Intraocular Pharmacokinetics of Intravitreal Aflibercept (Eylea) in a Rabbit Model

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PURPOSE. We determined the intraocular pharmacokinetic properties of intravitreally injected aflibercept (Eylea) in a rabbit model.

METHODS. Aflibercept was injected intravitreally in 21 eyes from New Zealand White rabbits. The eyes were enucleated 1, 24, 48, 120, 216, 360, and 720 hours (1, 2, 5, 9, 15, and 30 days, respectively) after injection and immediately frozen at −80°C. The concentrations of aflibercept in the vitreous, aqueous humor, and retina/choroid were determined by performing an indirect enzyme-linked immunosorbent assay, and analyzed to understand the pharmacokinetic properties of the drug.

RESULTS. The maximum concentration of aflibercept was observed 1, 48 (2 days), and 24 (1 day) hours after intravitreal administration in the vitreous, aqueous humor, and retina/choroid, respectively. The one-compartment model was selected as the final model for all three ocular tissues. In the vitreous, aqueous humor, and retina/choroid, the estimated half-lives of aflibercept were 94.1, 48.0, and 58.2 hours, and the estimated mean residence times (MRTs) were 135.8, 69.2, and 84.0 hours, respectively. The area under curve from time 0 to the end point (AUClast) was 135,810.6 hours × μg/ml for the vitreous, 13,889.7 hours × μg/ml for the aqueous humor, and 2453.1 hours × μg/ml for the retina/choroid.

CONCLUSIONS. In rabbits, the vitreous half-life of aflibercept is 94.1 hours (3.92 days). This is shorter than that of bevacizumab (6.99 days), and longer than that of ranibizumab (2.51 days) and VEGF-Trap (3.63 days).

Keywords: anti-vascular endothelial growth factor agent, aflibercept, eylea, intraocular pharmacokinetics

The introduction of anti-VEGF treatment has changed the paradigm of standard treatment for and improved visual prognosis of VEGF-related ocular diseases, such as exudative age-related macular degeneration (AMD), diabetic macular edema, and retinal vein occlusion. Intravitreal ranibizumab (Lucentis; Genentech, Inc., San Francisco, CA, USA) has been used widely as a first-line treatment for these vision-threatening conditions, along with the off-label use of intravitreal bevacizumab (Avastin; Genentech, Inc.). Recently, aflibercept (VEGF Trap-Eye, Eylea; Regeneron, Inc., Tarrytown, NY, USA and Bayer Healthcare Pharmaceuticals, Berlin, Germany) was approved for therapeutic use and has since been used widely. Aflibercept has greater binding affinity to VEGF than does ranibizumab or bevacizumab, which indicates longer duration of action for aflibercept in the eyes.1–3 However, unlike that for ranibizumab and bevacizumab, data for the intraocular pharmacokinetics (PK) of aflibercept are scarce. A rabbit model–based PK study reported intravitreal PK properties of intravitreally administrated I-124–labeled aflibercept by the use of positron emission tomography/computed tomography (PET/CT) imaging; the intravitreal half-life of I-124–aflibercept was 4.58 days, which was similar to the manufacturer data of 4.79 days.2,4 Meanwhile, another PK study using a conventional immunoassay-based macaques model showed aqueous humor PK properties of intravitreally administrated aflibercept in comparison with ranibizumab; intravitreally administrated aflibercept and ranibizumab have similar half-lives in aqueous humor (2.2 and 2.3 days, respectively).5 However, to our knowledge, no study has addressed detailed, comparable intraocular PK properties of aflibercept by the use of a conventional immunoassay-based rabbit model as did the previous PK studies for ranibizumab and bevacizumab.6–12 This resulted in our recent study investigating the detailed intraocular PK properties of intravitreally administrated VEGF-Trap, a prototype of VEGF Trap-Eye, by the use of a conventional immunoassay-based rabbit model.3 However, although the sequences of VEGF-Trap and VEGF Trap-Eye are similar and show differences in only 5.3% (25 of 476) of the amino acids, these differences...
might result in differences in their intraocular PK properties. Moreover, the commercially available VEGF Trap-Eye, Eylea (which is widely used in clinical practice) passes through several manufacturing processes, which also might affect its intraocular PK properties. Hence, in this study, we investigated the intraocular PK properties of aflibercept (Eylea) in the same experimental setting described in our previous PK studies for bevacizumab, ranibizumab, and VEGF-Trap to provide comparable PK data of aflibercept with that of bevacizumab, ranibizumab, and VEGF-Trap.

**METHODS**

**Animal Experiments**

The present study was approved by the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee, and all applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research. We also confirm adherence to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

A total of 21 eyes were obtained from 21 healthy New Zealand White rabbits weighing 1.5–2 kg. The experimental design for assessing intraocular PK of intravitreally injected aflibercept was similar to that in our previous studies. Briefly, the animals were anesthetized with an intramuscular injection of 15 mg/kg Zoletil (a mixture of tiletamine hydrochloride and zolazepam hydrochloride; Virbac Laboratories, Carros, France) and 5 mg/kg xylazine hydrochloride (Bayer Korea, Ltd., Seoul, South Korea). Topical anesthesia (1% proparacaine hydrochloride ophthalmic eye drops; Alcaine; Bayer Healthcare Pharmaceuticals) was intravitreally injected into the right eye, 1 mm behind the surgical limbus in the superotemporal quadrant, using a 30-gauge needle and a Hamilton syringe. Three rabbits were killed at each of the following time points: 1, 2, 5, 9, 15, or 30 days, respectively) after injection. The right eye, aflibercept (1.2 mg/0.03 mL; Eylea; Regeneron and Bayer Healthcare Pharmaceuticals) was intravitreally injected into the right eye, 1 mm behind the surgical limbus in the superotemporal quadrant, using a 30-gauge needle and a Hamilton syringe. Three rabbits were killed at each of the following time points: 1, 2, 5, 9, 15, or 30 days, respectively) after injection. The right eye was enucleated and immediately frozen at −80°C until dry. Next, the vitreous, aqueous humor, and retina/choroid were weighed and the weights of aflibercept (g) by that of the retina/choroid tissues (g).

**Pharmacokinetic Data Analysis**

The concentrations of aflibercept in the vitreous, aqueous humor, and retina/choroid samples were analyzed by one- and two-compartment models. In addition, a noncompartmental analysis was performed on the Phoenix WinNonlin software version 6.4 (Certara, Princeton, NJ, USA).

The following equation was used for the one-compartment model:

\[
C(t) = \frac{\text{Dose}}{V_{z/F}} \times e^{-k_{t} \cdot t},
\]

where \(C(t)\) denotes concentration (µg/mL) at time \(t\), \(V_{z/F}\) is the apparent volume of distribution, and \(k\) (1/h) indicates the elimination rate constant. For the vitreous, \(F\) was assumed to be 1. However, for the aqueous humor and retina/choroid, \(F\) was neither assumed nor estimated.

The following equation was used for the two-compartment model:

\[
C(t) = A \times e^{-\alpha \cdot t} + B \times e^{-\beta \cdot t},
\]

where \(A\) and \(B\) (both in µg/mL) are the back-extrapolated intercepts of the distribution and elimination phases, respectively, and \(\alpha\) (1/h) and \(\beta\) (1/h) represent the rate constants for the distribution and elimination phases, respectively. Half-life (\(t_{1/2}\) in hours), mean residence time (MRT in hours), maximum concentration (\(C_{\text{max}}\) in µg/mL), area under the concentration-time curve (AUC, hours × µg/mL), apparent volume of distribution (\(V_{z/F}\) in mL), and apparent clearance (CL/F in mL/hours) were estimated by post hoc analysis. After analysis, either the one- or two-compartment model was selected based on the following criteria: (1) the Akaike Information Criterion (AIC), (2) precision of parameter estimates, and (3) graphical analysis. The AIC was computed using the weighted residual sum of squares of model (WRSS), and the number of observations and parameters (N and P, respectively) during the modeling were given as follows:

\[
\text{AIC} = N \times \log(\text{WRSS}) + 2P.
\]

The AIC, precision-of-parameter estimate (standard error, presented as the coefficient of variation [CV]), and goodness-of-fit plot including the predicted versus observed concentrations were compared between the two models. In addition, the \(C_{\text{max}}\) time to \(C_{\text{max}}(T_{\text{max}})\), and AUC last in the vitreous, aqueous
humor, and retina–choroid samples also were calculated by following a noncompartmental method.

**RESULTS**

Data were collected for 21 eyes from 21 rabbits. There was no evidence of ocular inflammation or other adverse events following drug treatment. Changes in concentrations and estimated amounts of aflibercept in the vitreous, aqueous humor, and retina–choroid samples over time are provided in Table 1. The concentration of aflibercept in the vitreous was highest 1 hour after intravitreal administration ($C_{\text{max}} = 989.0 \mu g/mL$), and decreased through the subsequent time points. Approximately 40% of the amount of aflibercept at 1 hour remained at 120 hours (5 days), approximately 10% remained at 360 hours (15 days), and 0.7% remained at 720 hours (30 days) after intravitreal administration. In the aqueous humor, the highest concentration of aflibercept was observed 48 hours (2 days) after intravitreal administration ($C_{\text{max}} = 108.9 \mu g/mL$), while in the retina–choroid, the highest concentration was observed at the 24-hours (1-day) time point ($C_{\text{max}} = 21.9 \mu g/g$).

The one-compartment model was selected as the final model for all three eye tissues. The one- and two-compartment models fit both these tissues. Basic goodness-of-fit plots are presented in Figures 1A through 1F for the one-compartment model for each tissue.

The estimated one-compartment models for the vitreous, aqueous humor, and retina–choroid were as follows:

$$C(t) = \frac{1200}{T_{1/2}} \times e^{-0.0075t}$$

$$C(t) = \frac{1200}{T_{\text{MRT}}} \times e^{-0.0145t}$$

In the vitreous, aqueous humor, and retina–choroid, the estimated half-lives of aflibercept were 94.1, 48.0, and 58.2 hours, respectively, while the corresponding MRTs were 135.8, 69.2, and 84.0 hours. Further, the respective calculated AUCs were 135,810.6 hours $\times \mu g/mL$, 13,889.7 hours $\times \mu g/mL$, and 2453.1 hours $\times \mu g/g$. Detailed PK parameters for aflibercept are provided in Table 2. The observed concentration-time data for aflibercept at seven time points, as well as the models that fit the vitreous, aqueous humor, and retina–choroid are provided in Figure 2.

**DISCUSSION**

The present study investigated the intraocular distributions of intravitreally administered aflibercept in vitreous, aqueous humor, and retina–choroid tissues, as well as the corresponding PK properties of the drug, by a conventional immunomessay in a rabbit model using New Zealand White rabbits. The results showed that the half-life of intravitreally administrated aflibercept was 94.1 hours (3.92 days) in the vitreous, indicating that intravitreal aflibercept clears through the aqueous humor and retina–choroid. Substantial amounts of aflibercept could be measured just 1 hour after intravitreal administration in the aqueous humor and retina–choroid. Moreover, the aflibercept

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**Table 1.** Pharmacokinetics of Intravitreal Aflibercept

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Vitreous</th>
<th>Aqueous Humor</th>
<th>Retina–Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration, $\mu g/mL$</td>
<td>Amount, $\mu g$</td>
<td>Concentration, $\mu g/mL$</td>
</tr>
<tr>
<td>1 h</td>
<td>989.0 ± 34.35</td>
<td>1483 ± 513.53</td>
<td>12.87 ± 17.59</td>
</tr>
<tr>
<td>1 d</td>
<td>567.17 ± 215.73</td>
<td>850.76 ± 323.60</td>
<td>66.07 ± 51.90</td>
</tr>
<tr>
<td>2 d</td>
<td>577.57 ± 113.92</td>
<td>866.46 ± 170.88</td>
<td>108.89 ± 44.64</td>
</tr>
<tr>
<td>5 d</td>
<td>394.04 ± 58.97</td>
<td>591.06 ± 88.46</td>
<td>33.33 ± 11.34</td>
</tr>
<tr>
<td>9 d</td>
<td>209.04 ± 9.66</td>
<td>313.56 ± 14.49</td>
<td>18.40 ± 13.39</td>
</tr>
<tr>
<td>15 d</td>
<td>91.43 ± 6.30</td>
<td>137.15 ± 9.45</td>
<td>7.13 ± 1.22</td>
</tr>
<tr>
<td>30 d</td>
<td>6.44 ± 2.64</td>
<td>9.66 ± 3.96</td>
<td>0.84 ± 0.42</td>
</tr>
</tbody>
</table>

The volumes of the vitreous and aqueous humor of the rabbits were considered 1.5 and 0.2 mL, respectively, and the weight of the retina–choroids was considered 0.024 g.

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**Table 2.** PK Parameters of Aflibercept (Eylea) in the Vitreous, Aqueous Humor, and Retina–Choroid of Eyes From New Zealand White Rabbits

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Vitreous</th>
<th>Aqueous Humor</th>
<th>Retina–Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2}$, h*</td>
<td>94.1 ± 21.4</td>
<td>47.9 ± 7.1</td>
<td>58.2 ± 76.9</td>
</tr>
<tr>
<td>MRT, h*</td>
<td>135.8 ± 30.9</td>
<td>69.2 ± 10.2</td>
<td>84.0 ± 110.9</td>
</tr>
<tr>
<td>$C_{\text{max}}$, $\mu g/mL$†</td>
<td>989.0</td>
<td>108.9</td>
<td>21.9</td>
</tr>
<tr>
<td>$T_{\text{max}}$, h*</td>
<td>1</td>
<td>48</td>
<td>24</td>
</tr>
<tr>
<td>AUC_{last}, h $\times \mu g/mL$†</td>
<td>135,810.6</td>
<td>13,889.7</td>
<td>2453.1</td>
</tr>
<tr>
<td>$V/F$, mL*</td>
<td>1.4 ± 0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$CL/F$, mL/h*</td>
<td>0.01 ± 0.001</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* One-compartmental analysis; data presented as parameter estimate ± standard error.
† Noncompartmental method.
FIGURE 1. Plots show basic goodness-of-fit plot for one-compartment model for the vitreous, aqueous humor, and retina–choroid of eyes from New Zealand White rabbits. (A, B) Represent the vitreous. (C, D) The aqueous humor. (E, F) The retina–choroid.

FIGURE 2. Time-concentration plots for aflibercept (Eylea) concentrations in the eyes of New Zealand White rabbits after intravitreal administration. Points represent the observed concentrations, and lines represent the estimated concentrations determined by the one-compartmental model.
concentrations increased, and peaked 2 days after intravitreal administration in the aqueous humor and 1 day after that in the retina–choroid. Subsequently, the concentrations decreased through the rest of the study period in all the three eye compartments. These results indicate that intravitreally-administered aflibercept distributes rapidly to the aqueous humor and retina/choroid and remains in these tissues for considerable periods.

The vitreous half-life of aflibercept in the present study (3.92 days) was relative short in comparison with that given by the drug manufacturer (4.79 days) and that reported in the PET/CT study on the I-124–labeled drug (4.58 days), although the head-to-head comparison is not tractable due to the differences in measurement methodologies (PET/CT imaging versus conventional immunoassay) and rabbit breeds (Dutch-belted rabbit versus New Zealand White rabbit) between the PET/CT study and the present study. When compared to PK properties from the same experimental settings, this vitreous half-life is equivalent to, or even slightly longer than, that of VEGF-Trap in our previous study (3.63 days). Similarly, the half-lives of aflibercept in the aqueous humor and retina/choroid also were slightly longer than those for VEGF-Trap (47.95 vs. 36.8 hours in aqueous humor; 58.24 vs. 35.0 hours in retina/choroid for aflibercept and VEGF-Trap, respectively). These improvements in intraocular longevity might be attributed to the development of the drug from VEGF-Trap to VEGF Trap-Eye, although the differences in amino acid sequences are minor. We previously had conducted PK studies for bevacizumab and ranibizumab under the same experimental setting of the present study; the vitreous half-lives were 7.06 and 2.75 days, respectively. Since the applied experimental setting of the present study; the vitreous half-life of bevacizumab (Avastin; molecular weight = 145 kDa) might be the reason for their similar intraocular half-lives.3,14

In conclusion, the present study presents, for the first time to our knowledge, detailed PK properties of intravitreally administered, commercially available aflibercept (Eylea), by conventional immunoassay in a rabbit model. We expect that our results will aid aflibercept treatment strategies in the clinic, and will provide a foundation for future studies on such treatments.

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References


### Table 3. Comparison of the Vitreous PK Parameters of Aflibercept (Eylea), Ranibizumab (Lucentis), Bevacizumab (Avastin), and VEGF-Trap in Eyes From New Zealand White Rabbits

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Aflibercept</th>
<th>Ranibizumab</th>
<th>Bevacizumab</th>
<th>VEGF-Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight, kD</td>
<td>145</td>
<td>49</td>
<td>148</td>
<td>145</td>
</tr>
<tr>
<td>$T_{1/2}$, h</td>
<td>94.1</td>
<td>52.4</td>
<td>181.4</td>
<td>87.1</td>
</tr>
<tr>
<td>MRT, h</td>
<td>155.8</td>
<td>75.5</td>
<td>233.8</td>
<td>125.7</td>
</tr>
<tr>
<td>$V/F$, mL</td>
<td>1.4</td>
<td>2.7</td>
<td>3.06</td>
<td>3.8</td>
</tr>
<tr>
<td>$CL/F$, mL/h</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters were reestimated based on the concentrations at each time point in the published data. $T_{1/2}$, half-life; MRT, mean residence time; $V/F$, apparent volume of distribution; $CL/F$, apparent clearance.

* One-compartmental analysis for aflibercept, ranibizumab, and VEGF-Trap, and two-compartmental analysis for bevacizumab.


