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Evaluation of Interethnic Differences in Repinotan Pharmacokinetics by Using Population Approach

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Summary: Repinotan is a selective full serotonin receptor agonist at the 5-HT1A subtype which has been studied in phase I and II studies involving over 500 healthy subjects and patients. Repinotan is primarily metabolized by CYP2D6 which is known to be subject to polymorphism and ethnic differences in its quantitative and qualitative expression pattern. In order to investigate the effect of ethnicity on repinotan pharmacokinetics (PK) between a Caucasian and Japanese population and to explain PK variability, this population PK evaluation was conducted.

A population PK model was established based on the data of 1314 blood samples from 241 patients from 3 Phase II studies. This analysis has characterized the repinotan PK, with particular attention to ethnicity. Using the MIXTURE subroutine of NONMEM, evidence was provided for different CL groups. Repinotan plasma levels in the ‘High CL’ subgroup, which comprised the majority of patients, did not show relevant differences between a Japanese and Caucasian population. In the ‘Low CL’ subgroup, Japanese and Caucasian populations were different. These findings are consistent with the published literature, which reports ethnic differences in the distribution of CYP2D6 activity. The finding of a greater percentage of patients with intermediate CL in the Japanese population falling between poor and extensive metabolizers is consistent with the distribution pattern of CYP2D6 in the Japanese population. The results of this evaluation can be used to assist in designing future trials.

Key words: population pharmacokinetics; NONMEM; repinotan; interethnic evaluation

Introduction

Repinotan hydrochloride (repinotan) is a selective, high affinity, full serotonin receptor agonist at the 5-HT1A subtype. In animal models of acute stroke, significant neuroprotective effects including reductions in infarct volume were observed when repinotan was administered at intervals up to 5 hours after cerebral artery occlusion.1,2) Repinotan has been studied in phase I and II studies involving over 500 healthy subjects and patients.3–5) No significant safety and tolerability issues were observed, and adverse events were limited primarily to dose-related central nervous system effects.

Regarding the pharmacokinetics (PK) of repinotan, it has been demonstrated in non-clinical and phase I studies that repinotan is metabolized mainly by the cytochrome P450 2D6 (CYP2D6) isoenzyme [data on file, Bayer HealthCare AG]. This isoenzyme is known to exhibit a polymorphism which results in a bimodal distribution with a poor metabolizer (PM) and an extensive metabolizer (EM) population. The quantitative distribution of EM and PM subjects in a population also depends on ethnicity with the percentage of PM subjects estimated at about 7% in Caucasians and about 1% in Chinese, Japanese and Korean populations, respectively.6,7) Differences in genotype and/or phenotype would be expected to translate into interethnic differences in the PK of repinotan.

However, due to the acute nature of the target indication stroke it is not possible to conduct genotyping or phenotyping studies in patients in advance in order to select the appropriate dose for administration of repinotan. Therefore, before conducting large scale clinical studies, we evaluated the PK of repinotan in order to investigate whether it is subject to ethnic differences between Japanese and Caucasian patients, using plasma
concentration data from 3 clinical phase IIa studies and the population approach with NONMEM.8)

Methods

Study design and patients population: Data from 3 clinical phase IIa studies were used in this evaluation including a study in Caucasian patients with traumatic brain injury (STUDY I), a second study in Caucasian patients diagnosed with acute ischemic stroke (STUDY II) and a third study in Japanese patients with acute ischemic stroke (STUDY III).

STUDY I and II used three dosages in a group-wise design which were 0.5, 1.25 and 2.5 mg/day whereas study III did not include the dose of 2.5 mg/day. Doses are given in repinotan free base equivalents. In STUDY I the infusion lasted for 7 days, whereas in STUDY II and III the infusion was given over 3 days. The studies were conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study protocols and patient consent procedure were approved by an Institutional Review Board at each study centre.

The plasma samples were collected at predefined sampling times: in STUDY I at 0, 2, 6, 12, 24, 48 and 168 hours after start of infusion and 2, 6, 12 and 24 hours after stop of the infusion, in STUDY II at 0, 1, 2, 6, 10 and 72 hours after start of infusion and 6 and 24 hours after stop of the infusion and in STUDY III at 0, 6, 24, 48 and 72 hours after start of infusion.

Details of the respective study designs as well as the main demographic and biochemical characteristics of the dataset are given in Table 1.

Assay of repinotan in plasma: Blood samples were collected in 5 mL NH4-heparin tubes at each sampling point. After centrifugation, the plasma supernatant was added to a labeled polystyrene tube which was immediately frozen and stored at or below 20°C until analysis. The samples were analyzed by a validated gas-chromatography assay with electron capture
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detection following liquid/liquid extraction. To guarantee the comparability of the generated concentration data across the studies, the involved analytical laboratories had performed successful cross-validations prior to analysis of study samples. The range of the quantification was from 0.05 µg/L, which is the lower limit of quantification (LLOQ), to 100 µg/L. Inter-day accuracy was within ±10% and inter-day precision was <15% consistently. Since concentrations were measured as repinotan hydrochloride equivalents, the reported concentration data were converted to free base equivalents for the PK evaluation by multiplying with the factor 0.917. All concentrations below the LLOQ were removed and 7 subjects whose repinotan plasma-concentration at time = 0 were 3 times greater than the LLOQ were excluded.

Population Pharmacokinetic model: The data was analyzed with the software package NONMEM Version V level 1.0 with first order (FO) estimation method under IRIX 6.5 on a SGI workstation. All statistics and graphics were made with S-PLUS and Xpose.9) Two levels of random effects were used: The first level of random effects, inter subject variability, \( \eta \), having a mean of 0 and a variance of \( \omega^2 \) is modeled as having a constant coefficient of variation. The second level of random effects, the in-patient variability, \( \epsilon \), also has a mean of 0 and has a variance of \( \sigma^2 \). This intra subject variability or better-called residual error can include model misspecification, variation in analytical assay, variation in sampling or dosing times etc.

To build up the population PK model, Generalized Additive Models (GAM) and the output from the NONMEM posthoc estimation step were used. If the covariate had categorical values, the parameter (P) was modeled as:

\[
P = \Theta_1 \text{ (if covariate } = 1) \text{ or } P = \Theta_2 \text{ (if covariate } = 2)\]

If the covariate had continuous values, variables were centered to their mean values from the whole dataset. For example, the relationship between P and body weight (WGT) was modeled as:

\[
P = \Theta_1 + \Theta_2 \cdot (\text{WGT-Mean of WGT})\]

Where P is population parameter, \( \theta_1 \) is the value for P of a patient with the mean WGT, and \( \theta_2 \) is the fraction of WGT.

The influence of covariates on the PK model was investigated employing the Likelihood Ratio Test at a level \( p < 0.05 \). Regarding gender and race as covariates, they were evaluated after checking all other covariates since they are considered to be biased by other covariates (see Fig. 1). When no further improvement in fit was obtained, each covariate was tested by removing it from the model and assessing if the resulting NONMEM run was significantly worse at a level \( p < 0.01 \). If the model was not significantly worse, the covariate was left out; otherwise the covariate was left in the model. Using the ‘remaining’ model the next covariate was tested. Finally the model only included covariates being significant at \( p < 0.01 \).

The evaluation used a two compartment model with elimination from the central compartment (ADVAN3 TRANS4). In this evaluation, the effect of covariates, such as body size (body weight, calculated body surface area (BSA),10) calculated lean body mass (LBM) and calculated fat (FAT),11) AGE, GENDER, RACE and comedication on PK parameters was evaluated. Comedicated drugs were classified into the following categories according to their interaction with selected cytochrome isoenzymes; CYP2D6 substrate, CYP2D6

\[
\text{Fig. 1. Relationships among RACE, GENDER and BSA in the dataset.}
\]
inhibitor, CYP3A4 substrate, CYP3A4 inhibitor and CYP3A4 inducer. These classifications were based on the Flockhart’s list.\footnote{12)}

The MIXTURE subroutine in NONMEM allows mixture modeling to be carried out within the context of mixed-effects modeling. A mixture model assumes that the population consists of two or more subpopulations, each approximating a normal distribution, where each subpopulation may have its own model. For example, with two subpopulations it might be assumed that one fraction of the population has one set of typical values of the parameters while the remaining fraction has another set of typical values. The ratio of each fraction and the corresponding sets of typical values can be estimated, and NONMEM assigns individuals to one of the subpopulations.

**Results**

A total of 1314 plasma concentration data from 241 patients were obtained and Fig. 2 shows repinotan plasma concentrations through 3 studies. A two compartment PK model with first order elimination from the central compartment was found to optimally describe the data as a result of the comparison of individual data fitting and objective function values between a 1 compartment model (objective function value: 4016.902) and a 2 compartment model (objective function value: 3538.072). With respect to the error model for residual variability, additive, proportional and combined additive plus proportional error models were tested. Because of the objective function value and goodness-of-fit plots, inter subject variability was modeled as being proportional and intra subject variability was modeled as being a combination of additive and proportional. Because plots of the Bayesian estimates of CL and V2 for this model showed a possible correlation, a parameter estimating the covariance in the two parameters was added to the model.

On the basis of plots of Bayesian parameters, the following covariates in the full population PK model were selected for evaluation:
- LBM, FAT, BSA, comedication of CYP2D6 inhibitor, RACE and STUDY for CL
- BSA for V1
- STUDY, GENDER and comedication of CYP2D6 inhibitor for Q
- FAT and GENDER for V2.

During the backward elimination process, each covariate was tested separately. The final population PK model and its precision estimates are given in the following equations and in Table 2.

\[
CL_{\text{subgroup} A} = \Theta_{CL} + \Theta_{BSA} \cdot (BSA - 1.803)
\]

\[
CL_{\text{subgroup} B} = \Theta_{CL}^{\text{MIX}} \cdot (\Theta_{CL} + \Theta_{BSA} \cdot (BSA - 1.803) \cdot \Theta_{JPN})
\]

\[
V1 = \Theta_{V1}
\]

\[
Q = \Theta_{Q} \cdot \Theta_{\text{female}}
\]

\[
V2 = \Theta_{V2} + \Theta_{V2}^{\text{FAT}} \cdot (FAT - 21.7)
\]

In the goodness-of-fit of the final population PK model in the whole dataset and the two subgroups which are presented in Fig. 3, there were no trends and biases. Figure 4 shows that the individual weighted residuals (IWRES) vs individual predicted concentrations...
Table 2. Results from final population PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Change&lt;sup&gt;4)&lt;/sup&gt;</th>
<th>Estimate</th>
<th>SE</th>
<th>Inter subject variability Estimate&lt;sup&gt;2)&lt;/sup&gt;</th>
<th>SE</th>
<th>Residual variability Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>—</td>
<td>7.45 L/hr</td>
<td>0.342</td>
<td>46.8%&lt;sup&gt;3)&lt;/sup&gt;</td>
<td>0.17</td>
<td>73.5%&lt;sup&gt;3)&lt;/sup&gt;</td>
<td>0.35</td>
</tr>
<tr>
<td>% of Subgroup A</td>
<td>1587.215</td>
<td>76.6%</td>
<td>0.0286</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;BSA&lt;/sub&gt;</td>
<td>BSA for CL</td>
<td>41.999</td>
<td>6.22</td>
<td>0.893</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;MIX&lt;/sub&gt;</td>
<td>IF Subgroup B</td>
<td>1493.542</td>
<td>0.12</td>
<td>0.0168</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;PV&lt;/sub&gt;</td>
<td>IF JAPANESE</td>
<td>23.476</td>
<td>2.42</td>
<td>0.543</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;V1&lt;/sub&gt;</td>
<td>V1</td>
<td>—</td>
<td>11.1 L</td>
<td>1.58</td>
<td>42.2%</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;Q&lt;/sub&gt;</td>
<td>Q</td>
<td>—</td>
<td>15.3 L/hr</td>
<td>5.54</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;Gfemale&lt;/sub&gt;</td>
<td>IF FEMALE</td>
<td>32.335</td>
<td>0.309</td>
<td>0.0742</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;V2&lt;/sub&gt;</td>
<td>V2</td>
<td>—</td>
<td>9.12 L</td>
<td>1.74</td>
<td>85.6%</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>θ&lt;sub&gt;FAT&lt;/sub&gt;</td>
<td>FAT for V2</td>
<td>29.77</td>
<td>0.345</td>
<td>0.112</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation between parameters:
- CL for Subgroup A and V1: 21.51% | 0.17
- CL for Subgroup B and V1: 16.58% | 0.31

Residual error:
- additive error [μg/L]: 0.22 | 0.09
- Constant CV error (%): 21.2 | 0.108

<sup>1</sup>CV%, <sup>2</sup>Subgroup A, <sup>3</sup>Subgroup B, <sup>4</sup>—2 log likelihood difference from the values for the final model if a covariate was deleted.

Fig. 3. Goodness-of-fit plots for the final population pharmacokinetic model for repinotan. (A) Observed concentrations vs. individual predicted concentrations in whole dataset (B) Observed concentrations vs. individual predicted concentrations in each subgroup.
Fig. 4. Individual weighted residual plots for the predicted concentration (A) in whole dataset (B) in each subgroup.

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Race</th>
<th>Group</th>
<th>Population mean parameters (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>Caucasian</td>
<td>Subgroup A</td>
<td>7.80 L/hr (7.03–8.57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subgroup B</td>
<td>0.94 L/hr (0.61–1.31)</td>
</tr>
<tr>
<td></td>
<td>Japanese</td>
<td>Subgroup A</td>
<td>5.77 L/hr (5.57–5.97)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subgroup B</td>
<td>1.67 L/hr (0.66–3.18)</td>
</tr>
<tr>
<td>V1</td>
<td></td>
<td></td>
<td>11.1 L (8.0–14.2)</td>
</tr>
<tr>
<td>Q</td>
<td></td>
<td></td>
<td>15.3 L/hr (4.4–26.2)</td>
</tr>
<tr>
<td>V2</td>
<td>Caucasian</td>
<td></td>
<td>5.48 L (5.24–10.44)</td>
</tr>
<tr>
<td></td>
<td>Japanese</td>
<td></td>
<td>5.33 L (4.33–6.29)</td>
</tr>
</tbody>
</table>

Typical Caucasian: male, 75.5 Kg body weight, 168.8 cm body height and no concomitant drugs, Typical Japanese: male, 55.3 Kg body weight, 155.3 cm body height and no concomitant drugs. The typical values of body weight and body height are the respective mean values of Japanese and Caucasian data.

Table 3. The values in Table 3 are calculated for a typical male Caucasian patient of 75.5 Kg body weight and 168.8 cm body height as mean value in the Caucasian data who has no concomitant drugs and for a typical male Japanese patient of 55.3 Kg body weight and 155.3 cm body height as mean value in the Japanese data who also has no concomitant drugs.

In the final model, body size effect, such as FAT and BSA, were retained as covariates for CL and V2 and ethnicity remained as covariate for CL. The factors having the most pronounced influence on CL were body size and race, though the covariates of body size and race influences have multi correlation as shown in Fig. 1. Intra subject variability was 0.22 μg/L (additive error) and 21.2% (CV error).

Discussion

The population PK model of repinotan in Japanese and Caucasian patients was based on a two compartment model with first order elimination from the central compartment. NONMEM with MIXTURE subroutine revealed different CL groups, which are referred to as Subgroup A and B in the whole dataset. These subgroups can not be explained by other covariates as the two CL subgroups were not biased by demographic data or other covariates. This finding is consistent with previous phase I studies, which had also demonstrated the existence of two CL groups for repinotan in healthy volunteers. In the Subgroup B the CL was lower (0.894 L/h) than the Subgroup A (7.45 L/h) but the inter subject variability was also clearly increased; 73.5% for the Subgroup B and 46.8% for the Subgroup A. While overall 23.4% of the patients were assigned to Subgroup (IPRED) were symmetrically distributed in the whole dataset and the two subgroups. The model with MIXTURE option was better than the model without MIXTURE option (objective function value with MIXTURE option: 3538.072, objective function value without MIXTURE option: 5389.346). Since NONMEM results were unstable if three or more subgroups were adapted to this dataset with the MIXTURE option and in agreement with the known polymorphism of CYP2D6, we decided to use two subgroups, called Subgroup A (‘High CL’) and Subgroup B (‘Low CL’). The percentage in the whole dataset was 76.6% for Subgroup A and 23.4% for Subgroup B, respectively. The population PK parameters for each ethnic group are summarized in
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Fig. 5. Typical plasma repinotan concentration vs. time profiles for each group.

The curves were plotted for a Caucasian male patient with 75.5 Kg body weight, 168.8 cm body height using no concomitant drugs and for a Japanese male patient with 55.3 Kg body weight, 155.3 cm body height also not using concomitant drugs. Dosing regime; 0–2 hr: 0.1 mg/hr, 2–72 hr: 0.05 mg/hr. The black solid black line represents Caucasian subgroup A, dashed black line = Caucasian subgroup B, solid gray line = Japanese subgroup A and dashed gray line = Japanese subgroup B.

B, 22.4% of the Caucasian patients were estimated to belong to Subgroup B compared to 25.8% of the Japanese patients.

Although a gender effect on Q can not be explained by physiology, it can theoretically be due to a difference in sampling points and/or imbalance of data. Due to the small contribution of Q to the PK of repinotan, however, its overall impact is considered to be minor.

Based on the final model, population mean plasma concentration profiles for Subgroup B and Subgroup A in each race are presented in Fig. 5, and Fig. 6 shows the distribution of individual predicted CL values in each race. The distributions within Subgroup A (High CL) in the two ethnic groups were similar, but the distribution within the Japanese Subgroup B was different from the Caucasian Subgroup B. This suggests that the group of intermediate metabolizers (IM) with CYP2D6 activity falling between poor and extensive subjects is included in Subgroup B.

Pharmacogenomic (PG) data were not collected in this study i.e. these patients with acute ischemic disease were not genotyped (nor phenotyped) in view of the fact that prompt treatment was required. Therefore, we can not correlate the population PK results to PG investigations. However, the present findings are consistent with previous reports of a different distribution of the PM phenotype between the ethnic groups of Caucasians and Japanese whereby the percentage of PM subjects is estimated at about 7% in Caucasians and about 1% in Chinese, Japanese and Korean populations, respectively. Moreover, it was shown that a larger number of IM exists in the Japanese population.

A MIXTURE subroutine in NONMEM has been developed for the analysis of a subpopulation. The usefulness was shown in a simulation study of population PK analysis and population pharmacodynamics. Some applications to real clinical studies were also reported. In this evaluation the MIXTURE option was useful to divide different CL groups since it provided a significantly better fit to each dataset. Moreover, the existence of High and Low CL groups is consistent with the metabolic pathway of repinotan. Actually the NONMEM estimated proportion of subgroups was not fully consistent with the reported EM/PM ratio of CYP2D6 in Caucasians (7% PM) and Japanese (1% PM), respectively. This discrepancy would appear to be due to the fact that other factors than CYP2D6 phenotype also impact on repinotan plasma concentrations, especially in patients who have varied covariable factors.

To investigate the interethnic differences in PK, the plasma repinotan concentrations at the end of infusion were simulated (n = 1000) based on the final PK model and the distribution is shown in Fig. 7. The simulations are based on the following dosing regime which appears to be appropriate for clinical use based on preclinical data; 0–2 hr: 0.1 mg/hr, 2–72 hr: 0.05 mg/hr. It can be seen that the Japanese Subgroup B mainly results in intermediate concentrations (mean = 37.6 µg/L SD = 28.3 µg/L) while in the Caucasian population a higher percentage of concentrations above 50 µg/L is present. This is supported by the evaluation with an estimated mean CL for the Caucasian Subgroup B more than 2 times lower than that estimated for the Subgroup B of Japanese origin.
Since genotyping and phenotyping are not applicable as diagnostic tools to identify low CL patients, repinotan plasma concentrations should be monitored at the bed side i.e. as patients are receiving the drug in order to avoid high plasma concentrations in this group. A device allowing therapeutic drug monitoring (TDM) of repinotan in plasma has been developed [data on file, Bayer HealthCare AG]. Based on the read-out of this device a dose adaptation can be performed i.e. infusion rate and dose are reduced in low CL patients in order not to reach unduly high plasma concentrations. A higher proportion of Caucasian patients than Japanese patients are expected to benefit from such a device as a relatively greater number of poor metabolizers are present in the Caucasian population (see also simulation in Fig. 5).

In conclusion, this population PK analysis has characterized the repinotan PK, especially with regard to the influence of ethnicity. Using the MIXTURE subroutine of NONMEM, it was demonstrated that there are different CL groups and that the ratio of the two identified subgroups in each race is different. Since PG data could not be collected in the clinical trials, the relationships between subgroups and CYP2D6 geno- or phenotype could not be investigated. Although the ratio of EM/PM for CYP2D6 was not exactly consistent with the published ratio based on other CYP2D6 substrates, it can be concluded that the Japanese population comprises a relevant portion of metabolizers of the intermediate type which stands between poor and extensive metabolizers.

The results of this population PK evaluation can be used to assist in designing future clinical trials, which comprise patients of different ethnic origin.

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References

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