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. When a definite cause cannot be demonstrated, the condition is termed primary pulmonary hypertension (PPH), which predominantly affects women most commonly in their third decade of life. No ethnic predisposition is apparent in the National Institutes of Health registry, and the proportions by ethnic group parallel those in the general population. 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. 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.

Recently, it was shown that PAH is associated with increased concentrations of endothelin (ET)-1, a potent vasoconstrictor, in plasma and the lungs, 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. 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. Therefore, prior to the start of the clinical trial, the multiple-dose pharmacokinetics of bosentan were compared in Caucasian and Japanese patients.

**Methods**

**Comparative Study of the Ethnic Differences in the Pharmacokinetics of Bosentan**

This part of the study was performed at FOCUS GmbH.
(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.13 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 (Cmax) and the time to Cmax (tmax) 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 tmax, the median and range.

The study was powered to detect with 90% power a difference of 50% in AUC and range.

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/cm5, (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

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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 arithmetic means ± SEM.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Cmax (ng/ml)</th>
<th>tmax (h)</th>
<th>AUC (ng·h/ml)</th>
<th>t1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosentan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>1,434</td>
<td>3.5</td>
<td>6,046</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(1,137, 1,808)</td>
<td>(2.0, 4.0)</td>
<td>(−49,997,311)</td>
<td>(5.3, 9.3)</td>
</tr>
<tr>
<td>Japanese</td>
<td>1,212</td>
<td>3</td>
<td>4,640</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>(940, 1,564)</td>
<td>(1.0, 4.0)</td>
<td>(3,641, 5,914)</td>
<td>(4.6, 6.9)</td>
</tr>
<tr>
<td>Ro 48-5033</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>175</td>
<td>4</td>
<td>859</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>(138, 221)</td>
<td>(3.0, 5.0)</td>
<td>(3,641, 5,914)</td>
<td>(4.6, 6.9)</td>
</tr>
<tr>
<td>Japanese</td>
<td>136</td>
<td>4</td>
<td>721</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>(92, 201)</td>
<td>(3.9, 5.0)</td>
<td>(532, 977)</td>
<td>(7.7, 11.8)</td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean (95% confidence limits).

AUC, area under curve; Cmax, peak plasma concentration; tmax, time to Cmax.

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

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

*aSensitive 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.
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/cm5 (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.²²,²³ The increased expression of ET-1 in the lungs of patients with pulmonary hypertension²⁴ or idiopathic pulmonary fibrosis²⁵ 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,²⁶ 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;²⁷,²⁸,²⁹ 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.³⁰⁻³² 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.³³ The International Conference on Harmonization guideline (ICH) 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.³⁴ 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.³⁵ 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 adenosine 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 inter racial/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,³³ 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.³⁴ However, increased doses led to a frequent elevation of aminotransferase concentrations in accord with the known incidence of abnormal hepatic function.³⁵ 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 patient 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.³⁶ 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.³⁷ 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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References


Appendix 1

Investigators in the Study

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