Role of the Fc Region in the Vitreous Half-Life of Anti-VEGF Drugs

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PURPOSE. To identify the role of the fragment crystallizable (Fc) region in determining intraocular protein drug pharmacokinetics.

METHODS. We generated a new VEGF-Trap lacking the Fc region (Fc/VEGF-Trap, MWt = 100 kDa) by replacing the Fc region of native VEGF-Trap (MWt = 145 kDa) with a dimerized coiled-coil domain. Forty-two rabbits were injected intravitreally with VEGF-Trap or Fc/VEGF-Trap (n = 21 each) in one of the eyes, harvested at six time points (1 hour and 1, 2, 4, 14, and 50 days after injections). VEGF-Trap and Fc/VEGF-Trap concentrations in the vitreous, aqueous humor, and retina/choroid were measured, and drug pharmacokinetic properties were analyzed.

RESULTS. In all three ocular compartments, the maximal concentrations for both Fc/VEGF-Trap and VEGF-Trap were observed at 1 hour after injection. Half-lives of Fc/VEGF-Trap in the vitreous and retina/choroid (145.02 and 102.12 hours, respectively) were 1.39 and 2.30 times longer than those of VEGF-Trap (103.99 and 44.42 hours, respectively). Total exposure of the aqueous humor and retina/choroid to Fc/VEGF-Trap was 13.2% and 39% of the vitreous exposure, respectively, whereas VEGF-Trap concentrations were 25.2% and 26.2%, indicating that Fc/VEGF-Trap shows a preference for posterior distribution and elimination.

CONCLUSIONS. Fc/VEGF-Trap, despite its lower molecular weight, showed longer half-lives in vitreous and retina/choroid than VEGF-Trap did, suggesting that Fc receptors in ocular tissues contribute to anti-VEGF drug elimination. Truncation or mutation of the Fc region can prolong the intraocular residence time of VEGF-Trap and possibly reduce the number of VEGF-Trap injections required in clinical practice.

Keywords: VEGF-Trap, Fc/VEGF-Trap, Fc free VEGF-Trap, Fc receptor, Fc region, anti-VEGF, ocular pharmacokinetics

The use of anti-vascular endothelial growth factor (anti-VEGF) agents has revolutionized the treatment of retinal vascular diseases associated with abnormal neovascularization or vascular permeability, including exudative age-related macular degeneration, diabetic macular edema, and macular edema secondary to retinal vein occlusion.1 Despite the success of potent anti-VEGF agents in treating diverse retinal disorders associated with the overproduction of VEGF problems such as short half-lives and a high injection frequency remain unresolved.

Three types of anti-VEGF antibodies are currently used for the treatment of age-related macular degeneration and retinal vascular disorders: the Food and Drug Administration–approved drugs ranibizumab and aflibercept, and the off-label drug bevacizumab.2 The molecular weights of bevacizumab (149 kDa) and ranibizumab (48.39 kDa) are considerably different because ranibizumab does not have a fragment crystallizable (Fc) region and bevacizumab is N-glycosylated in its Fc region.3

Aflibercept (VEGF Trap-Eye, Eylea) is not a monoclonal antibody, but a recombinant fusion protein consisting of portions of the human VEGF receptor (VEGFR) 1 and VEGFR2 extracellular domain fused to the Fc region of human IgG1.4 Generally, drugs with larger molecular weights are thought to
have prolonged vitreous half-life. In fact, it was previously reported that the vitreous half-life of ranibizumab (2.75 days) was shorter than that of bevacizumab (7.06 days) and aflibercept (3.63 days) in rabbits. Based on our experience and skills in ocular PK, we comparatively studied the ocular PK of VEGF-Trap and a new compound, VEGF-Trap–based fusion proteins is a relatively new advance, and whether their elimination or transport depends on FcRn and/or the blood–retina barrier is unknown. Recent studies reported that the elimination of intravitreally administered IgG across the blood–retina barrier is mediated by the neonatal Fc receptor (FCn), which is expressed in the retinal pigment epithelium and endothelial cells of the retinal and choroidal vasculature. Ocular application of the Fc-based fusion proteins is a relatively new advance, and whether their elimination or transport depends on FCn and/or the molecular weight remains unclear. Understanding of the role of the Fc region and FCn in intraocular pharmacokinetics (PK) is important in determining the duration of therapeutic efficacy of drugs as well as in anticipating the systemic exposure of intravitreally injected anti-VEGF agents, which can potentially cause systemic complications.

During the past few years, we have reported the intraocular PK of bevacizumab, ranibizumab, VEGF-Trap, and aflibercept, which are representative anti-VEGF agents currently used in the clinic. Based on our experience and skills in ocular PK, we comparatively studied the ocular PK of VEGF-Trap and a newly synthesized Fc region–free VEGF-Trap (Fc-VEGF-Trap) to uncover the role of Fc region in ocular PK.

**MATERIALS AND METHODS**

**Generation of Fc-VEGF-Trap, a VEGF-Trap–Based Protein Obtained by Replacing the Fc Region of Human IgG1 With a Dimerized Coiled-Coil Domain**

Previously, we generated VEGF-Trap, a fusion protein containing human VEGFR1-Ig2 and VEGFR2-Ig3 and the human Fc domain. The generated VEGF-Trap consisted of 476 amino acids with a sequence similarity of approximately 95% to commercially available VEGF-Trap-Eye, aflibercept (Eylea; Regeneron, Inc., and Bayer Healthcare Pharmaceuticals, Berlin, Germany), which consisted of 458 amino acids. We reported the results of ocular PK of VEGF-Trap in our previous study. Fc-VEGF-Trap was generated based on VEGF-Trap sequences. Instead of the Fc domain of VEGF-Trap, we comparatively studied the ocular PK of VEGF-Trap, which is a fusion protein containing human VEGFR1-Ig2 and VEGFR2-Ig3, and the human Fc domain. (B) Fc-VEGF-Trap is composed of 309 amino acids, and the predicted molecular weight of Fc-VEGF-Trap is two-thirds of that of VEGF-Trap. (C) The model structure of Fc-VEGF-Trap/VEGF-A complex.

**Role of the Fc Region of Anti-VEGF Drugs**

**FIGURE 1.** Protein structures and amino acid sequences of anti-VEGF analogues: conventional VEGF-Trap and Fc-truncated VEGF proteins (Fc-VEGF-Trap). (A) Fc-VEGF-Trap contains a dimerized coiled-coil domain (AP-1, UniProtKB ID: P05412, 276R) instead of the Fc domain in VEGF-Trap, which is a fusion protein containing human VEGFR1-Ig2 and VEGFR2-Ig3 and the human Fc domain. (B) Fc-VEGF-Trap is composed of 309 amino acids, and the predicted molecular weight of Fc-VEGF-Trap is two-thirds of that of VEGF-Trap. (C) The model structure of Fc-VEGF-Trap/VEGF-A complex.
A total of 42 eyes from 42 New Zealand White rabbits weighing approximately 1.5 to 2.0 kg were randomly assigned to VEGF-Trap (n = 21) or Fc/VEGF-Trap (n = 21) groups. The intraocular PK of VEGF-Trap and Fc/VEGF-Trap were evaluated using same experimental design as in our previous studies.6–9 Intramuscular injection of 15 mg/kg Zoleftic (a mixture of tiletamine and zolazepam; Virbac Laboratories, Carros, France) and 5 mg/kg xylazine, and a topical ophthalmic anesthetic (1% proparacaine hydrochloride, Alcaine; Alcon Laboratories, Inc., Fort Worth, TX, USA) were used for anesthesia. Eyes under investigation were dilated with eye drops containing a mixture of phenylephrine and tropicamide. Povidone iodine solution (5%) was applied for periocular skin and conjunctival antisepsis. Next, all intravitreal injections of VEGF-Trap (0.3 mg/0.03 mL) or Fc/VEGF-Trap (0.2 mg/0.03 mL) were performed in the right eye. To administer the same molar dose of Fc/VEGF-Trap and VEGF-Trap, the dose of Fc/VEGF-Trap was matched to two-thirds of the dose of VEGF-Trap because the molecular weight of Fc is 45 kDa while VEGF is 165 kDa. Three or four rabbits were killed at each of the following time points: 1, 24, 48, 120, 356, and 720 hours (1 hour and 1, 2, 5, 14, and 30 days) after injection. Enucleated eyes were immediately stored at −80°C until analysis. Before immunoassay, eyes were divided into vitreous, aqueous humor, and retina/choroid compartments. To solubilize the vitreous, samples were soaked in PBS with 1% BSA at 4°C overnight and centrifuged at 387g for 10 minutes. Tissue proteins were extracted from the lysates of homogenized retina/choroids using lysis reagent (Celllytic MT; C3228; Sigma-Aldrich Corp., St. Louis, MO, USA) and a homogenizer.

Drug Immunoassay

We measured the concentration of Fc/VEGF-Trap in each compartment at serial time points by an indirect enzyme-linked immunosorbent assay (ELISA) in accordance with previous reports.8,15 The diluted 165 amino acid antigen solution of human recombinant VEGF (rVEGF) (10 ng/mL) in a 50 mM carbonate buffer (pH 9) was divided into aliquots in 96-well plates at 100 μL/well. The plates were incubated with rVEGF antigens at 4°C overnight, washed with PBS, blocked with PBS containing 1% BSA at 4°C for 4 hours, and dried at 4°C in an incubator. Vitreous, aqueous humor, and retina/choroid samples were diluted in PBS with 0.1% BSA, put into a 96-well plate, and incurred at 4°C overnight to form antigen and antibody (VEGF-Trap or Fc/VEGF-Trap) complexes. We incubated Strep-Tactin conjugated with horseradish peroxidase (HRP) (1:5000; Bio-Rad, Hercules, CA, USA, no. 1610381) to detect rVEGF-Fc/VEGF-Trap complex, and 1:20,000 goat anti-human IgG/Fc antibody labeled with HRP (Abcam, Cambridge MA, USA) to detect rVEGF-VEGF-Trap, in 96-well plates for 2 hours at room temperature. 3,3′,5,5′-Tetramethyl benzidine substrate was used to detect HRP activity, and the optical density of the color change, which reflects the VEGF-Trap or Fc/VEGF-Trap concentration, was determined using a standard ELISA plate reader (Bio-Rad) and embedded software, SoftMax Pro 5.4.1 (Molecular Devices, Sunnyvale, CA, USA). Using standard curves, the concentrations of VEGF-Trap and Fc/VEGF-Trap in our samples were calculated. The drug concentration in retina/choroid was defined as the weight of drugs (mg) to the weight of the retina/choroid tissue (g).

Pharmacokinetic Data Analysis

Quantitative analysis of all samples was performed using a four-parameter logistic (4PL) curve, which is widely used in curve-fitting analysis for typical ELISAs, and is more reasonable for describing biological systems than linear curve or semilog plot.16 The changes in concentrations of VEGF-Trap and Fc/VEGF-Trap in the vitreous, aqueous humor, and retina/choroids were analyzed by one- and two-compartment models. The equations and detailed parameters used for pharmacokinetic modeling and analysis were described previously.8,17 The half-life of elimination (t1/2), mean residence time (MRT), observed maximum concentration (Cmax), area under the concentration–time curve (AUC), apparent volume of distribution (Vd/F), and apparent clearance (CL/F) were estimated. Using the AUC for each compartment, the total exposure of the aqueous humor and retina/choroid to VEGF-Trap and Fc/VEGF-Trap from the vitreous was calculated. Statistically, the mean values and standard deviation of drug concentration at each time point were calculated, and the estimated curves were plotted using these values. Estimated pharmacokinetic data are presented as parameter estimate (CV%) values. A low CV value indicates reliable parameter without large variability.

RESULTS

No adverse events or signs of ocular inflammation were observed after intravitreal injection of either VEGF-Trap or Fc/VEGF-Trap. The changes in estimated amounts and concentrations over time for VEGF-Trap and Fc/VEGF-Trap in the vitreous, aqueous humor, and retina/choroids are shown in Table 1. The estimated concentration–time curves with observed concentrations at the six time points for VEGF-Trap and Fc/VEGF-Trap are shown in Figure 2. The concentrations of VEGF-Trap and Fc/VEGF-Trap in the aqueous humor and retina/choroids, as well as the vitreous, declined in a biexponential fashion. For the vitreous, one-compartment model could explain the PK of Fc/VEGF-Trap and VEGF-Trap, while data fitting could not be achieved in other models (Fig. 3). For the aqueous humor and retina/choroids, the two-compartment model was selected, considering physiological compartment as well as AIC and CV values. For the two-compartment model, Akaike’s information criterion (AIC) values of Fc/VEGF-Trap in the aqueous humor and retina/choroids were 20.42 and 12.54, and those of VEGF-Trap were 19.72 and 32.55, respectively. The Cmax of VEGF-Trap and Fc/VEGF-Trap in the vitreous were 67.57 and 57.44 μg/mL at 1 hour after injection of equal molar dose of Fc/VEGF-Trap (0.2 mg/0.05 mL) and VEGF-Trap (0.3 mg/0.05 mL). Similarly, the Cmax of both drugs in the aqueous humor and retina/choroid was reached at 1 hour (Table 2). The estimated half-lives of Fc/VEGF-Trap in the vitreous and retina/choroid were 1.59 and 2.30 times longer (145.02 and 102.12 hours, respectively) than those of VEGF-Trap (103.99 and 44.42 hours, respectively). The MRT of Fc/VEGF-Trap and VEGF-Trap was 209.22 and 150.02 hours, respectively. Likewise, the dose-normalized AUC of Fc/VEGF-Trap in the vitreous was 1.162 times higher than that of VEGF-Trap. In addition, the total exposure of the aqueous humor and retina/choroid to Fc/VEGF-Trap was approximately 13.2% and 39% that of the vitreous exposure, respectively, whereas VEGF-Trap concentrations in the aqueous humor and retina/choroid were approximately 25.2% and 26.2% that of the vitreal exposure, respectively. These results indicate that the anterior excretion of Fc/VEGF-Trap is relatively low and the posterior excretion is relatively high, compared to that of VEGF-Trap, suggesting that Fc/VEGF-Trap shows a preference for posterior excretion. The Vd/F values of Fc/VEGF-Trap were higher than those of VEGF-Trap in the vitreous (5.34 vs. 4.45 mL) and the retina/choroid (22.84 vs. 15.25 mL), but not in the aqueous humor (22.26 vs. 53.62 mL). These results indicate that Fc/VEGF-Trap is mainly distributed in the posterior...
TABLE 1. The Concentrations and Amounts of VEGF-Trap and Fc Region of Anti-VEGF Drugs

<table>
<thead>
<tr>
<th></th>
<th>Vitreous</th>
<th>Aqueous Humor</th>
<th>Retina/Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc., µg/mL</td>
<td>Amount, µg</td>
<td>Conc., µg/mL</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>3.48 ± 1.08</td>
<td>16.06 ± 0.61</td>
<td>13.60 ± 0.98</td>
</tr>
<tr>
<td>2 d</td>
<td>3.32 ± 0.95</td>
<td>14.80 ± 0.61</td>
<td>16.20 ± 0.98</td>
</tr>
<tr>
<td>5 d</td>
<td>2.53 ± 0.95</td>
<td>14.80 ± 0.61</td>
<td>16.20 ± 0.98</td>
</tr>
<tr>
<td>14 d</td>
<td>1.88 ± 0.12</td>
<td>1.26 ± 0.35</td>
<td>1.51 ± 0.17</td>
</tr>
<tr>
<td>30 d</td>
<td>1.51 ± 0.17</td>
<td>1.26 ± 0.35</td>
<td>1.51 ± 0.17</td>
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</table>

The values of the vitreous and aqueous humor of the rabbit were considered 1.5 and 0.2 µl, respectively, and the weight of the retina/choroid was considered 0.0024 g.

DISCUSSION

In this study, we investigated and analyzed the ocular PK of an Fc region–free VEGF-Trap and compared it with that of VEGF-Trap. The lower molecular weight of the Fc/VEGF-Trap (compared to native VEGF-Trap) may promote initial elimination from the vitreous. However, the replacement of the Fc region with a dimerized coiled-coil domain may enhance the long-term intraocular retention of Fc/VEGF-Trap, which was found to be approximately 40% longer than that of the conventional VEGF-Trap in this study.

According to previous reports, molecular weight is one of the determinant factors for ocular PK. The rate of diffusion is approximately inversely proportional to the cube root of the molecular weight; therefore, high molecular weights are thought to prolong vitreous half-life. However, in our study, the low molecular weight Fc/VEGF-Trap showed a 1.39 times longer vitreous half-life than VEGF-Trap (145.02 vs. 103.99 hours). This suggests that the elimination of vitreous VEGF-Trap is not merely through a molecular weight–dependent mechanism and that Fc region is associated with the prolongation of intraocular half-life. Considering the smaller molecular weight of Fc/VEGF-Trap (100 kDa) compared to VEGF-Trap (145 kDa), the actual amount of FcRn-dependent elimination of VEGF-Trap is estimated to be larger than our result.

The elimination of an intravitreally injected drug is achieved through two main routes: anterior and posterior. Anterior elimination refers to the diffusion of vitreous drugs into the posterior chamber and turnover via aqueous and uveal flow. Posterior elimination is achieved through permeation across the posterior blood–eye barrier. The initial concentration (1 hour after intravitreal administration) of Fc/VEGF-Trap was relatively lower than that of VEGF-Trap in all three compartments, even allowing for the dosage difference between the two drugs to match the molar dose (Fig. 2). There was a sharp decrease in Fc/VEGF-Trap levels, which was more than that for VEGF-Trap, in the early time period. Although the exact mechanism for this sharp decrease could not be identified, it might be associated with the low molecular weight of Fc/VEGF-Trap. The low molecular weight Fc/VEGF-Trap could achieve early dynamic equilibrium, or a large amount of drug could be transported into the anterior chamber. The rapid decline phase of drug concentration, a second phase was observed in which the relative concentration of Fc/VEGF-Trap was lower in the aqueous humor and higher in the retina/choroid than that of VEGF-Trap, which may be correlated with the amount of drug distributed into the tissue, was significantly higher in the posterior compartment and lower in the anterior compartment, compared to that of VEGF-Trap. This indicates that Fc/VEGF-Trap is more readily distributed in the posterior segment of the eyeball than VEGF-Trap, whereas a larger portion of intravitreally injected VEGF-Trap is distributed in the anterior segment than is Fc/VEGF-Trap. This higher preference for second-phase distribution of Fc/VEGF-Trap in the posterior compartment may be associated with the replacement of the Fc region with the coiled-coil domain. Our previous study illustrated that the AUC of ranibizumab in aqueous humor and retina/choroid were 3.72% and 20.46% that of vitreous, respectively, suggesting that a monoclonal antibody fragment (Fab) lacking an Fc region had similar preference for posterior distribution. Eventually, the preference for posterior elimina-
tion possibly enhances the efficacy of Fc/VEGF-Trap by increasing the delivery of anti-VEGF molecules into the retina.

The common anti-VEGF agents, including bevacizumab and aflibercept, are Fc-containing proteins. The main receptors for these proteins are the Fcy receptors (FcγR) on cell membrane and FcRn. The expression of FcγR has been previously demonstrated using an RPE model: the ARPE-19 cell line. FcRn is vital for increasing the serum circulating half-life of antibodies and for protecting them from lysosomal degradation. Hence, it is an important factor in systemic PK of antibodies. However, the function of FcRn in intraocular PK was not reported until recently, when studies showed the presence of FcRn on RPE cells; moreover, recent studies showed that FcRn, which is involved in intracellular uptake and transport of Fc-containing molecules in the retina, played an essential role in eliminating intravitreally administered IgGs across the blood–retina barrier into the systemic circulation. 12 Despite recent studies regarding FcRn, the function of the Fc region in ocular PK remains controversial. Some reports showed that inhibition of FcRn increased apical to choroidal transport of bevacizumab, indicating a role of FcRn in the recycling of the molecule. Another study showed that protein molecular weight and Fc region did not play critical roles in ocular PK as they do systemically. 22 According to our results, VEGF-Trap showed Fc region–dependent properties in ocular PK. Therefore, we suggest that the Fc region of VEGF-Trap may play a role in vitreoretinal-specific elimination of VEGF-Trap and that the Fc region is a potential factor that diminishes VEGF-Trap concentration in the posterior compartment of the eyeball. By considering these characteristics of VEGF-Trap associated with Fc region–dependent clearance, we can advance our understanding of ocular PK of anti-VEGF agents.

We generated a new Fc/VEGF-Trap by replacing for the Fc region of VEGF-Trap and VEGF-Trap in the rabbit eyes. Points represent observed concentrations and lines represent estimated concentrations by models.

**FIGURE 2.** Concentration of Fc/VEGF-Trap and VEGF-Trap in the rabbit eyes. Points represent observed concentrations and lines represent estimated concentrations by models.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Compartment</th>
<th>K_{e}</th>
<th>Terminal t_{1/2} (h)</th>
<th>MRT (h)</th>
<th>C_{max} (pg/mL)</th>
<th>AUC (h*pg/mL)</th>
<th>V_d/F (ml/h)</th>
<th>CL/F (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc/VEGF-Trap</td>
<td>Vitreous humor</td>
<td>0.0048 (26.11)</td>
<td>145.02 (26.09)</td>
<td>209.22 (26.09)</td>
<td>37.44 (8.05)</td>
<td>7832.22 (22.46)</td>
<td>5.34 (8.06)</td>
<td>0.026 (22.48)</td>
</tr>
<tr>
<td></td>
<td>Aqueous humor</td>
<td>0.0161 (76.24)</td>
<td>43.02 (76.17)</td>
<td>114.92 (328.86)</td>
<td>16.46 (8.38)</td>
<td>1032.68 (75.50)</td>
<td>22.26 (59.59)</td>
<td>0.194 (75.58)</td>
</tr>
<tr>
<td>200 μg Retina/choroids</td>
<td>0.0028 (26.86)</td>
<td>102.12 (26.83)</td>
<td>234.40 (53.63)</td>
<td>13.83 (6.00)</td>
<td>2052.42 (25.97)</td>
<td>22.84 (32.02)</td>
<td>0.097 (25.99)</td>
<td></td>
</tr>
<tr>
<td>VEGF Trap</td>
<td>Vitreous humor</td>
<td>0.0067 (25.67)</td>
<td>105.99 (25.64)</td>
<td>150.02 (25.64)</td>
<td>67.37 (8.36)</td>
<td>10107.73 (21.69)</td>
<td>4.45 (8.36)</td>
<td>0.029 (21.71)</td>
</tr>
<tr>
<td>300 μg Aqueous humor</td>
<td>0.0088 (57.55)</td>
<td>78.89 (57.50)</td>
<td>285.36 (183.96)</td>
<td>22.20 (5.79)</td>
<td>2546.34 (56.70)</td>
<td>33.62 (132.37)</td>
<td>0.118 (56.76)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retina/choroids</td>
<td>0.0156 (58.11)</td>
<td>44.42 (58.06)</td>
<td>200.47 (161.33)</td>
<td>60.58 (5.47)</td>
<td>3944.30 (57.70)</td>
<td>15.25 (109.07)</td>
<td>0.076 (57.75)</td>
</tr>
</tbody>
</table>

Data are presented as parameter estimate (CV%). Ke, elimination rate constant; t_{1/2}, half-life; MRT, mean residence time; C_{max}, observed maximum concentration; AUC, area under concentration-time curve; V_d/F, apparent volume of distribution; CL/F, apparent clearance.
One of the limitations of our study is that the molecular weight of Fc/VEGF-Trap is lower than that of VEGF-Trap, and this may affect ocular PK, although these two drugs are predicted to have similar chemical properties. However, the longer half-life of Fc/VEGF-Trap despite its lower molecular weight emphasizes the role of Fc region in ocular PK. We replaced the Fc region with the coiled-coil domain, which was presumed not to affect the chemical properties of VEGF-Trap; but the coiled-coil domain itself might affect ocular PK by changing the chemical properties of the drug. Furthermore, we neither investigated the stability of Fc/VEGF-Trap nor performed a functional comparison of Fc/VEGF-Trap and VEGF-Trap. Despite these limitations, our study is the first to investigate the effect of Fc region on ocular PK by generating a novel molecule of Fc region–free VEGF-Trap.

In conclusion, Fc-region–deficient VEGF-Trap showed significantly longer vitreous and retina/choroid half-lives than conventional VEGF-Trap despite its lower molecular weight, indicating that Fc receptors in ocular tissues contribute to drug elimination. Our findings may be useful in future development of new anti-VEGF agents for intraocular administration. Truncation or mutation of the Fc region of protein drugs can prolong the intraocular residence time, reducing the number of injections and the systemic exposure to intraocular drugs.

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