entry and end of study in the two groups

	Pravastatin ($n=757$)	Diet ($n=455$)	P-value
Cholesterol (mg/dl)			
Entry	259 ± 28	236 ± 23	<0.001
End	217 ± 32*	232 ± 31*	<0.001
Change	−42 ± 35	−4 ± 34	<0.001
Triglyceride (mg/dl)			
Entry	163 ± 101	137 ± 68	<0.001
End	149 ± 88*	148 ± 100	0.69
Change	−15 ± 96	11 ± 83	<0.001
LDL (mg/dl)			
Entry	177 ± 29	158 ± 25	<0.001
End	133 ± 26*	150 ± 23*	<0.001
Change	−44 ± 28	−8 ± 25	<0.001
HDL (mg/dl)			
Entry	52 ± 14	51 ± 14	0.57
End	57 ± 16*	55 ± 16*	0.071
Change	5 ± 12	4 ± 12	0.057

LDL, low-density lipoprotein; HDL, high-density lipoprotein.
* $P<0.01$ vs. entry.

Table 3
Relative risk of ECG events during 5-year follow-up

	No. of events	Age-adjusted incidence rate[a]	Relative risk (95% CI)[b]	P-value
All				
Diet group ($n=455$)	20	11.2	1.000	
Pravastatin group ($n=757$)	18	5.4	0.610 (0.318–1.177)	0.14
Primary prevention cohort				
Diet group ($n=374$)	16	11.2	1.000	
Pravastatin group ($n=599$)	11	4.2	0.418 (0.189–0.922)	0.031
Secondary prevention cohort				
Diet group ($n=81$)	4	11.4	1.000	
Pravastatin group ($n=158$)	7	10.3	2.264 (0.569–9.004)	0.25

CI = confidence interval.
[a] Incidence rate per 1000 person-years. Calculated by the direct method using the person-years by 10-year age class in the whole subjects as standard.
[b] Adjusted for gender, age, diabetes mellitus, smoking, serum HDL cholesterol and diastolic blood pressure.

pravastatin group (Table 3). Relative risk (RR) adjusted for gender, age, diabetes mellitus, smoking, serum HDL cholesterol, and diastolic blood pressure did not differ between the two groups ($P=0.14$). However, adjusted RR was significantly lower in the pravastatin group than in the normal diet group in the primary prevention cohort ($P=0.031$). In contrast, adjusted RR did not differ between the two groups in the secondary prevention cohort ($P=0.25$).

When analysis was limited to patients who had both stress ECGs at entry and at the end of the study ($n=761$, Fig. 1), the pravastatin group had a lower rate of worsening on ECG compared with the normal diet group (6 [1.3%] vs. 13 [4.2%], respectively, adjusted RR = 0.353 [95% CI 0.131–0.957], $P=0.041$). In particular, worsening was found more frequently in the normal diet group in the primary prevention cohort (4 [1.1%] in the pravastatin group vs. 11 [4.3%] in the diet group, adjusted RR = 0.235 [95% CI 0.072–0.771], $P=0.017$), while the rate of worsening was not different between the normal diet and pravastatin

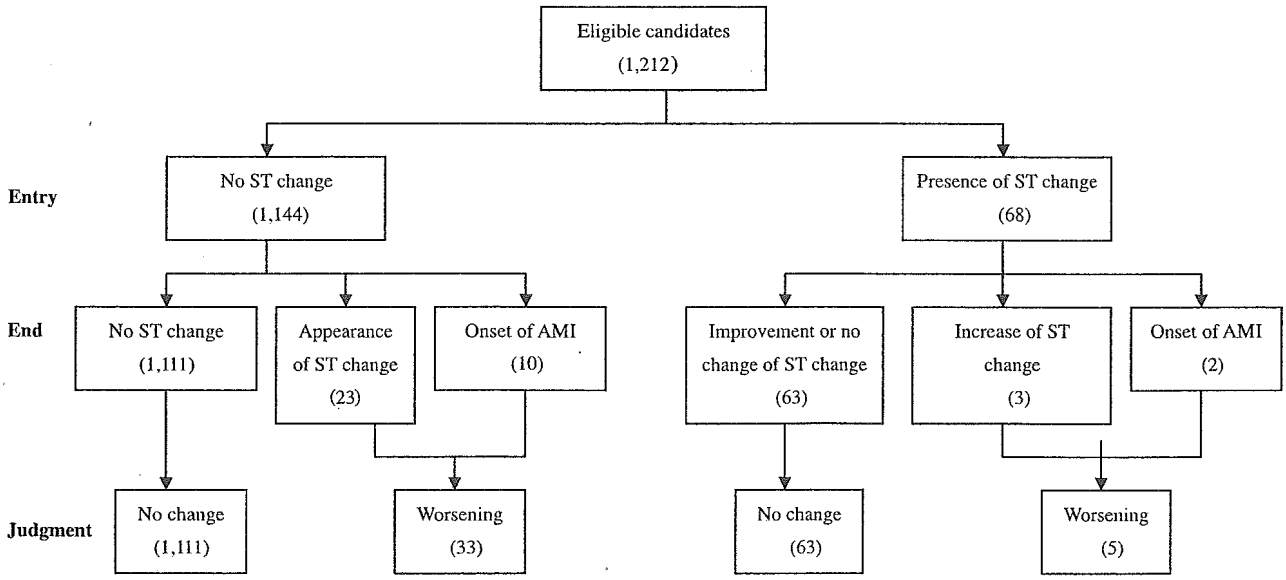

Fig. 2. Judgment profile.

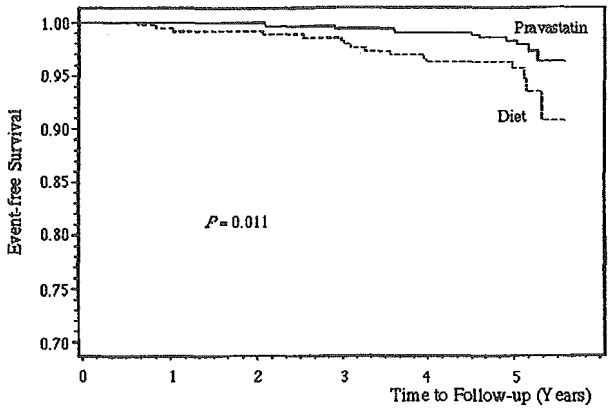

Fig. 3. Event-free survival curves in the primary prevention cohort in two groups.

groups in the secondary prevention cohort (2 [3.9%] vs. 2 [2.2%] respectively, $P=0.71$).

3.4. Event-free survival

Event-free survival was better in the pravastatin group than in the normal diet group ($P=0.035$). In particular, event-free survival in the primary prevention cohort was better in the pravastatin group ($P=0.011$), as shown in Fig. 3. In the secondary prevention cohort, event-free survival did not differ between the two groups ($P=0.99$).

4. Discussion

The present study demonstrates that the pravastatin group had less evidence of worsening in terms of ECG changes than the normal diet group. Furthermore, this was especially clear in the primary prevention cohort.

In many previous reports [2–6], coronary heart disease events were evaluated in terms of the occurrence of myocardial infarction and/or sudden cardiac death. However, such an approach does not identify mild coronary artery lesions, nor does it allow intervention early in the development of coronary heart disease. In addition, diagnosis of angina pectoris is difficult in some patients, some of whom may complain of chest pain even in the absence of angina. When the coronary artery is occluded, functional abnormalities, ECG abnormalities, and chest pain occur sequentially [16]. Accordingly, transient myocardial ischemia due to mild-to-moderate coronary atherosclerosis may not cause chest pain (silent myocardial ischemia). For these reasons, we sought to evaluate the effect of cholesterol reduction therapy with pravastatin in protecting against coronary heart disease, using the ECG. This study has provided evidence that an ECG-based method is effective in identifying a role for pravastatin in reducing the incidence of coronary heart disease.

Pravastatin, one of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, potently reduces serum cholesterol levels and inhibits coronary events [3,8,11,12,17]. In the present study, total cholesterol and LDL-cholesterol levels at the end of the study were lower in the pravastatin group than in the normal diet group. In addition to their ability to reduce cholesterol levels, various pleiotropic effects of statins have been reported, such as an improvement of endothelium-dependent coronary vasomotion [18], a reduction of inflammation in atheroma [19–22], worsening of myocardial contractile dysfunction [23], and the stabilization of vulnerable plaques [24]. Beattie et al. [21] reported that in patients treated with statins, exercise-induced ischemia was frequently found in the group with the highest C-reactive protein levels, and that an association between C-reactive protein and ischemia was not found. Although we unfortunately did not measure C-reactive protein levels in the present study, anti-inflammatory effects of pravastatin might also affect the induction of ischemia. Cholesterol reduction therapy may produce a greater effect on the incidence of coronary heart disease than on coronary vessel morphology [25] via such preferable actions of statins.

In the present study, there were differences in the proportion of males, the frequencies of diabetes mellitus and smoking, and diastolic blood pressure between the two groups at baseline. Male gender, diabetes mellitus, smoking, and blood pressure are risk factors for coronary heart disease, and these factors might affect the occurrence of coronary heart disease in the normal diet group. Even when adjustment was made for these factors in statistical analyses, the occurrence of such events was significantly lower in the pravastatin group than in the diet group in the primary prevention cohort. Accordingly, it is hypothesized that cholesterol reduction by pravastatin may be the most important factor responsible for improvement in this case.

In contrast to our results, Sasaki et al. [8] reported that pravastatin did not improve exercise ECG findings. These authors hypothesized that quantitative changes in coronary atheromatous plaques, i.e. changes in the severity of coronary artery stenosis might not be sufficient to improve ECG findings. On the other hand, De Divitiis et al. [13] reported an improvement in myocardial effort ischemia with simvastatin, even though the number of patients in that study was very small. The differences in these observations may have been due to the differences in the degree of coronary atherosclerosis. In the present study, the deterioration rate of ECG findings was lower in the pravastatin group than in the normal diet group. This result was clear in the primary, but not in the secondary prevention cohort. These findings suggest that pravastatin may affect the progression of mild-to-moderate atherosclerosis, more than in moderate-to-severe atherosclerosis. Alternatively, pravastatin may not be able to further improve endothelial function in severe stenotic lesions. It

is also possible, however, that the small numbers in the secondary prevention cohort may cast doubt on this conclusion.

5. Study limitations

This study had several limitations. First, the study was not a randomized trial. If lipid levels in both groups at entry were the same, differences in event ratios and event-free survival in the two groups might have been larger than in the present study. Second, all patients did not undergo an exercise stress study. However, even though analysis was only carried out in patients with stress ECGs, the results were almost the same. Therefore, we hypothesize that inclusion of patients who were evaluated by rest ECGs might not have had a large effect on our results. Third, the secondary prevention cohort comprised relatively small numbers. Further investigations are necessary to establish the validity of the present approach in the secondary prevention cohort.

Acknowledgements

The identities of the collaborators and committees involved in the conduct of the study were previously published [15]. We are grateful to Hiroko Kodama, Sankyo, for statistical analysis of the data. This study was funded Sankyo, Tokyo, Japan.

Appendix A. Subcommittee for ECG judgment

Masami Shimizu, Hiroyuki Yoshio, Kazuyasu Okeie, Masato Yamaguchi, and Toshihiko Yasuda, Kanazawa University.

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Original Articles

Risk Evaluation of Coronary Heart Disease and Cerebrovascular Disease by the Japan Atherosclerosis Society Guidelines 2002 Using the Cohort of the Holicos-PAT Study

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Our purpose in this study was to evaluate the new JAS guidelines as a risk assessment tool in Japanese patients with hypercholesterolemia, using the cohort of the Holicos-PAT study. The Holicos-PAT study was designed as a prospective observational study. 2039 patients were followed with or without pravastatin for 5 years. We assessed coronary heart disease (CHD) and cerebrovascular disease (CVD) risks by the patient categories described in the JAS guidelines. In the Holicos-PAT study, the primary endpoints were CHD, and the secondary endpoints were CVD and total mortality. CHD event includes onset and worsening of angina pectoris, performing CABG or PTCA, non-fatal and fatal myocardial infarction, and death from CHD including heart death and sudden death. CVD events are onset or recurrence of cerebral infarction, onset of cerebral hemorrhage, and death from cerebral infarction or hemorrhage. The event rates were calculated by the person-years method, and the differences in event rates between category groups were analyzed by chi-square test. The event rates of CHD in Category A, B1, B2, B3, B4 and C, were 1.1, 4.0, 2.8, 5.7, 18.2 and 38.8 per 1,000 person-years. The rates of CHD events in the higher risk category groups, Category B4 group ($p = 0.004$ in whole patients) and C group ($p < 0.001$ in whole patients), were significantly higher than that in the combined category groups A + B1 + B2. The event rates of CVD in Category A, B1, B2, B3, B4 and C, were 2.1, 1.8, 1.8, 0.6, 10.8 and 6.4 per 1,000 person-years. The event rates of CHD in men were significantly higher than those in women, in categories B4 ($p < 0.001$) and C ($p < 0.001$). From these results, each category classified by accumulation of risk factors, showed increasing event rates of CHD and CVD. The categories in the JAS guidelines are useful to assess CHD and CVD risk in Japanese patients with hypercholesterolemia. However, the risk evaluation by the JAS guideline categories may underestimate the risk in men and overestimate it in women. *J Atheroscler Thromb, 2005; 12: 48–52.*

Key words: Risk assessment, Coronary heart disease, Cerebrovascular disease, Guidelines

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