Absorption, distribution, metabolism, and excretion of macitentan, a dual endothelin receptor antagonist, in humans

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Abstract
1. Macitentan is a tissue-targeting, dual endothelin receptor antagonist, currently under phase 3 investigation in pulmonary arterial hypertension.
2. In this study the disposition and metabolism of macitentan were investigated following administration of a single oral 10 mg dose of ¹⁴C-macitentan to six healthy male subjects.
3. The total radioactivity in matrices was determined using liquid scintillation counting. The proposed structure of metabolites was based on mass spectrometry characteristics and, when available, confirmed by comparison with reference compounds.
4. Mean (± SD) cumulative recovery of radioactivity from faeces and urine was 73.6% (±6.2%) of the administered radioactive dose, with 49.7% (±3.9%) cumulative recovery from urine, and 23.9% (±4.8%) from faeces. In plasma, in addition to parent macitentan, ACT-132577, a pharmacologically active metabolite elicited by oxidative depropylation and the carboxylic acid metabolite ACT-373898 were identified. In urine, four entities were identified, with the hydrolysis product of ACT-373898 as the most abundant one. In faeces, five entities were identified, with the hydrolysis product of macitentan and ACT-132577 as the most abundant one. Concentrations of total radioactivity in whole blood were lower compared to plasma, which indicates that macitentan and its metabolites poorly bind to or penetrate into erythrocytes.

Keywords: Mass balance, metabolite profiling, pharmacokinetics, Phase I

Introduction
Macitentan (ACT-064992, N-[5-(4-bromophenyl)-6-(2-(5-bromopyrimidin-2-yl)ethoxy)pyrimidin-4-yl]-N’-propylsulfuric diamide)) is a tissue-targeting, dual endothelin receptor antagonist, under phase 3 investigation in pulmonary arterial hypertension. Macitentan is a lipophilic compound, which inhibits endothelin receptors ET₄ and ET₂. It has been hypothesised that by targeting both endothelin receptors with a high tissue affinity, macitentan could constitute a new therapeutic approach in the management of pulmonary arterial hypertension or other diseases in which the tissue endothelin system is activated (Iglarz et al. 2008; Sidharta et al. 2011). Maximum plasma concentrations of macitentan are achieved approximately 8 hours after oral dosing in humans. The terminal elimination half-life (t1/2) is approximately 16 hours. After multiple dosing the pharmacokinetics (PK) are proportional over the tested dose range (1–30 mg); steady state is achieved by Day 3 and macitentan accumulates approximately 1.5 fold. In plasma, besides macitentan, ACT-132577, a pharmacologically active metabolite of macitentan has a t₁/₂ of about 48 h (Sidharta et al. 2011; Kummer et al. 2009; Sidharta et al. 2008). The t₁/₂ of macitentan and its main metabolite is in accordance with a once daily dosing regimen. Formation of ACT-132577 in
man is predominantly catalyzed by cytochrome P450 (Sidharta et al. 2010). There are no notable differences in pharmacokinetic parameters of macitentan and its active metabolite between males and females and/or between Caucasian and Japanese subjects (Bruderer et al. 2011). In addition, macitentan can be given to subjects irrespective of food intake.

The objectives of this study were to determine the mass balance, to identify the clearance routes of macitentan and its metabolites, and to detect the compound-related entities in plasma, urine, and faeces. Comparison of metabolite profiling in human with animal toxicity studies will help to identify possible unique human metabolites. We report the identification of metabolites of macitentan in humans by liquid chromatography followed by on-line mass spectrometry (MS) and fraction collection in 96-well solid scintillation plates (SSC) for off-line scintillation counting (TopCount). The structures of metabolites were established or confirmed by high resolution MS and/or comparison with synthetic standards. The concentrations of macitentan and its active metabolite ACT-132577 were determined by a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assay.

Materials and methods

Subjects and study design

The clinical part of this study was conducted at Covance Clinical Research Unit AG, Allschwil, Switzerland, after approval of the study protocol by the Ethics Committee of both Cantons of Basel, Switzerland. The study was conducted in full conformity with the principles of the Declaration of Helsinki and the EMA Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95).

After providing informed consent, six subjects were screened for eligibility between 14 and 3 days prior to dosing. Demography, previous and concomitant medications, and a medical history were documented, and safety assessments were performed. Male subjects aged 45–65 years, with Body Mass Index between 18 and 28 kg/m² (inclusive) were eligible for this study. In addition, previous treatment with any prescribed or over-the-counter medications within 2 weeks prior to screening and exposure to artificial ionizing radiation (e.g. X-ray, thyroid scan) in the 12-month period before screening were not permitted.

It was planned that all subjects remain in the study centre from the evening of Day -1 until at least 14 days thereafter. Subjects received on Day 1 a single dose of 10 mg ¹⁴C-labelled macitentan (specific radioactivity 0.164 MBq/mmol (4.43 μCi/mmol), radiochemical purity 95.3%, manufactured by Aptuit Edinburgh UK), as one capsule. Target radioactivity was 2.78 MBq, which is equivalent to 970 μSv (Sievert). This target radioactivity was based on tissue exposure data in rats and PK in humans. According to the International Commission on Radiological Protection, this additional radiation exposure poses no relevant risk for human subjects (Valentin 2007). The subjects could be discharged from the study centre only if the amount of plasma radioactivity on Day 13 did not exceed 6% of the maximum radioactivity measured in plasma (attained between 0 to 72 hours post-dose). The criterion of 6% of maximum radioactivity in plasma was set based on the fact that after 4 elimination half-lives of a drug following one-compartment PK, approximately 94% of its maximum concentration in plasma is eliminated. C_max of macitentan and its active metabolite ACT-132577 are attained approximately 8 h and 48 h after dose administration, respectively. It was estimated that maximum plasma radioactivity would be attained between 0 to 72 h post-dose and 94% of it should be eliminated before Day 13. In case a subject would have to remain longer in the study centre, the collection of blood (once daily sampling), urine, and faeces continued until the radioactivity in plasma would be below the predefined threshold of 6%. During their residence in the study centre, subjects received standardized meals.

An End of Study (EOS) examination was performed immediately after the last blood, urine, and faeces samplings had been performed. From screening until EOS examination, the subjects had to refrain from strong physical exercise and strenuous sports activities (endurance sports), and were not allowed to consume any alcoholic beverages, grapefruit, or grapefruit juice. Drinking of xanthine-containing beverages was not permitted during the time spent in the clinic.

Sample collection

Blood samples were drawn through an indwelling catheter or by direct venipuncture for at least 14 days at 1, 3, 5, 6, 7, 8, 9, 10, 12, 16, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, and 312 h after macitentan dosing for analysis of total radioactivity and pharmacokinetics in blood and plasma, and metabolite profiling in plasma.

At each sampling time point a total of 18 mL blood was collected in ethylene diamine tetra-acetic acid (EDTA)-containing tubes. 3 mL of collected blood was transferred to pre-labelled tubes for total radioactivity measurement in whole blood. Within 30 min after collection, the remaining blood was centrifuged at 4°C for 1500g for 10 min, and plasma was separated. All plasma and blood tubes were stored in an upright position at −20°C or below until transferred to the analyzing laboratory.

All urine and faeces were collected after dosing for analysis of total radioactivity and metabolite profiling.

Two samples of 10 mL of each collected 24-hour urine volume were stored in an upright position at −20°C or below until transferred to the analyzing laboratory.

Faecal samples collection started after study drug intake and continued until the end of the hospitalization period at EOS. Faecal samples were frozen as soon as possible and stored at −80°C in an upright position until transferred to the analyzing laboratory.
Sample preparation and bioanalytical methods

Measurement of total radioactivity in plasma, whole blood, faeces, and urine

Total radioactivity was determined using a liquid scintillation counter, TriCarb 2800TR (PerkinElmer Life and Analytical Sciences, Downers Grove, IL, USA).

Pre-dose plasma, whole blood, urine, and faeces samples were measured and served as background radiation. For determination of total radioactivity in plasma, whole blood, urine, and faeces, the limit of quantification was set to 10 disintegrations per minute (dpm) after subtraction of background.

Faeces: Each faeces sample, delivered deep-frozen to the analytical laboratory, was separately freeze-dried (Alpha 2–4 LD freeze-dryer from Christ GmbH, Osterode, Germany) for at least 72 h, and weighed. The dried samples were homogenized and four aliquots (approximately 20–30 mg) were transferred to glass scintillation vials. 300 μL water (Milli-Q Plus, Volketswil, Switzerland) and then 1 mL of tissue solubilizer (Soluene 350®, PerkinElmer Life and Analytical Sciences, Downers Grove, IL, USA) were added and incubated for one hour at 50°C, followed by addition of 0.5 mL isopropanol (Mallinckrodt Baker, Deventer, The Netherlands) and a further incubation time of two hours at 50°C. After cooling at room temperature for 30 min, 500 μL of hydrogen peroxide (30%, Fluka, Buchs, Switzerland) were added drop wise and the solution was allowed to stand for at least 30 min at 50°C to complete the reaction. A volume of 0.25 mL hydrochloric acid (1.0 M, Fluka, Buchs, Switzerland) was added. Finally, 10 mL of the scintillation cocktail (Hionic Fluor, PerkinElmer Life and Analytical Sciences, Downers Grove, IL, USA) were added and the samples were incubated for at least 12 hours in the dark. Each faeces sample was analysed in triplicate. Generally, the measurements were performed for a counting time of 10 min.

Whole blood: Three aliquots of 300 μL (weighed) were transferred into glass vials. Under stirring a mixture of 0.75 mL of isopropanol and tissue solubiliser (Soluene 350®) (2/1, v/v) was added and incubated for one hour at approximately 50°C. After cooling at room temperature 0.5 mL of hydrogen peroxide (30%) was added drop wise and the solution was allowed to stand for at least 15 min to complete the reaction. Thereafter, the vial was capped and placed for at least 30 min in a water bath (water bath SBK 25 D, Salvis AG, Reussbühl, Switzerland) at approximately 50°C. After cooling at room temperature, 0.25 mL hydrochloric acid (1.0 M) and 10 mL of the scintillation cocktail (Ultima Gold, PerkinElmer Life and Analytical Sciences, Downers Grove, IL, USA) were added. Prior to scintillation counting, samples were allowed to adapt for temperature and light for one hour. Each whole blood sample was analysed in triplicate.

Plasma: Three aliquots of 300 μL (weighed) were transferred into glass vials. The samples were mixed with 1 mL of water and then with 10 mL of the scintillation cocktail (Ultima Gold) while vortexing (Vortex mixer IKA, IKA-Works Inc., Wilmington, NC, USA, and/or Vortex Genie 2, Scientific Industries Inc., Bohemia, NY, USA). Each plasma sample was analysed in triplicate.

Urine: Two aliquots of 300 μL (weighed) were transferred into glass vials. The samples were mixed with 10 mL of the scintillation cocktail (Ultima Gold) while vortexing. Each urine sample was analysed in duplicate.

Metabolite profiling in plasma, urine, and faeces

For metabolite profiling, selected samples were pooled. Plasma pools were prepared by combining a fixed volume of samples collected at the following time points from all six subjects, pool 1: time points 1 and 3h, pool 2: 5 and 6h, pool 3: 7 and 8h, pool 4: 9 and 10h, pool 5: 12 and 16h, pool 6: 24 and 48 h, pool 7: 72 and 96 h, pool 8: 120 and 144 h, pool 9: 168 and 192 h, pool 10: 216 and 240 h, pool 11: 264–336 h. The pooled plasma samples were precipitated by ACN (acetonitrile, Mallinckrodt Baker, Deventer, The Netherlands)/EtOH (ethanol, Mallinckrodt Baker, Deventer, The Netherlands). Each sample supernatant was analysed by separation with HPLC followed by fraction collection and on-line MS-detection. The fractions were collected in 96-well SSC plates and dried for subsequent off-line scintillation counting (TopCount NXT, PerkinElmer Life and Analytical Sciences, Downers Grove, IL, USA). A Luna C18 (2), 4.6 × 250 mm ID, 5 μm analytical column (Phenomenex, Aschaffenburg, Germany) was used. The mobile phases consisted of water containing 1% formic acid (v/v, purchased from Riedel de Haen, puriss (RdH Laborchemikalien GmbH), Seelze Germany) (phase A), and ACN (phase B). The flow rate was 1 mL/min. Fixed percentages (by volume) of selected time intervals of all six subjects were combined to prepare urine sample pools. The pooled urine samples were injected without further work-up onto the HPLC column, followed by online MS-detection as well as on-line fraction collection in 96-well SSC plates for off-line scintillation counting (TopCount).

Fixed percentages (by weight) of freeze-dried, homogenized faeces samples of selected time intervals of all six subjects were combined to prepare faeces sample pools. The time intervals used for preparation of each urine and faeces pool samples are as follows: pool 1: 0–24 h, pool 2: 24–48 h, pool 3: 48–72 h, pool 4: 72–96 h, pool 5: 96–120 h, pool 6: 120–168 h, pool 7: 168–216 h, pool 8: 216–264 h, pool 9: 264–336 h.

Each dried faeces pool was extracted, by using approximately 10 v/w (i.e. 4 mL per 400 mg faeces) of ACN/H2O (50/50, v/v). Following the addition of the solvent, samples were vortex-mixed for 30 min and allowed to stand. Thereafter, samples were centrifuged for 10 min at 4000 rpm and 8°C. After centrifugation the supernatant was decanted off. This process was repeated one more time. Both supernatants were combined and evaporated to dryness by nitrogen gas (Messer Schweiz
AG, Lenzburg, Schweiz) and dissolved in 200 μL of a mixture of ACN/H₂O (30/70, v/v). 50 μL were injected onto the HPLC column followed by on-line MS-detection as well as on-line fraction collection in 96-well SSC plates for off-line scintillation counting (TopCount).

The recovery of radioactivity after sample preparation was determined for all matrix samples. For one representative faeces sample, the recovery of radioactivity was also determined after extraction and before reconstitution of the sample. In addition, the column recoveries after chromatography were determined for each matrix with one representative sample.

A LTQ (linear trap quadrupole) mass spectrometer (ThermoFisher Scientific, San Jose, CA, USA) was used for structure identification of the metabolites of macitentan in plasma, urine, and faeces. The ESI (electrospray ionization) source was set in positive and negative ion mode and analytical conditions were set as follows: ESI voltage 4.0 kV, capillary temperature 350°C, mass-to-charge ratio range 100–1000 Thomson.

In order to gain more information about the identity of selected metabolites a high resolution LTQ Orbitrap was used at a resolving power of at least 30,000.

The synthetic standards, which were used as reference to confirm retention times (RTs) and structures of macitentan, ACT-132577, and ACT-373898, were synthesized at Actelion Pharmaceuticals Ltd.

**Analysis of macitentan and ACT-132577 in plasma**

The concentration of macitentan and its active metabolite ACT-132577 in plasma was determined using a validated LC-MS/MS method, as described previously (Kummer et al. 2009). K₃-EDTA was used as an anticoagulant. The lower limit of quantification (LOQ) in plasma was 1 ng/mL for both analytes.

The concentrations of the analytes were determined using the internal standardization method. Tetraederated internal standards were used (Actelion Pharmaceuticals Ltd). The square of the correlation coefficient (R²) of calibration curves was at least 0.9986 for macitentan, and 0.9934 for ACT-132577 in all runs. For macitentan, the inter-day precision (coefficient of variation) of quality control samples was <5.6% and the inter-day inaccuracy was −5.3 to −0.2%. For ACT-132577, inter-day precision was <9.9% and inter-day inaccuracy was −4.0 to −0.7%. All samples were analysed within the period, over which the analytes had been shown to be stable.

**Pharmacokinetic analysis of macitentan and ACT-132577 in plasma, and total radioactivity in plasma and whole blood**

Maximum plasma concentration (Cₘₐₓ), time to reach maximum plasma concentration (tₘₐₓ), t₁/₂, area under plasma concentration-time curve from zero to time t of the last measured concentration above the LOQ (AUC₀ₜ), area under plasma concentration-time curve from zero to infinity (AUC₀₋∞) of macitentan and ACT-132577 in plasma, total radioactivity in plasma and whole blood were determined with model-independent methods, using WinNonlin professional version 5.0 (Pharsight Corporation, Mountain View, CA, USA).

Based on the actual radioactive doses administered to each subject, the specific activity of ¹⁴C-macitentan in individual administered capsules (dpm/mg) was calculated, which in turn was used to calculate ng equivalents of total radioactivity in plasma. Pharmacokinetic parameters were descriptively analysed, calculating geometric means and 95% confidence limits.

**Tolerability and safety**

Tolerability and safety were evaluated descriptively by recording of Serious Adverse Events (SAEs), Adverse Events (AEs), clinical laboratory variables, vital signs, and 12-lead ECG during the study. Clinical laboratory variables and 12-lead ECG were assessed before study drug administration and at EOS, vital signs were assessed before study drug administration, at different time points on Day 1, and every second day until EOS.

**Results**

Six healthy male subjects (age range: 47–62 years, BMI range: 24.7–27.4 kg/m²) were enrolled and all completed the study as per protocol.

The amount of radioactivity of individual capsules administered to each subject was between 2.750 and 2.754 MBq.

Subjects 101, 104, and 106 were discharged in the morning of Day 14, whereas subjects 102, 103, and 105 were discharged in the morning of Day 15.

**Excretion profiles in faeces and urine**

The cumulative excretion of radioactivity in faeces and urine is shown in Figure 1. Mean (± SD) total recovery of radioactivity from faeces and urine was 73.6% (± 6.2%) of the administered dose. The urinary excretion, accounting for a mean (± SD) recovery of 49.7% (± 3.9%) of the administered dose at the end of study, was a more

![Mean cumulative excretion profile of total radioactivity in faeces and urine (expressed as % of administered dose) until Day 14, after a single oral administration of 10 mg ¹⁴C-macitentan.](image-url)
important route of elimination of drug-related material than faeces in humans.

**Metabolite profiles in plasma, urine, and faeces**

Representative HPLC radiochromatograms of plasma pool 1 (time points 1 and 3 h), 3 (time points 7 and 8 h) and 7 (time points 72 and 96 h), a urine pool collected during the interval 48–72 h, and from a faeces pool sample collected during the interval 48–72 h are presented in Figure 2. In plasma, apart from parent macitentan, and the known oxidative depropylated metabolite, ACT-132577, the carboxylic acid ACT-373898 was identified. The retention times (RTs) and chemical structures of macitentan, ACT-132577, and ACT-373898 were confirmed with the synthetic standards as reference.

ACT-373898, which was first identified in this study, represented more than 20% of radioactivity of macitentan and about 8% of the total plasma radioactivity in the plasma pools analysed (estimated in terms of total counts per minute [CPM] measured). ACT-132577 represented about 190% of macitentan and 60% of the total plasma radioactivity.

In urine, four entities were identified: the conjugate of ACT-132577 with glucose (M 706 u), ACT-080803 resulting from hydrolysis of the sulfamide moiety of either macitentan or ACT-132577, ACT-373898, and its hydrolysis product (M 323 u).

![Figure 2. Metabolite profiles for plasma pool 1 (time points 1 and 3 h), 3 (time points 7 and 8 h), and 7 (time points 72 and 96 h), urine pool 3 (time interval 48 to 72 h), and faeces pool 3 (time interval 48 to 72 h).](image-url)
ACT-373898 and M 323 u accounted for 22.9% and 26.0%, respectively, of radioactivity excreted in urine. M 706 u and ACT-080803 accounted for 24.8% and 7%, respectively, of radioactivity excreted in urine. Neither macitentan nor ACT-132577 was present in urine.

In faeces, five entities were identified: macitentan, ACT-132577, ACT-373898, its hydrolysis product (M 323 u), and ACT-080803.

ACT-080803 was the major product present in faeces, accounting for 37.7% of radioactivity excreted therein. Macitentan and ACT-132577 represented 16.9% and 14.0%, respectively, of radioactivity excreted in faeces. ACT-373898 and M 323 u accounted for 3.6% and 13.1%, respectively, of radioactivity excreted in faeces.

In addition to the five above-mentioned products, one minor metabolite, an oxidation product of macitentan with a molecular weight of 602 u but unidentified chemical structure, was also identified in faeces. M 602 u accounted for 2% of radioactivity excreted in faeces.

The proposed metabolic pathways of macitentan in humans and chemical structure of metabolites are presented in Figure 3.

The LC-MS/MS spectra of identified entities are presented in Figure 4 and the fragmentation analysis is presented in Table 1.

The recovery after extraction of 14C from plasma and faeces was 100%, and 93.2%, respectively. The recovery after sample injection and separation on the HPLC column was around 100% for all matrices. The recovery after reconstitution for faeces was around 50%. The low recoveries after reconstitution are more likely explained by the high concentrations of suspended sediment in faeces samples, which may have hindered the complete dissolution of the radioactive compounds in the dried faeces extracts. The peaks resulting from radioactivity profiling were found to be intensive. In general, a plausible and conclusive time profile was found. Therefore, it is not expected that the recovery after reconstitution, has an influence on the results of the metabolic profiling.

**Analysis of macitentan, ACT-132577, and total radioactivity in plasma and whole blood**

Mean ± SD plasma concentration-time profiles of total radioactivity, macitentan, and ACT-132577 are shown in Figure 5.

Macitentan showed a slow absorption with a geometric mean \( C_{\text{max}} \) of 170 ng/mL, attained 6 h after dosing (ranging between 5 and 10 h) and a geometric mean terminal \( t_{\text{1/2}} \) of 15 h. For ACT-132577, the \( C_{\text{max}} \) (geometric mean 121 ng/mL) was attained 48 h after dosing and its geometric mean terminal \( t_{\text{1/2}} \) was 44 h. The plasma concentration-time profile of total radioactivity (ng equiv./mL) was also characterized by slow oral absorption. \( C_{\text{max}} \) of total radioactivity (geometric mean 235 ng equiv./mL) was attained after approximately 12 h (ranging between 7 and 24 h). Total radioactivity concentrations in plasma were higher than the sum of macitentan and ACT-132577 concentrations at most of the time points. The geometric mean terminal \( t_{\text{1/2}} \) of radioactivity in plasma was 103 h, which was longer compared to terminal \( t_{\text{1/2}} \) of macitentan (geometric mean 15 h) and ACT-132577 (geometric mean 44 h). \( \text{AUC}_{0-\infty} \) of total radioactivity in plasma was about 25% greater than the sum of \( \text{AUC}_{0-\infty} \) of macitentan and ACT-132577. For \( \text{AUC}_{0-t} \) this difference was approximately 19% (Table 2).

In whole blood, maximum total radioactivity (geometric mean 131 ng equiv./mL) was attained at 14 h (ranging between 6 and 24 h). At 264 h, the amount of radioactivity...
Geometric mean terminal t½ of total radioactivity in whole blood was 43 h which was shorter than the terminal t½ of total radioactivity in plasma (geometric mean 103 h). Concentrations of total radioactivity in whole blood were lower compared to plasma. Cmax and AUC0–t values of total radioactivity in plasma were about 80% and 96%, respectively, greater than in whole blood. For AUC0–∞ this difference was 105%.

Whole blood / plasma ratio of total radioactivity (ng equiv./mL) at 12 h post-dose was 0.55.

Safety and tolerability
There were no clinically relevant findings in vital signs, physical examination, electrocardiogram recording, and clinical laboratory tests. There were no SAEs or AEs
leading to study discontinuation. In total, three AEs of mild intensity were reported during the study, i.e. two cases of hematochezia (presence of blood in faeces) and one case of headache. No concomitant medication was received during the study. All AEs resolved spontaneously and without sequelae before the end of study.

**Discussion**

**Mass balance:** Mean (± SD) cumulative recovery of radioactivity from faeces and urine was 73.6% ± 6.2% of the administered dose. Excretion involved both renal and faecal routes and urine represented a more important elimination route for macitentan’s metabolites than faeces. A recent clinical study showed no clinically relevant differences in PK of macitentan, ACT-132577, and ACT-373898 between patients with mild, moderate or severe hepatic impairment due to liver cirrhosis and healthy subjects following a single oral dose of 10 mg 14C-labelled macitentan (n = 6). Data are expressed as arithmetic means on a linear scale.

Another possible explanation would be the long half-life of total radioactivity in the circulation. It has been reported that many compounds with a radioactivity half-life in plasma longer than 50 h do not reach the 80% threshold of radioactive dose recovery (Roffery et al. 2007). The long plasma half-life of total radioactivity in this study (103 h) and slow elimination of radioactive material might have contributed to the incomplete recovery of radioactivity in excreta. This is consistent with the small amount of radioactivity excreted each day, especially in faeces. Maximum mean faecal excretion was on Day 6 with only 4.3% of administered dose (data not shown). Since the amounts of radioactivity excreted during the last days of sampling were very low, with extended sample collections the amount of excreted radioactivity would have reached soon the
Disposition of macitentan in man 9

Table 2. Summary statistics of pharmacokinetic parameters of macitentan and ACT-132577 in plasma, and total radioactivity (ng equivalent) in plasma and whole blood, after a single oral administration of 10 mg 14C-labelled macitentan.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>N</th>
<th>t_{max} [h]</th>
<th>C_{max} [ng/mL]</th>
<th>t_{1/2} [h]</th>
<th>AUC_{0-∞} [ng.h/mL]</th>
<th>AUC_{0-t} [ng.h/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactivity in whole blood</td>
<td></td>
<td>6</td>
<td>14.0 (6.0–24.0)</td>
<td>131 (119, 144)</td>
<td>42.9 (31.6, 58.1)</td>
<td>11385 (10405, 12457)</td>
<td>11149 (10293, 12076)</td>
</tr>
<tr>
<td>Radioactivity in plasma</td>
<td></td>
<td>6</td>
<td>12.0 (7.0–24.0)</td>
<td>235 (208, 266)</td>
<td>103.0 (93.9, 112.9)</td>
<td>23232 (20238, 26873)</td>
<td>21885 (18971, 25248)</td>
</tr>
<tr>
<td>Macitentan</td>
<td></td>
<td>6</td>
<td>6.0 (5.0–10.0)</td>
<td>170 (134, 214)</td>
<td>15.0 (13.6, 16.5)</td>
<td>5541 (4950, 6204)</td>
<td>5494 (4903, 6156)</td>
</tr>
<tr>
<td>ACT-132577</td>
<td></td>
<td>6</td>
<td>48.0 (48.0–48.0)</td>
<td>121 (108, 135)</td>
<td>44.0 (37.9, 51.2)</td>
<td>13055 (11543, 14767)</td>
<td>12924 (11455, 14581)</td>
</tr>
</tbody>
</table>

aData are expressed as geometric mean (95% confidence limit).

LOQ and an accurate measurement would not be possible. Possible enterohepatic recirculation of macitentan and ACT-132577 has been investigated using the tandem rat model. In these experiments enterohepatic recirculation of macitentan and ACT-132577 was not evident. Also in humans there were no indications of enterohepatic recirculation of either compound. In the rat and dog mass balance studies, excretion of radioactivity was complete at the end of the sample collection period (216 h for rat, and 336 h for dog), which indicates a rather slow excretion. On the other hand, the tissue distribution of 14C-radiolabelled macitentan investigated in rats indicated extensive distribution, with a slow disposition into tissues. Slow disposition to tissues could be a possible explanation for the long half-life of radioactivity observed in the present study (Actelion Pharmaceuticals, data on file).

Metabolite profiling: There were three, four, and five entities in plasma, urine, and faeces, respectively. The results of this study indicate that macitentan undergoes two major metabolic reactions:

1. oxidative depropylation to form ACT-132577. This metabolite subsequently undergoes conjugation with glucose to form M 706 u, which is eliminated in urine. M 706 u was not present in faeces.

2. oxidative cleavage of the ethylene glycol linker to form the carboxylic acid, ACT-373898, which in turn undergoes hydrolysis to form M 323 u. Both are present in urine and faeces and constitute the major radio-labelled products in urine.

Both macitentan and ACT-132577 can be chemically hydrolyzed to the aminopyrimidine ACT-080803, which is present in both urine and faeces, and is the major radio-labelled product in faeces. Both macitentan and ACT-132577 are present in faeces. Macitentan in faeces could reflect the non-absorbed fraction of the dose.

The metabolites identified in humans had also been detected in animal studies and the latter provided sufficient coverage for the human situation. In addition, in vitro metabolic patterns with hepatocytes and liver microsomes of rat, mouse, dog, and man were similar (Actelion Pharmaceuticals, data on file). Therefore, no unique human metabolite was identified in this study. In this study no metabolite structure was identified, which would be indicative of a safety concern. Based on in vitro and in vivo toxicity studies there was no evidence of genotoxicity with macitentan. In addition, in different clinical studies with more than 300 healthy subjects, and 850 patients treated with different doses and for different durations with macitentan no safety concern has been identified so far.

PK in plasma and blood: The plasma concentration-time profiles of macitentan and ACT-132577 were comparable with those in previous studies and are consistent with a once-a-day dosing (Kummer et al. 2009; Sidharta et al. 2011).

Concentrations of total radioactivity in plasma were greater compared to whole blood. Ratio of total radioactivity concentration blood / plasma of 0.55 is close to the theoretical minimum blood-to-plasma distribution ratio, \( l^- \) haematocrit = 0.57 (based on the haematocrit measurements performed at screening and EOS). This indicates that macitentan and its metabolites poorly bind to or penetrate into erythrocytes. This is also in accordance with in vitro data on blood / plasma cell partitioning, which showed no notable uptake of either macitentan or ACT-132577 into human erythrocytes (Actelion Pharmaceuticals, data on file).

Conclusion

In this study the disposition of macitentan in man has been well characterized and its metabolic pathways were proposed, leading to the identified metabolites. Urine was the major route of elimination for macitentan’s metabolites in humans. In plasma, parent compound macitentan, ACT-132577, a pharmacologically active metabolite, and the carboxylic acid metabolite ACT-373898 were identified.

Declaration of interest

This study was funded by Actelion Pharmaceuticals Ltd, Allschwil, Switzerland. Bruderer, Sidharta, Treiber, and Dingemanse are employees of Actelion Pharmaceuticals Ltd. Hopfgartner was member of the Scientific Advisory Board and Wank was employee at Swiss BioAnalytics and received financial compensation for their work related to the study. Seiberling was the principal investigator of the clinical trial and received financial compensation for the clinical costs associated with conducting the study.
References


