Tolerability, Safety, Pharmacokinetics, and Pharmacodynamics of Macitentan, a New Endothelin Receptor Antagonist, in Healthy Japanese Male Subjects

Yoshinari YOKOYAMA*1, Jasper DINGEMANSE*2, Motonori HATTA*1 and Hiroyuki FUKASE*3

Abstract

Tolerability, safety, pharmacokinetics, and pharmacodynamics of orally administered macitentan at 3 and 10 mg once daily for 10 days were investigated in 16 healthy Japanese male subjects in a randomized, placebo-controlled, double-blind, single-center study. Plasma concentrations of macitentan were found to peak at 5 hr, with a mean terminal elimination half-life of approximately 11 hr, for both doses of macitentan. Furthermore, the active metabolite of macitentan, ACT-132577, demonstrated a longer elimination half-life (approximately 48 hr). Area under the plasma concentration–time curve (AUC) of endothelin-1 was significantly higher in the macitentan 10-mg group compared with the placebo group (exploratory p value, 0.0154). No critical issues regarding the safety and tolerability of macitentan were observed, including time-matched electrocardiographic (ECG) evaluations. The results of this study in healthy Japanese male subjects corroborate with previous studies in Caucasian and Korean subjects. In conclusion, the macitentan dose range from 3 to 10 mg had a good safety and tolerability profile in healthy Japanese male subjects, and its pharmacokinetics and pharmacodynamics were dose-dependent without ethnic differences.

Key words: macitentan, pharmacokinetics, pharmacodynamics, intensive QT study, endothelin-1

Introduction

Macitentan is a dual endothelin (ET) receptor antagonist (ERA) with a high binding affinity and has been shown to cause sustained inhibition of ET_a and ET_b receptors. The physicochemical properties of macitentan allow high penetration into tissues. Moreover, macitentan has significantly slower receptor dissociation kinetics resulting in sustained receptor occupancy with binding time 15 times longer compared with currently approved ERAs. These characteristics are consistent with the possibility of greater efficacy of macitentan compared with bosentan, another ERA, as shown by significantly reduced mean pulmonary artery pressure, inhibition of pulmonary arterial thickening and right ventricular hypertrophy, and prolonged survival in a rat model of pulmonary hypertension.

The pharmacokinetic (PK) profile of macitentan permits once daily dosing without affecting metabolizing enzymes or transporters, thereby avoiding significant drug–drug interactions. The lack of propensity of macitentan to interfere with intrahepatic bile salt transport results in reduced risk of elevation of liver enzymes, which is a well known adverse event of bosentan. Macitentan inhibits ET_b receptors and reportedly improves reduced reabsorption of alveolar fluid, thereby preventing pulmonary edema which is one of the complications of pulmonary hypertension.

In the SERAPHIN trial, the first event-driven, randomized, placebo-controlled outcome study of pulmonary arterial hypertension (PAH) conducted outside Japan, long-term administration of 3 or 10 mg macitentan significantly reduced the risk of morbidity and mortality.

Previous reports have shown no substantial difference in the standard of medical care for PAH between Japan and Western countries. The PK of a single dose of 10 mg macitentan in healthy Japanese and Caucasian subjects were evaluated in a previous study, but the dose-response, safety, and tolerability of repeated dosing of macitentan in the Japanese population have yet to be elucidated. In the context of safety evaluations, the effect of macitentan on ECG parameters has been comprehensively evaluated in Caucasian populations.

Therefore, this study was designed to determine the tolerability, safety (including ECG evaluations), PK, and pharmacodynamics (PD) of repeated 3 and 10 mg doses of macitentan in 16 Japanese subjects.

Methods

1. Subjects and study design

This was a single-center, randomized in each dose group, double-blind, placebo-controlled, phase I study. Japanese males aged between 20 and 50 years assessed as healthy by medical history, previous medication, 12-lead ECG, physical examination, and laboratory tests; non-smokers; with a body mass index (BMI) of 18.0–28.0 kg/m^2 were eligible as subjects.

In each of the 3-mg and 10-mg macitentan groups, eight healthy male subjects were randomly assigned to receive either oral macitentan (n = 6) or matching placebo (n = 2) for 10 days.

---

*1 Research and Development Division, Actelion Pharmaceuticals Japan Ltd, Japan  **1 Department of Clinical Pharmacology, Actelion Pharmaceuticals Ltd, Switzerland  *2 CPC Clinical Trial Hospital, Medipolis Medical Research Institute, Japan

Address for correspondence : YOKOYAMA Y, Research and Development Division, Actelion Pharmaceuticals Japan Ltd, Ebisu Prime Square Tower 1-1-39 Hiroo Shibuya-ku, Tokyo 150-0012, Japan  E-mail : yoshinari.yokoyama@actelion.com

Fast track publication : Manuscript received March 10, 2016 : revised May 17, 2016 and June 6, 2016 : accepted June 7, 2016

ISSN 0388–1601  Copyright: ©2016 the Japanese Society of Clinical Pharmacology and Therapeutics (JSCPT)
Subjects were admitted to the trial center 2 days prior to the first administration of the study drug and were discharged 24 hr after the last administration. The study drug was administered at the same time in the morning before breakfast from days 1 to 10. All meals consumed during the study were controlled by the center. Subjects were discharged on day 11 following a safety assessment. Subjects revisited the study center on days 12, 13, 14, 16, 18, 20, and 40 for safety assessments and PK sampling.

This study was conducted at the Clinical Trial Hospital, Medipolis Medical Research Institute (Toso, Kagoshima Prefecture, Japan) in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines of the International Conference on Harmonization, and Japanese Good Clinical Practice. The study protocol and informed consent forms were approved by the Institutional Review Board of the study center. Signed informed consent was obtained from all subjects.

2. Study endpoints and assessments

2.1. Tolerability and safety

Tolerability was assessed according to the occurrence of adverse events (AE; classified by MedDRA version 14.0); occurrence of serious adverse events (SAEs); and changes in physical examination findings, vital signs, body weight, ECG, and clinical laboratory test results from baseline values. AEs were monitored up to day 20, and SAEs up to day 40 ± 1.

2.2. Pharmacokinetic and pharmacodynamic analysis

The maximum plasma concentrations (Cmax) and times to reach Cmax (tmax) of macitentan and its active metabolite, ACT-132577, were determined from actual measured data on days 1, 8, and 10. The elimination half-life (t1/2) of macitentan from the systemic circulation was estimated using individual log-linear plasma concentration-time data obtained following the last dose on day 10. Venous blood samples for measurements of macitentan, ACT-132577, and ET-1 concentrations were drawn before drug administration and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 16 hr after drug administration on days 1 and 8. For the last dose on day 10, blood samples were obtained before drug administration and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 24, 48, 72, 96, 144, 192, and 240 hr after drug administration. The areas under the plasma concentration-time curves (AUC) on days 1 and 10 were calculated using the trapezoidal method. AUC was calculated as AUC (0→last) + Clast/kel, where Clast is the plasma concentration at the last measurement point, and kel is the elimination rate constant from plasma. The accumulation ratio, Ra, for exposure was defined as the ratio of AUC (0→24) at steady state to AUC (0→24) after the first dose. Linear regression was used to analyze the relationships between PK parameters of macitentan (including active moiety parameters) and PD parameter (change in ET-1 concentration from baseline). Furthermore, the total active moiety was calculated in terms of molar concentrations, with ACT-132577 assumed to have 20% of the macitentan activity (molar concentration of macitentan (MW, 588.27 g/mol) + molar concentration of ACT-132577 (MW, 546.19 g/mol) × 0.2), because ACT-132577 contributes to the drug effect. As macitentan and ACT-132577 are both highly (>99%) protein bound, we did not adjust for plasma protein binding while calculating the active moiety concentration.

2.3. Twelve-lead and Holter ECG evaluations

Twelve-lead ECGs were performed only in the 10-mg group, and were evaluated by exploratory data analysis. Recorded waveform data were sent to a central ECG laboratory. Individual data were analyzed at Cardiocore Lab (Rockville, USA). Holter ECG measurements (PR, QRS, RR, QT, and QTc) were obtained at the time of blood sampling for macitentan and ACT-132577 measurements (denoted as time-matched ECG). QT values were corrected with Fridericia's formula (QTcF) during the primary ECG analysis of the present study. ΔQTcF was corrected for baseline value.

3. Analytical method

Concentrations of macitentan and its active metabolite, ACT-132577, were measured using a validated liquid chromatography/tandem mass spectrometry (LC-MS/MS) method at Swiss BioAnalytics AG (Birsfelden, Switzerland). The inter-day accuracy (coefficient of variation) of quality control samples was 88.3%–98.0% and 91.3%–97.3% and the inter-day precision was <5.9% and <5.2% for macitentan and ACT-132577, respectively. For both analytes, the lower limit of quantification (LLOQ) was 1.00 ng/mL using 10 μL of plasma.

Plasma concentrations of ET-1 were measured using a commercially available luminescent immunoassay kit (QET00B; R&D Systems, Inc., Abingdon, UK) at the LSI Medience Corporation (Tokyo, Japan). The quantification range of the assay was 0.34–250 pg/mL.

4. Statistical analyses

Subjects who received at least one dose of the study drug were included in the analysis data set for tolerability and safety. Statistical Analysis System software version 9.1.3 (SAS Institute, Cary, NC, USA) was used in all statistical analyses and reporting of clinical and PK data. PK and PD parameters were analyzed using descriptive statistics. The dose–response relationships of PK parameters were evaluated using a linear or power model. Normal distribution was assumed for log-transformed PK data. Statistical testing was not performed in this study because there was no statistical hypothesis. However, 95% confidence interval (CI) was reported for estimates. Missing data were not imputed or replaced. Exploratory statistical analysis for AUC (0→24) of ET-1 was performed by t-tests.

Table 1 Summary of subject demographics characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo n = 4</th>
<th>3 mg macitentan n = 6</th>
<th>10 mg macitentan n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>31.5</td>
<td>27.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Min, Max</td>
<td>22.48</td>
<td>21.38</td>
<td>25.38</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>62.90</td>
<td>58.35</td>
<td>61.15</td>
</tr>
<tr>
<td>Min, Max</td>
<td>55.8, 69.7</td>
<td>53.4, 73.7</td>
<td>52.6, 80.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>21.5</td>
<td>21.0</td>
<td>21.7</td>
</tr>
<tr>
<td>Min, Max</td>
<td>18.0, 22.5</td>
<td>18.2, 23.0</td>
<td>18.5, 27.0</td>
</tr>
</tbody>
</table>

n = number of subjects; Min, Max = minimum, maximum; BMI = body mass index.
Results

1. Subjects
A total of 16 healthy Japanese males, 8 subjects each for the 3-mg and 10-mg groups (macitentan: placebo = 3:1), were enrolled in the present trial. All subjects were evaluated for tolerability, safety, PK, and PD. The age, weight, and BMI of subjects ranged from 21 to 48 years, 52.6 to 80.7 kg, and 18.0 to 27.0 kg/m², respectively. No notable differences in baseline characteristics were observed between groups (Table 1).

2. Pharmacokinetics
Plasma concentrations of macitentan peaked at 5–6 hr (median tₘₐₓ) after both single and repeated doses of 3 and 10 mg of macitentan (Figure 1). The mean (95% CI) terminal elimination t½ on day 10 was 11.5 (9.1, 14.5) hr and 11.1 (8.8, 13.9) hr in the 3-mg and 10-mg groups, respectively. Macitentan concentrations in both dose groups reached steady state by day 4. By day 16 (6 days after the last dose), plasma concentrations of macitentan were below the LLOQ. Exposure over the dosing interval [AUC(0–24)] on day 10 was 1.4-fold higher than that on day 1 for the 3-mg group and 1.5-fold higher for the 10-mg
Table 2  Plasma pharmacokinetic parameters of macitentan and its metabolite, ACT-132577 in healthy Japanese male subjects who received multiple doses of 3 or 10 mg macitentan once daily for 10 days

<table>
<thead>
<tr>
<th></th>
<th>Macitentan</th>
<th>ACT-132577</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-mg group (n=6)</td>
<td>10-mg group (n=6)</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>73.0 (62.3, 85.7)</td>
<td>193.4 (141.0, 265.3)</td>
</tr>
<tr>
<td>tmax (hr)</td>
<td>6.00 (4.00, 7.00)</td>
<td>6.00 (5.00, 8.00)</td>
</tr>
<tr>
<td>AUC (0→24) (ng·hr/mL)</td>
<td>1070.9 (967.6, 1185.4)</td>
<td>2802.4 (2195.1, 3577.7)</td>
</tr>
<tr>
<td>Day 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>99.7 (79.3, 125.2)</td>
<td>280.0 (230.6, 339.9)</td>
</tr>
<tr>
<td>tmax (hr)</td>
<td>6.00 (5.00, 8.00)</td>
<td>5.00 (5.00, 10.00)</td>
</tr>
<tr>
<td>AUC (0→24) (ng·hr/mL)</td>
<td>1539.0 (1271.6, 1862.7)</td>
<td>4028.7 (3384.2, 4796.0)</td>
</tr>
<tr>
<td>Ra AUC (0→24)</td>
<td>1.4 (1.3, 1.6)</td>
<td>1.4 (1.2, 1.7)</td>
</tr>
<tr>
<td>AUC (0→24)/dose (ng·hr/mL/mg)</td>
<td>513.0</td>
<td>402.9</td>
</tr>
<tr>
<td>Day 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>106.7 (90.4, 125.9)</td>
<td>291.2 (220.1, 385.3)</td>
</tr>
<tr>
<td>tmax (hr)</td>
<td>5.00 (4.00, 7.00)</td>
<td>5.00 (5.00, 10.00)</td>
</tr>
<tr>
<td>AUC (0→24) (ng·hr/mL)</td>
<td>1551.0 (1243.4, 1934.6)</td>
<td>4190.1 (3426.2, 5124.5)</td>
</tr>
<tr>
<td>AUC (ng·hr/mL)</td>
<td>2202.5 (1654.3, 2932.4)</td>
<td>6359.8 (4943.8, 8181.4)</td>
</tr>
<tr>
<td>t1/2 (hr)</td>
<td>11.5 (9.1, 14.5)</td>
<td>11.1 (8.8, 13.9)</td>
</tr>
<tr>
<td>Ra AUC (0→24)</td>
<td>1.4 (1.2, 1.7)</td>
<td>1.5 (1.2, 1.9)</td>
</tr>
</tbody>
</table>

n = number of subjects
Data expressed as the means (95% CI) and the median (range) for tmax.
AUC (0→24), area under the plasma concentration–time curve from 0 to 24 hr; AUC, area under the plasma concentration–time curve from 0 to infinity
Ra, refers to the accumulation ratio compared to day 1

The AUC (0→24) of macitentan on day 8 was 513.0 in the 3-mg group and 402.9 in the 10-mg group. In contrast, the AUC (0→24) of ACT-132577 was 2175.6 in the 3-mg group and 1835.0 in the 10-mg group. The mean terminal elimination t1/2 of ACT-132577 was 48.3 hr in the 3-mg group and 46.6 hr in the 10-mg group. The AUC (0→24) of ACT-132577 on day 10 was approximately 4-fold greater than that of macitentan (Figure 1). ACT-132577 concentrations reached steady state by day 7. The AUC-132577 accumulation ratio, Ra, was between 6.8- and 7.3-fold. By day 40, all measured ACT-132577 plasma concentrations were below LLOQ (data not shown).

3. Pharmacodynamics
Macitentan administration increased plasma ET-1 concentrations after the 10-day administration period, whereas ET-1 levels did not change in the placebo group (Figure 2). The plasma ET-1 concentration, as a PD parameter, increased after repeated once daily dosing of macitentan for 10 days. When comparing ET-1 levels of the 3-mg and 10-mg groups versus the placebo group, a significant difference was observed only between the 10-mg and placebo groups (p value from exploratory t-tests, 0.0154).

4. Pharmacokinetic-Pharmacodynamic relationship
We calculated the concentration of the active moiety in plasma in order to consider the contribution of the active metabolite to the overall pharmacological effect of macitentan. A statistically significant relationship between the change in plasma ET-1 concentration from baseline and the plasma active moiety concentration was observed (R² = 0.3915; p < 0.0001; Figure 3).
5. Tolerability and safety

During this study, 12 AEs were reported in six subjects. None of the AEs were serious, and none led to discontinuation of the study drug. In the placebo group, two AEs occurred in two subjects: one case of constipation and one case of increased ALT (an increase from 40 at baseline to 53 IU/L on day 4; the latter value was just above the upper limit of the normal range). For the laboratory values shown hereafter, the value at baseline and that classified as abnormal are given. In the 3-mg group, nine AEs occurred in three subjects [subject 1: diarrhea; subject 2: headache, oropharyngeal pain and tonsillitis; subject 3: increased ALT (35 and 62 IU/L on day 20), increased AST...
reported during this study. ECG data of individual subjects were analyzed at a central ECG laboratory. Categorical analyses of ΔQTcF intervals were performed using all available Holter data of day 1 and day 8 during the administration period. No increases in QTcF to >480 ms were observed during the study period. Only one subject in the 10-mg group exceeded 450 ms, but not 480 ms, at one time point. All other values in all subjects remained <450 ms (data not shown). In general, baseline-corrected ΔQTcF values in the 10-mg group were <10 ms. No significant relationship between Holter ECG variables and plasma drug concentrations was observed (Figure 4). QT values were not associated with individual peak plasma drug levels.

**Discussion**

A previous study of single-dose macitentan indicated no significant differences, in particular PK parameters, between Japanese and Caucasian individuals\(^{10}\). This study was designed to evaluate the tolerability and safety including time-matched ECG-PK evaluations, and ET-1 concentrations following repeated 3 and 10 mg doses of macitentan in healthy Japanese male subjects.

In this study, \(t_{1/2}\) observed for macitentan (approximately 11 hr) and ACT-132577 (approximately 48 hr) corroborated with a previous macitentan study in healthy Japanese subjects\(^{10}\).

On day 10, the \(R_{0}\) of ACT-132577 was approximately 7, which was approximately two-fold larger than the theoretical accumulation ratio \(|1/(1-\exp(-\ln2/t_{1/2} \times \tau))|\) of 3.4 determined on the basis of \(t_{1/2}=41.2 \) hr obtained in a previous study of single-dose macitentan\(^{10}\) and a dosage interval \(\tau=24\) hr. This apparent discrepancy in the accumulation of ACT-132577 was considered to be due to two factors. First, the PK of ACT-132577 are affected by the rate of macitentan metabolism and its accumulation. Second, macitentan does not follow one-compartmental PK because it has a high affinity for the lipophilic phase\(^{2}\), shows sustained binding to receptors\(^{3}\), and has high protein binding (>99\%). Therefore, the accumulation ratios of macitentan and ACT-132577 shown in this study were calculated from observed data.

After oral administration of 10 mg macitentan for 10 days, plasma ET-1 concentrations were significantly increased compared with placebo administration. Plasma concentrations of macitentan and ACT-132577 reached steady state after 4 and 7 days, respectively, in both dose groups. However, in the 3-mg group, plasma ET-1 concentrations did not increase significantly compared with the placebo group. These results indicate that 10 mg macitentan (\(C_{\text{min}}: 495 \text{nM}, C_{\text{avg}}: 157 \text{nM}\)) substantially inhibits the binding of ET-1 to ET\(_B\) receptors (\(IC_{50}: 391 \text{nM}\)) which are responsible for ET-1 clearance\(^{16}\). Such levels also inhibit the binding of ET-1 to ET\(_A\) receptors (\(IC_{50}: 0.5 \text{nM}\)). Although this study was exploratory, the increased plasma ET-1 concentrations observed in this study indicate that drug exposure following 10 mg macitentan reaches pharmacologically effective concentrations.

PK and PD of macitentan in healthy Japanese male subjects obtained in this study were similar to those reported previously in Caucasian and Korean subjects\(^{17,18}\). The results of this study indicate a lack of significant variation in PK and PD of macitentan between ethnicities. In addition, similarity in PK between Caucasian healthy subjects and Caucasian PAH patients.
has been reported\(^\text{13}\). The results of the SERAPHIN study indicated that the therapeutic dose of macitentan in Caucasian PAH patients is 10 mg once daily. These are strong indications that the therapeutic dose of macitentan in Caucasian PAH patients can be also applied to Japanese PAH patients.

No subjects required withdrawal from the study, and all AEs were of mild intensity except for one case (tonsillitis). Elevated liver enzymes were observed in all groups including placebo. Similar incidence of liver enzyme elevation has been reported for liver enzymes were observed in all groups including placebo. Elevated tonsillitis (\(3, \text{13, 19}\)) these results suggest that the safety of macitentan with respect to liver function is similar to that of placebo also in Japanese subjects. Further studies including long-term administration of macitentan in Japanese patients are required to investigate its efficacy and safety under clinically relevant conditions, because the current study only investigated a small number of healthy subjects for 10 days.

The effect of macitentan on cardiac function has been evaluated previously in healthy Caucasian subjects in a thorough QT study\(^\text{11}\) according to the ICH-E14 guidelines\(^\text{12}\). As no clinically relevant issues were reported in the previous study, which had an active control group and statistical robustness, we performed a small scale evaluation of QT intervals in healthy Japanese male subjects with concurrent ECG recordings and measurements of plasma macitentan concentrations. No clinically relevant changes in intensive QT assessments were observed in this study.

In conclusion, repeated administration of 3 or 10 mg macitentan for 10 days increased exposure to macitentan and plasma ET-1 concentrations in a dose-dependent manner with good tolerability and safety in healthy Japanese male subjects. Although this study was exploratory and involved a small number of healthy male subjects, the therapeutic dose in Japanese patients is expected to be similar to that in other ethnicities.

**Sponsorship and Declaration of Interest**

This study was sponsored by Actelion Pharmaceuticals Japan Ltd (APJ) and Nippon Shinyaku Co., Ltd. Medical writing support was funded by APJ. Yoshinari Yokoyama and Motonori Hatta are employees of APJ, and Jasper Dingemanse is employee of Actelion Pharmaceuticals Ltd. Hiroyuki Fukase is the principal investigator at Medipolis Medical Research Institute that was commissioned by APJ to conduct this study. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict.

**Acknowledgments**

This study was designed by the sponsor, Actelion Pharmaceuticals Japan Ltd, with advice provided by Dr. Sasayama, Director of Uji Hospital.

**Reference**


324.


