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Population Pharmacokinetic-Pharmacodynamic Modeling and Simulation of Platelet Decrease Induced by Peg-interferon-alpha 2a

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Summary: Peg-interferon-alpha-2a (PEG-IFN) has been used all over the world including Japan as the standard of care for chronic hepatitis C (CHC). PEG-IFN causes platelet count decrease, while CHC patients with compensated liver cirrhosis have a low baseline of platelets. To use PEG-IFN more safely in these patients, we analyzed the effect of PEG-IFN on the longitudinal platelet profile with a pharmacokinetic-pharmacodynamic model. Platelet count and serum PEG-IFN concentration obtained from a Japanese clinical study on 40 patients were analyzed. The serum PEG-IFN concentration profile was fitted with an open 1-compartment model and the platelet profile was fitted with a turnover model. After the final model was fixed, the platelet profiles were simulated with various platelet baselines. The simulation revealed that according to PEG-IFN administration platelets decreased gradually and reached steady state within 12 weeks, and almost subjects would not have a lower platelet count than the criteria for discontinuation of the treatment. Once administration was discontinued, platelets recovered up to the baseline within several weeks. In conclusion, platelet count was predicted to be about a 30% (5th–95th percentiles in 1,000 simulation: 11–66%) decrease and to return to the baseline value in 4 to 8 weeks after the last administration of PEG-IFN.

Keywords: Peg-interferon-alpha-2a; PK-PD; modeling; simulation; platelet; liver cirrhosis; chronic hepatitis C

Introduction

Concurrent medication with Peg-interferon alpha-2a (PEG-IFN)/rivavirin is a standard of care for chronic hepatitis C (CHC). The medication with PEG-IFN/rivavirin has also been used for the treatment of CHC with compensated liver cirrhosis (CHCLC). Although this concurrent medication is shown to be effective, physicians have to use it while ensuring patient safety by means of dose reduction and discontinuation, because the medication causes many adverse events. In particular, since interferons have an inhibitory effect on the bone marrow, the dose has to be controlled by monitoring the decrease of erythrocytes, neutrophils, and platelets.

In Japan, the concurrent medication with PEG-IFN/rivavirin is also the standard of care for CHC patients both with and without compensated liver cirrhosis. It has been known that CHCLC patients have a low platelet baseline; thus, the platelet count is particularly important from a safety point of view. However, no model has been reported to date that quantitatively describes how the platelet count is affected by PEG-IFN in Japanese CHCLC patients. Accordingly, we developed a pharmacokinetic-pharmacodynamic (PK-PD) model to predict the decrease in platelet count for this patient population during and after PEG-IFN therapy.

Methods

Subjects and study design: A multicenter, randomized, parallel-group comparison study was performed with 40 (male 22, female 18) CHCLC patients. The median (minimum–maximum) age, body weight, and platelet baseline were 60 (38–75) years old, 63.55 (42–94) kg, and 101 (75–217) × 10^9/L, respectively. PEG-IFN was administered at a dose of 90 or 180 µg subcutaneously every week for 48 weeks. From the safety point of view, appropriate dose reduction and discontinuation were done when hemoglobin, neutrophils or platelets decreased, or ALT increased. For example, in the case where platelets decreased to less than 50 or 35 × 10^9/L, half or a quarter...
amount of PEG-IFN was administered. When platelets decreased to $25 \times 10^9/L$, the administration was discontinued. When the platelets recovered to more than $50 \times 10^9/L$, the dose returned to the initial level.

This clinical study was performed in accordance with the Declaration of Helsinki, and local laws and regulations, and informed consent was obtained from all patients in written form. Blood was collected to determine the serum concentrations of PEG-IFN. The trough concentration was obtained 168 h after administration in week 12. After the plate was washed, primary antibody was added and incubated on the plate. After the plate was washed again, and a secondary antibody was added and incubated. After a final wash, a substrate solution was added. The intensity of oxidation was measured by a sandwich enzyme-linked immuno-sorbent assay (ELISA), in which rabbit anti-PEG-IFN was coated onto microtiter plates according to the method previously reported.12 The samples were added and incubated on the plate. After the plate was washed, primary antibody (mouse AGP3) was added followed by incubation. The plate was washed again, and a secondary antibody (peroxidase-conjugated goat anti-mouse IgM) was added and incubated. After a final wash, a substrate solution was added. The intensity of color developed in proportion to the amount of PEG-IFN in the sample. The detection limit of the assay was 250 pg/mL.

The inter-assay reproducibility and intra-assay reproducibility of serum concentrations of PEG-IFN were examined in the concentration range from 250 pg/mL to 5,000 pg/mL; the accuracy and precision in the intra-assay reproducibility were $-5.5\%$ to $13.2\%$ and $2.5\%$ to $8.4\%$, respectively, and the accuracy and precision in the inter-assay reproducibility were $0.4\%$ to $9.0\%$ and $11.7\%$ to $16.6\%$, respectively.

**Model analysis:** NONMEM® Version 7.1.0 (ICON Development Solutions, Ellicott City, MD, USA) was used for the population PK and PK-PD analyses. CPU of the computer, OS, and fortran used for analyses were Intel® Xeon® 2.53 GHz, Windows XP professional service pack 3, and Intel® fortran compiler version 11.1, respectively. The model parameters were estimated by a nonlinear mixed effects analysis with FOCE with the interaction method. The estimated population model parameters were the fixed effects ($\theta$), related to the typical individual, and the random effects, with magnitudes of inter-individual variability in parameters ($\omega^2$) and magnitudes of residual variability ($\sigma^2$).

A logarithmic normal distribution expressed by the following equation is assumed for the inter-individual variability

$$\theta_i = \theta \exp (\eta)$$

where $\eta$ is a random variable distributed normally with a mean of zero and variance of $\omega^2$.

In the residual variability, $Y_{ij}$ is defined as the $j$th observation of the $i$th subject, $\hat{Y}_{ij}$ hat is defined as the $j$th predicted value of the $i$th subject, and the relation between $Y_{ij}$ and $\hat{Y}_{ij}$ is expressed by the following equations:

**Additive error model**

$$Y_{ij} = \hat{Y}_{ij} + \epsilon_{ij}$$

**Proportional error model**

$$Y_{ij} = \hat{Y}_{ij}(1 + \epsilon_{ij})$$

**Combined error model**

$$Y_{ij} = \hat{Y}_{ij}(1 + \epsilon_{ij}) + \epsilon_{2ij}$$

where $\epsilon_{ij}$, $\epsilon_{1ij}$, and $\epsilon_{2ij}$ are random variables distributed normally with a mean of zero and variance of $\sigma^2$.

**Population PK analysis:** A PK model analysis was performed using the patients whose serum concentration of PEG-IFN was measured. The dataset above the lower limit of quantification was used for the analysis. Since it has been reported that an open 1-compartment model was used for the PEG-IFN,12,13 and the serum PEG-IFN concentration-time profile did not show biphasic elimination in this dataset, the 1-compartment model was applied. The estimated parameters are population mean values of absorption rate constant ($k_a$), apparent clearance (CL/F), apparent distribution volume (V/F), inter-individual variability of CL/F, V/F and residual errors. NONMEM subroutine ADVAN2 was used for these calculations.

**Population PK-PD analysis:** The population PK-PD model was performed for the subjects whose serum PEG-IFN concentration and platelet counts had been measured. After the PK parameters of individual subjects were fixed, the following 4 models were examined to describe the decrease of platelet count: basic life span indirect model, modified life span indirect model, turnover model with input inhibition and turnover model with output stimulation.14,15 Figure 1 shows the PK-PD model structure.

The population mean values, inter-individual variability, and residual errors were calculated for PD parameters. NONMEM subroutine ADVAN 6 was used for these calculations. The differential equations of each PD model are as follows:

**Basic life span indirect model**

$$\frac{dA}{dt} = R_{in} \left(1 - I_{max} \frac{C_{PEG-IFN}}{IC_{50} + C_{PEG-IFN}}\right) - T_R$$

**Modified life span indirect model**

$$\frac{dA_{pre}}{dt} = R_{pre} \left(1 - I_{max} \frac{C_{PEG-IFN}}{IC_{50} + C_{PEG-IFN}}\right) - k_{in} A_{pre}$$

$$\frac{dA}{dt} = k_{in} A_{pre} - T_h$$

**Turnover model with input inhibition**

$$\frac{dA}{dt} = R_{in} \left(1 - I_{max} \frac{C_{PEG-IFN}}{IC_{50} + C_{PEG-IFN}}\right) - k_{out}$$

**Turnover model with output stimulation**
where $A$: amount of platelet, $R_{ni}$: production rate of platelet, $T_R$: production rate delayed by the platelet life span, $C_{PEG-IFN}$: serum PEG-IFN concentration, $I_{max}$: maximum inhibitory effect, $IC_{50}$: $C_{PEG-IFN}$ associated with 50% maximum inhibition, $A_{pre}$: amount of precursor platelet, $R_{pre}$: production rate of precursor platelet, $k_{in}$: input rate constant of platelet, $k_{out}$: output rate constant of platelet, $S_{max}$: maximum stimulatory effect, $SC_{50}$: $C_{PEG-IFN}$ associated with 50% maximum stimulation.

Model evaluation: After the final model was constructed, the population predicted values or individual predicted values were plotted against the observed values to evaluate the appropriateness of the model.

Visual predictive check (VPC) was done with one thousand simulations with the final model and the result was compared to the original dataset. The 5th–95th percentiles and the median of the predictive values at each sampling point were plotted over time and the observed values and 90% confidence interval of observed values were overlaid to help evaluate the model appropriateness.

Using the final model, the median, 2.5th and 97.5th percentile confidence range of each model parameter were estimated by the bootstrap method.

Simulation: By using the population PK-PD model, the simulation was performed in the following cases: PEG-IFN was administered at doses of 90 and 180 µg once weekly for 48 weeks to patients whose baseline platelet counts were 75, which is the lower limit of PEG-IFN treatment initiation, 80, 90, 100, 110, and 120 $\times 10^9$/L. The number of simulations for each case was one thousand.

Results

Population PK analysis: Serum PEG-IFN concentrations at 339 points were obtained from 40 patients. These data were fitted to the 1-compartment model. The minimum objective function value (OBJ) was obtained when the combined model [Eq. (4)] was used for the residual variability. Table 1 shows the population PK parameters and the bootstrapped estimates (median, 2.5th and 97.5th percentiles) of the model. The scatterplots of the observed values against predicted values is shown in Figures 2a and 2b and the results of VPC in Figure 3a. The estimated values of CL/F, V/F and $k_a$ were 0.064 (6.28), 9.81 L (20.9), and 0.0101/h, respectively. The value of $\omega_{\text{max}}$ was quite small (<0.00001); then the value was fixed as 0 and OBJ was confirmed not to change, and so $\omega_{\text{max}}$ was ignored. The model explained the observed values well. The estimated population PK parameters were well consistent with the population PK parameters estimated by the bootstrap method. Age, sex, height, weight, lab values related to liver function and serum creatinine were used as potential covariates for the PK model but no one was included in the model.

Population PK-PD analysis: A population PK-PD analysis was performed using the platelet values at 1994 points from 40 patients. The proportional and the combined error models were both examined as the residual error model but no significant decrease of OBJ was obtained at $p = 0.05$ (degree of freedom 1) on the assumption that there is a $\chi^2$ distribution in the difference of OBJ even when the additive term is added. Therefore, the proportional error model was adopted. The OBJ of the basic life span indirect model, modified life span indirect model, turnover model

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Table 1. Parameters of the final PK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (SE%)</th>
<th>Median (2.5th–97.5th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (L/h)</td>
<td>0.0643 (6.28)</td>
<td>0.0641 (0.0564–0.0725)</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>9.81 (20.9)</td>
<td>9.65 (5.92–13.8)</td>
</tr>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>0.0101 (18.9)</td>
<td>0.010 (0.00665–0.0138)</td>
</tr>
<tr>
<td>$\omega_{\text{CL}}$ (%)</td>
<td>38.7 (27.0)</td>
<td>38.0 (28.3–48.3)</td>
</tr>
<tr>
<td>$\omega_{\text{V}}$ (%)</td>
<td>48.6 (38.1)</td>
<td>48.8 (32.5–67.9)</td>
</tr>
<tr>
<td>$\omega_1$ (ng/mL)</td>
<td>0.378 (45.6)</td>
<td>0.371 (0.113–0.519)</td>
</tr>
<tr>
<td>$\omega_2$ (%)</td>
<td>15.2 (18.2)</td>
<td>15.2 (12.3–18.6)</td>
</tr>
</tbody>
</table>

CL/F: Apparent clearance, V/F: Apparent distribution volume, $k_a$: Absorption rate constant, $\omega$: Inter-individual variability, SE: Standard error.
with input inhibition and turnover model with output stimulation were 11420.07, 11419.65, 11353.72 and 11383.96, respectively. The minimum OBJ was obtained when the turnover model with input inhibition was used for the structure model and we selected this model as the final PK-PD model of PEG-IFN. Table 2 shows population PD parameters and the bootstrapped estimates (median, 2.5th and 97.5th percentiles) of the final model. The scatterplots of the observed values against predicted values are shown in Figures 2c and 2d and the results of VPC in Figure 3b. The estimated platelet baseline, $k_{out}$, $I_{max}$ and $IC_{50}$ were $112 \times 10^9/L$, 0.0124/h, 35.5% and 1.28 ng/mL. The model explained the observed values well. The estimated PD parameters were well consistent with the PD parameters estimated by the bootstrap method.

**Simulation:** The longitudinal platelet profile by initial platelet baseline in the administration of PEG-IFN was simulated with the final PK-PD model (Fig. 4). When PEG-IFN was continuously administered once a week at the doses of 90 and 180 µg for 48 weeks, there was a rapid decline in the first 4 weeks followed by a slower decline in the next 8 weeks. Thereafter the platelet count reached an approximately constant level. The platelet count decreased approximately 27% (8–64%) and 29% (11–66%) (median (5th–95th percentiles)) from the baseline at 12 weeks of PEG-IFN administration with 90 and 180 µg doses, respectively.

![Fig. 2. Scatterplot of observed against predicted values](image)

(a): Observed serum PEG-IFN concentration against population predicted serum PEG-IFN concentration, (b): Observed serum PEG-IFN concentration against individual predicted serum PEG-IFN concentration, (c): Observed platelet count against population predicted platelet count, (d): Observed platelet count against individual predicted platelet count.

![Fig. 3. Visual predictive check with the final PK-PD model](image)

(a): Serum PEG-IFN concentration over time at the steady state (week 12) when PEG-IFN was administered subcutaneously, (b): Platelets over time. Circle: Observed values, Dashed line: 90% confidence interval with observed values, Straight line and shaded area: Median and 90% prediction interval from the simulation with the final model, respectively.

**Table 2. Parameters of the final PD model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (SE%)</th>
<th>Bootstrapped estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (2.5th–95th percentile)</td>
<td></td>
</tr>
<tr>
<td>Baseline (10^9/L)</td>
<td>112 (47.1)</td>
<td>113 (104–110)</td>
</tr>
<tr>
<td>$k_{out}$ (1/h)</td>
<td>0.0124 (31.9)</td>
<td>0.0137 (0.00645–0.0266)</td>
</tr>
<tr>
<td>$I_{max}$ (%)</td>
<td>35.5 (11.9)</td>
<td>35.8 (28.9–46.1)</td>
</tr>
<tr>
<td>$IC_{50}$ (ng/mL)</td>
<td>1.28 (35.7)</td>
<td>1.21 (0.602–2.88)</td>
</tr>
<tr>
<td>$\sigma_{Baseline}$ (%)</td>
<td>28.3 (23.3)</td>
<td>28.1 (21.1–33.7)</td>
</tr>
<tr>
<td>$\sigma_{k_{out}}$ (%)</td>
<td>197 (26.8)</td>
<td>194 (140–262)</td>
</tr>
<tr>
<td>$\sigma_{I_{max}}$ (%)</td>
<td>48.0 (40.1)</td>
<td>45.7 (26.2–74.8)</td>
</tr>
<tr>
<td>$\sigma_{IC_{50}}$ (%)</td>
<td>151 (34.1)</td>
<td>141 (0.148–211)</td>
</tr>
<tr>
<td>$\sigma$ (%)</td>
<td>11.4 (3.41)</td>
<td>11.5 (10.7–12.3)</td>
</tr>
</tbody>
</table>

$k_{out}$: Output rate constant of platelets, $I_{max}$: Maximum inhibitory effect, $IC_{50}$: PEG-IFN concentration associated with 50% maximal inhibition, $\sigma$: Inter-individual variability, $\sigma$: Residual variability, SE: Standard error.
After the administration of PEG-IFN had been completed, the platelet count recovered to the baseline in 4 to 8 weeks after the last administration. With a platelet count of $75 \times 10^9/L$, which was the minimum platelet baseline under simulation conditions, the median value of the nadir was simulated to be $55 \pm 5$th-95th percentiles: $27 \pm 69 \times 10^9/L$ provided that $90 \mu g$ was administered. The value of the nadir was simulated to be $53 \pm 5$th-95th percentiles: $24 \pm 67 \times 10^9/L$ provided that $180 \mu g$ was administered under the same baseline.

**Discussion**

In this analysis, the 1-compartment model was used and the model fitted well based on the evaluation of both the scatterplot of the observed values against predicted values and VPC. Hence, we concluded that the PK model of PEG-IFN can be also expressed as the 1-compartment model in CHCLC patients.

Shiomi et al. estimated PK parameters as follows: $CL/F$, $V/F$ and $k_s$ were 0.0907–0.167 L/h, 7.97–11.2 L and 0.0220–0.00407/h, respectively. The values of $CL/F$ and $k_s$ were smaller in this study than those in the report of Shiomi et al. The reasons for this difference could be considered as the differences of subjects and sampling points. Namely, the subjects adopted in the Shiomi study were healthy subjects, while CHCLC patients were the subjects in the analysis result. The sampling points and the number of doses were different. The Shiomi study design involved a single dose and there were 12 sampling points, while our study had 48 administrations and there were 6 trough and 6 additional sampling points. In addition, there is a possibility that the metabolic capacity of the CHCLC patients who were generally elderly was somewhat lower than that of the healthy adults, since PEG-IFN was reported to be eliminated after metabolic conversion. Moreover, Bressler et al. estimated $CL/F$ of PEG-IFN for CHC patients and it was reported to be $0.102 \pm 0.051 L/h$ (mean and standard deviation). The 2.5th and 97.5th percentiles of bootstrapped estimates of $CL/F$ are within 95% confidence interval of Bressler’s results.

In the analysis, 4 models were examined as the population PK-PD model of the decrease in platelet count. The basic and modified life span indirect models have been often used to describe the dynamics of blood cells. We also examined the turnover model with input inhibition, as myelosuppression has been observed in interferon treatment. In addition, since it has been reported that the elimination of platelets was accelerated by the effect of interferons on autoimmune response, we also examined the turnover model with output stimulation. The turnover model with input inhibition showed the minimum OBJ and decided it as the final PK-PD model.

The $I_{\text{max}}$ was 35.5% (28.9–46.1%), the 2.5th and 97.5th percentiles of bootstrapped estimates as the population

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**Fig. 4. Simulated longitudinal platelets of PEG-IFN administered every week up to 48 weeks**

(a): 90 µg, (b): 180 µg. Straight line and shaded area: Median and 90% prediction interval from the simulation with the final model, respectively. Simulation was examined with 75, 90 and $120 \times 10^9/L$ as baseline of platelets (left, middle and right panel).
mean. The result indicates maximally 35.5% inhibition in the platelets on average, even at an excessively high serum PEG-IFN exposure. The value of IC$_{50}$ was 1.28 (0.602–2.88), the 2.5th and 97.5th percentiles of bootstrapped estimates) ng/mL as the population mean. The serum trough concentration of PEG-IFN at the steady state was 10.4 and 20.7 ng/mL at 90 and 180 µg, respectively. The C$_{max}$ of PEG-IFN at the steady state was 12.4 and 24.9 ng/mL at 90 and 180 µg, respectively. The inhibitory effects at the concentration were approximately 89–91% and 94–95% of the maximum effects at 90 and 180 µg, respectively and thus the inhibitory effect at the steady state was suggested to be close to the maximum effect at those doses.

The simulation results demonstrated that the platelet count decreased with the administration of PEG-IFN at the doses of 90 or 180 µg, attained approximately a constant value around 4 to 8 weeks after the initiation of the treatment, and thereafter was not observed to change further.

In the simulation at the doses of 90 and 180 µg, the nadir of platelets after administration of 180 µg was 53 × 10⁹/L, and 55 × 10⁹/L at the dose of 90 µg. No obvious difference in the platelet profile between 90 and 180 µg was observed in the time to the nadir or the time to return to the baseline.

If the platelet count of a patient is 75 × 10⁹/L or lower, the initiation of the treatment with PEG-IFN to CHCLC patients is currently prohibited in Japan and if the platelet count is 25 × 10⁹/L or lower during the treatment of PEG-IFN, the administration of PEG-IFN must be discontinued.²¹ By taking these administration conditions in Japan into consideration, we performed a simulation on the assumption that the dosing starts at the baseline value of 75 × 10⁹/L. As a result, even at the time point when the platelet count decreased to the minimum value, it was predicted that in 94% of subjects the platelet count would not reach 25 × 10⁹/L or lower, which is the condition for discontinuation of administration of PEG-IFN. The platelet count could, however, decrease to the level of 25 × 10⁹/L or lower in the remaining 6% of subjects whose care should not be neglected. Although a rapid platelet decrease has been reported in some subjects during the course of PEG-IFN treatment,²²,²³ no subject developed such a decrease in the analysis; hence the simulation was unable to describe the phenomenon.

In conclusion, a platelet profile affected by PEG-IFN was modeled and a platelet count decrease induced by PEG-IFN was simulated. The platelet count was predicted to decrease by about 30%, to show a rapid decline in the first 4 weeks followed by a slower decline in the next 8 weeks and to return to the baseline value in 4 to 8 weeks after the last administration of PEG-IFN.

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References


