Intraocular Pharmacokinetics of Ranibizumab in Vitrectomized Versus Nonvitrectomized Eyes

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Purpose. To analyze the intraocular pharmacokinetic properties of intravitreally injected ranibizumab in vitrectomized and nonvitrectomized rabbit eyes.

Methods. A procedure consisting of 25-gauge pars plana vitrectomy without lensectomy and posterior vitreous detachment was performed in 18 rabbit eyes, and 18 nonvitrectomized rabbit eyes served as controls. Ranibizumab (0.25 mg/0.025 mL) was intravitreally injected in all the vitrectomized and nonvitrectomized eyes. The eyes were enucleated at 1 hour or 1, 2, 5, 14, or 30 days after the intravitreal injections and frozen at −80°C. Ranibizumab concentrations in the vitreous, aqueous humor, and retina were determined using indirect enzyme-linked immunosorbent assay.

Results. Vitreous clearance of ranibizumab showed a 2-phase elimination. The vitrectomized and nonvitrectomized eyes showed comparable rates of vitreous clearance of ranibizumab. The vitreous half-life of ranibizumab for up to 14 days was 2.51 and 2.75 days in vitrectomized and nonvitrectomized eyes, respectively. Throughout the 30-day period after intravitreal injection, there were no statistically significant differences between the concentrations of ranibizumab in the vitreous, aqueous humor, and retina of vitrectomized eyes and those in nonvitrectomized eyes. Concentrations of ranibizumab in the vitreous peaked 1 hour after injection, with a mean concentration of 118.01 and 91.61 μg/mL in vitrectomized and nonvitrectomized eyes, respectively. The elimination rate constant of intravitreal ranibizumab in 1-phase analyses showed only a 9% increase in vitrectomized eyes compared to nonvitrectomized eyes.

Conclusions. Overall intraocular pharmacokinetic properties of ranibizumab in vitrectomized eyes were similar to those in nonvitrectomized eyes. Our data do not support the use of different dosing regimens of ranibizumab in vitrectomized eyes.

Keywords: pharmacokinetics, ranibizumab, vitrectomy

Antivascular endothelial growth factor (anti-VEGF) agents such as ranibizumab and bevacizumab have been used widely in the field of ophthalmology. Ranibizumab (Lucentis; Genentech, Inc., San Francisco, CA) is a recombinant, humanized monoclonal IgG1 isotype antibody fragment that inhibits VEGF.[1] The United States Food and Drug Administration has approved its intravitreal injection for treatment of age-related macular degeneration[1,2] and macular edema secondary to retinal vein occlusion.[3] Ranibizumab has shown remarkable efficacy in improving vision in patients with age-related macular degeneration and macular edema due to retinal vein occlusion and also in those with diabetic retinopathy.[1–7]

Ranibizumab is injected directly into the vitreous cavity to achieve the most effective therapeutic drug concentrations in retinal tissues. Eyes with age-related macular degeneration, macular edema due to diabetic retinopathy, or retinal vein occlusion often require repeated intravitreal anti-VEGF injections. Therefore, it is crucial to determine the pharmacokinetic (PK) properties of intravitreally injected ranibizumab when establishing a dosing regimen. Accordingly, the PKs and distribution of anti-VEGF agents after intravitreal injection have been investigated in several studies.[8–14]

In eyes undergoing treatment, clinicians frequently encounter the challenge of the requirement of anti-VEGF injection for vitrectomized eyes. One can easily speculate that the clearance of intravitreal anti-VEGF in vitrectomized eyes is different from that in nonvitrectomized eyes. However, few studies have compared intravitreal anti-VEGF concentrations of vitrectomized with those of nonvitrectomized eyes. Our group recently reported that the overall PK properties of intravitreal bevaci-

zumab in vitrectomized rabbit eyes were not substantially different from those in nonvitrectomized eyes.[11] However, Christoforidis et al.[9] and Kakinoki et al.[13] showed that intravitreal retention of bevacizumab and ranibizumab was significantly reduced after vitrectomy and lensectomy. Christoforidis et al.[9] used radiolabeled agents and measured radioactivity emission to calculate the half-life of either ranibizumab or bevacizumab. However, that approach results in some limitations when drawing conclusions, such as necessity for validation of a novel method used for a PK experiment, relatively small number of rabbit eyes (six vitrectomized eyes and six nonsurgical controls) used in the study, and a large variance observed in the study. Therefore, differences between PK properties of intravitreal ranibizumab in vitrectomized and those in nonvitrectomized eyes should be confirmed using a larger sample size and conventional methods.

In this study, we used a conventional immunoassay to determine the PK properties of intravitreal ranibizumab in vitrectomized and nonvitrectomized rabbit eyes. By comparing the PK profiles of intravitreally injected ranibizumab between vitrectomized and nonvitrectomized eyes, we elucidated the role of the vitreous gel in the clearance of intravitreal ranibizumab. We also measured the ranibizumab concentration in the retina in order to understand the effect of vitrectomy on drug delivery to target ocular tissues.

Methods

Animal Experiments

Approval for the study was obtained from the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee, and all procedures were performed with adherence to the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research in animals. A total of 36 eyes were obtained from 36 healthy New Zealand white rabbits weighing 1.5 to 2 kg, with 18 eyes included in the vitrectomized eye group and 18 in the nonvitrectomized eye group. The animals were anesthetized with an intramuscular injection of 15 mg/kg of a mixture of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil; Virbac Laboratories, Carros Cedex, France) and 5 mg/kg of xylazine hydrochloride (Fort Dodge Laboratories, Fort Dodge, IA). Furthermore, topical anesthesia consisting of 1% proparacaine hydrochloride ophthalmic eye drops (Alcaine; Alcon Laboratories, Inc., Fort Worth, TX) was induced after dilation with phenylephrine hydrochloride and tropicamide eye drops (Mydrin-P; Santen Pharmaceutical, Co., Osaka, Japan). Povidone-iodine 5% was placed on the conjunctiva of all the treated eyes (treatment consisting of intravitreal injection with or without vitrectomy), and all procedures were conducted using aseptic technique with surgical draping. The right eyes were treated in all the rabbits, whereas the left eyes received no intravitreal injections or vitrectomy.

For pars plana vitrectomy, 2-port 25-gauge sclerotomies were performed by trocar insertion (Alcon Laboratories, Inc.); one port was used as the infusion cannula and was connected to a bottle of balanced salt solution (BSS), and the other port was used for the surgical instrument. A wide-field fundus contact lens (Super Quad 160 lens; Volk, Mentor, OH) was placed after clear gel application on the cornea to enable a wide view of the peripheral retina, and the light from the operating microscope served as the illumination source. Total vitrectomy was performed (Accurus Surgical System; Alcon Laboratories, Inc.) meticulously to remove as much vitreous as possible. Fluid–air exchange was conducted to confirm the almost complete (more than 80%) removal of vitreous, after which the air was replaced with BSS. After cannula removal, the eyeball was palpated to ensure normal intraocular pressure. Eyes were monitored weekly for signs of inflammation.

After a recovery period of 3 weeks for the vitrectomized eyes, intravitreal ranibizumab was administered by injections to all 36 right eyes by using identical procedures on the same day. For intravitreal injections, 0.25 mg/0.025 mL ranibizumab was injected intravitreally 1 mm behind the surgical limbus in the superotemporal quadrant by using a 30-gauge needle. Three rabbits in each of the vitrectomized and nonvitrectomized eye groups were killed at each of the following 6 time points: 1 hour or 1, 2, 5, 14, or 30 days after injection. The right eyes were enucleated and immediately frozen and maintained at −80°C until the immunoassay. Before the immunoassay, the frozen eyes were separated into three compartments: vitreous, aqueous humor, and retina.

Aqueous humor samples were defrosted, and the volume of each sample was measured. Vitreous samples were defrosted and solubilized in 1.0 mL of PBS containing 1% BSA on a rotator overnight at 4°C. The samples were then centrifuged at 387*g* for 10 minutes as performed by Bakri et al.[8] The volume of each sample after centrifugation was measured. The frozen retinas were weighed, and the samples were defrosted and homogenized (product no. C3228, CellLytic MT; Sigma-Aldrich, St. Louis, MO) as described by Nomoto et al.[14]

Ranibizumab Immunoassay

Ranibizumab concentrations were measured using an indirect ELISA as described in previous reports.[8,11] Briefly, the 165-amino-acid variant of human recombinant VEGF (R&D Systems, Minneapolis, MN) was immobilized on 96-well flat bottom plates (Corning, Inc., Corning, NY). The human recombinant VEGF (rVEGF) was diluted to a concentration of 1.0 μg/mL in a 50 mM carbonate buffer (pH 9) and then divided into aliquots in 96-well plates at 100 μL/well. After incubating overnight at 4°C, the plates were washed with 1× PBS and blocked for 2 to 4 hours at 4°C with 1% BSA in 1× PBS. After a final washing procedure, the plates were stored and left to dry at 4°C.

Vitreous, aqueous humor, and retina samples were diluted in 0.1% BSA in 1× PBS to be within the linear range of the assay, divided into aliquots on a VEGF plate at 100 μL/well, and then incubated overnight at 4°C. For each individual plate, a standard curve of known ranibizumab concentrations ranging from 10.0 to 0.031 ng/mL was included. The bound ranibizumab was detected with goat antihuman IgG/F(ab′)2 antibody (1:20,000 dilution) labeled with horseradish peroxidase (Pierce Biotechnology, Inc., Rockford, IL). The diluted secondary antibody was incubated on the human rVEGF plate for 45 minutes at room temperature with agitation, followed by washing. The optical density was measured by detecting absorbance after the 3,3′,5,5′-tetramethyl benzidine substrate was triggered with hydrogen peroxide. The concentration of ranibizumab in our samples was calculated from the standard curve, which was constructed using the relative light signal from solutions of ranibizumab with known concentrations. The retinal concentration of ranibizumab was calculated by dividing the weight of ranibizumab (μg) by that of the retinal tissue (g). Data analysis was performed with SoftMax Pro version 5.4.1 software (Molecular Devices, Sunnyvale, CA).

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Pharmacokinetic Data Analysis

2-Phase PK Analysis. Vitreous concentration data were fitted to the following 2-phase exponential decay equation by using SAAMII version 1.2.1 software (Saam Institute, Seattle, WA): $C(t) = C_1\exp(-k_1t) + C_2\exp(-k_2t)$, where C (mg/mL) denotes concentration at any time, t (hours), and C_1 (mg/mL) and C_2 (mg/mL) are the back-extrapolated intercepts of the distribution and elimination phases, respectively. Both k_1 (h^{-1}) and k_2 (h^{-1}) represent rate constants at the distribution and elimination phases, respectively.

Overall (1-Phase) PK Analysis. To determine the overall PK properties of the two groups, vitreous concentration data were also fitted to the following single exponential decay expression (1-phase-exponential decay equation) by using the same software: $C(t) = C_0\exp(-kt)$, where C (mg/mL) and C_0 (mg/mL) denote concentrations at any time, t (h), and at $t = 0$, respectively; k (h^{-1}) indicates the elimination rate constant. The half-lives ($T_{1/2}$) of ranibizumab in the vitrectomized and nonvitrectomized eyes were calculated using the following equation: $T_{1/2} = 0.693/k$.

Results

In the 36 tested rabbit eyes, including 18 vitrectomized and 18 nonvitrectomized eyes, no adverse events or signs of ocular inflammation were noted after either vitrectomy or intravitreal ranibizumab injection.

Changes in total concentration of the ranibizumab protein over time in aqueous humor, vitreous, and retinas of vitrectomized and nonvitrectomized eyes are shown in Table 1. Throughout the 30-day period after intravitreal injection, there were no statistically significant differences among concentrations of ranibizumab in the vitreous, aqueous humor, and retinas of vitrectomized and those in nonvitrectomized eyes (all $P > 0.05$ using the Mann-Whitney U test). The maximum concentrations of ranibizumab in the aqueous humor, vitreous, and retina were obtained 1 hour after intravitreal injection. At that point, 0.25 mg of intravitreally injected ranibizumab was distributed to the aqueous humor at 4.34 μg (1.7%), 177.02 μg (70.8%) to the vitreous, and 23.63 μg (9.5%) to the retina in vitrectomized eyes. The corresponding amounts of ranibizumab in the nonvitrectomized eyes at the same time point were 4.08 μg (1.6%), 137.42 μg (55.0%), and 22.85 μg (9.1%), respectively. The amount of ranibizumab detected in the vitreous over a 1-month period is shown in Figure 1A, by the line representing a 2-phase exponential decay. Table 2 summarizes the rate constants of transfer for the first and second phases of the 2-phase analysis and those for the 1-phase analysis in both the vitrectomized and nonvitrectomized eyes. When the rate constants of transfer in the vitrectomized were compared with those in nonvitrectomized eyes, the elimination rate constant of ranibizumab in the vitreous in the first phase showed an increase of only 7% in the vitrectomized eyes, whereas a 187% increase was observed in the second phase.

However, our data showed a 1-phase exponential decay except on day 30, which had little impact on the determination of ranibizumab half-life, because only 1.3% of the initially injected ranibizumab remained within the vitreous on day 14. Thus, as shown in Figure 1B, the best-fit, 1-phase exponential decay equation was fitted to the data obtained from 1 hour to 14 days and was used to calculate the overall PK parameters. This calculation also showed PK properties of ranibizumab in vitrectomized eyes were comparable to those in nonvitrectomized eyes (Table 2). The half-lives of intravitreal ranibizumab were 2.75 and 2.51 days in the nonvitrectomized and

Table 1. Amounts of Ranibizumab in the Three Compartments (Aqueous Humor, Vitreous, and Retina) of 18 Vitrectomized and 18 Nonvitrectomized Rabbit Eyes at 1 Hour or at 1, 2, 5, 14, or 30 Days After Intravitreal Injection

	Vitrectomized Eyes						Nonvitrectomized Eyes					
	Aqueous Humor		Vitreous		Retina		Aqueous Humor		Vitreous		Retina	
Time	Total, μg	Conc, μg/mL	Total, μg	Conc, μg/mL	Total, μg	Conc, μg/g	Total, μg	Conc, μg/mL	Total, μg	Conc, μg/mL	Total, μg	Conc, μg/g
1 h	4.34 ± 0.41	21.7 ± 2.07	177.02 ± 10.62	118.01 ± 7.08	23.63 ± 6.61	984.53 ± 275.40	4.08 ± 0.47	20.38 ± 2.37	137.42 ± 14.64	91.61 ± 9.76	22.85 ± 0.08	952.25 ± 3.31
1 d	2.88 ± 0.27	14.4 ± 1.36	136.70 ± 8.55	91.13 ± 5.70	17.95 ± 3.21	748.08 ± 133.95	2.81 ± 0.10	14.04 ± 0.49	100.01 ± 1.74	66.67 ± 1.16	14.00 ± 1.28	583.54 ± 53.28
2 d	2.45 ± 0.16	12.3 ± 0.78	90.86 ± 6.60	60.57 ± 4.40	10.69 ± 3.42	445.58 ± 142.35	2.35 ± 0.10	11.75 ± 0.48	80.75 ± 4.35	53.83 ± 2.90	8.48 ± 1.70	353.19 ± 70.74
5 d	0.69 ± 0.00	3.43 ± 0.01	26.58 ± 3.00	17.72 ± 2.00	7.93 ± 3.49	330.49 ± 145.49	0.61 ± 0.03	3.03 ± 0.16	22.92 ± 3.51	15.28 ± 2.34	5.94 ± 0.62	247.64 ± 25.95
14 d	0.24 ± 0.09	1.20 ± 0.43	3.29 ± 0.05	2.19 ± 0.03	1.46 ± 0.55	60.80 ± 23.02	0.43 ± 0.09	2.13 ± 0.45	3.80 ± 0.56	2.53 ± 0.37	2.05 ± 0.20	85.25 ± 8.21
30 d	0.02 ± 0.07	0.12 ± 0.33	0.96 ± 0.29	0.64 ± 0.19	0.72 ± 0.21	29.89 ± 8.57	0.15 ± 0.06	0.75 ± 0.28	2.22 ± 0.26	1.48 ± 0.17	0.67 ± 0.27	28.07 ± 11.06

Data are means ± SD. Conc, concentration; d, day(s); h, hours; total, total amount in the compartment.

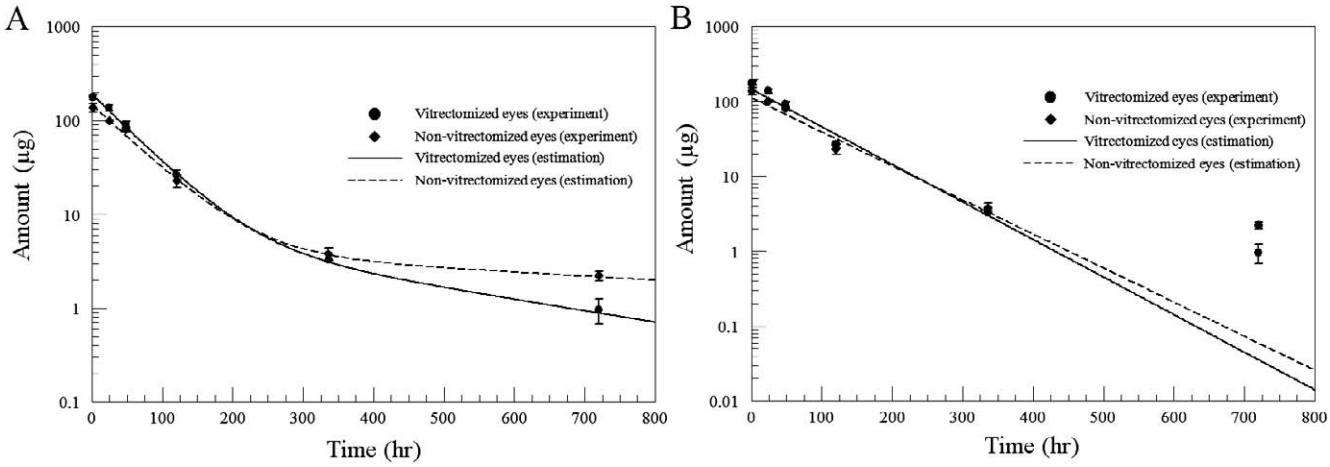

Figure 1. The amount of intravitreal ranibizumab over time in both vitrectomized and nonvitrectomized rabbit eyes following intravitreal injection (0.25 mg) is shown. (**A**) 2-phase exponential decay equation was fitted to each of the two data sets; (**B**) 1-phase equation was fitted to our data (except those at Day 30), which was used for the overall pharmacokinetic analysis including the calculation of the half-life of intravitreal ranibizumab.

vitrectomized eyes, respectively. The elimination rate constant of intravitreal ranibizumab in 1-phase analyses showed only a 9% increase in vitrectomized eyes compared to nonvitrectomized eyes.

The amount of ranibizumab in the aqueous humor peaked at 1 hour after intravitreal injection (4.34 and 4.08 μg in the vitrectomized and nonvitrectomized eyes, respectively) and declined in a mono-exponential fashion for up to 5 days following intravitreal injection (Fig. 2). However, from 5 days after intravitreal injection, elimination of ranibizumab from the aqueous humor was slower. From that time point, vitrectomized eyes showed greater elimination than the nonvitrectomized eyes did; however, the mean ranibizumab amounts in the aqueous humor at day 30 were 0.02 and 0.15 μg, respectively, and were not significantly different ($P = 0.100$ using the Mann-Whitney U test).

The ranibizumab concentrations in the retinas of vitrectomized and nonvitrectomized eyes were also measured. Figure 3 shows the mono-exponential decay of ranibizumab in the retinas of both the vitrectomized and nonvitrectomized eyes. The concentration peaked at 1 hour after intravitreal ranibizumab injection (984.53 and 952.25 μg/g in the retina in the vitrectomized and nonvitrectomized eyes, respectively), and subsequent measurements were comparable between the vitrectomized and nonvitrectomized eyes. At 30 days after injection, the ranibizumab concentrations in vitrectomized and nonvitrectomized eyes were 29.89 and 28.07 μg/g, respectively.

Discussion

In this study, we demonstrated no significant differences in intraocular (aqueous humor, vitreous, and retina) PK properties of intravitreally injected ranibizumab between vitrectomized and nonvitrectomized eyes. Several studies have been conducted on the PK properties of intravitreal ranibizumab, most of which were conducted in nonvitrectomized eyes. Bakri et al.[8] showed that elimination of intravitreal ranibizumab followed a mono-exponential function, with a half-life of 2.88 days. The half-life of intravitreal ranibizumab in previous studies varied from 2.63 to 2.9 days[8,10,12]; these findings are comparable to our findings in nonvitrectomized eyes (2.75 days). The half-life of intravitreal ranibizumab in vitrectomized eyes observed in our study (2.51 days) is shorter than those in all of the previously reported findings in nonvitrectomized eyes. However, the differences between vitrectomized and nonvitrectomized eyes (0.24 days or 5.8 hours) were not remarkable.

A few studies have shown a differences between PK properties of intravitreal drugs in vitrectomized and those in nonvitrectomized eyes. For example, Lee et al.[15] showed significantly faster intravitreal elimination of the 165 amino acid splice variant of human recombinant VEGF ($hVEGF_{165}$) in vitrectomized eyes (12.5 minutes) than in nonvitrectomized eyes (2.46 hours). Chin et al.[16] demonstrated that intravitreal triamcinolone acetonide decreases more rapidly in the vitrectomized eye than in the nonvitrectomized eye. Because VEGF is inherently present in the eye and its clearance may be modulated by mechanisms other than

Table 2. Comparison of the Pharmacokinetics of Intravitreal Ranibizumab in Vitrectomized Versus Nonvitrectomized Eyes by Using a 2-Phase Pharmacokinetic Analysis

	Vitrectomized Eye		Nonvitrectomized Eye		
Phase	k_{vit}, h^{-1}	$T_{1/2}$, h	$k_{non\text{-}vit}$, h^{-1}	$T_{1/2}$, h	**Percent Increase in Elimination Rate Constant, $k_{vit}/k_{non\text{-}vit} - 1$, %**
2-Phase analysis					
First phase	0.01755	39.5	0.01634	42.4	7
Second phase	0.00280	247.5	0.00098	707.1	187
1-Phase analysis	0.0115	60.3	0.0105	66	9

k_{vit}, rate constant of transfer in a vitrectomized eye; $k_{non\text{-}vit}$, rate constant of transfer in a nonvitrectomized eye; $T_{1/2}$, half-life.

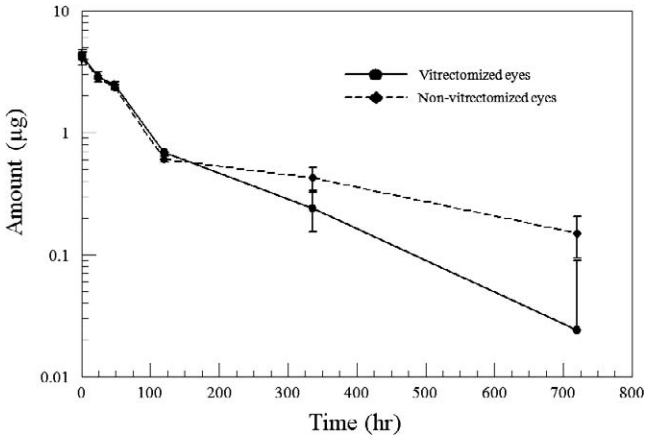

FIGURE 2. Ranibizumab concentrations in the aqueous humor over time in both vitrectomized and nonvitrectomized rabbit eyes following intravitreal injection (0.25 mg) are shown.

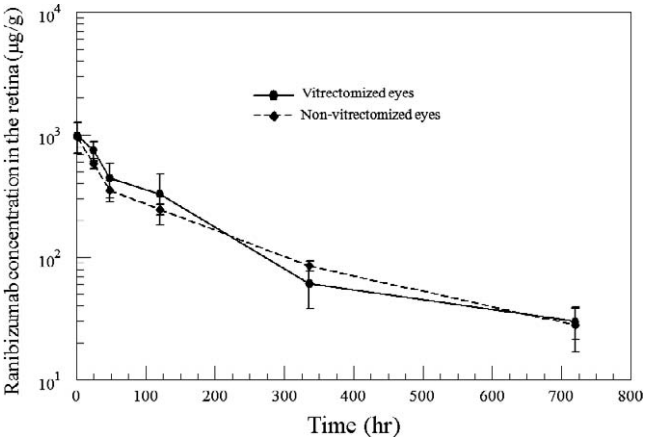

FIGURE 3. Ranibizumab concentrations in the retina (micrograms of ranibizumab/g of the retina) over time in vitrectomized and nonvitrectomized rabbit eyes following intravitreal injection (0.25 mg) are shown.

simple diffusion, the half-life of intravitreally injected VEGF in vitrectomized eyes might be affected by unknown mechanisms in addition to vitrectomy. Furthermore, triamcinolone acetonide aggregates in the vitreous to form visible crystalline microparticles, which may show different PK properties from those of the soluble form of triamcinolone in vitrectomized eyes. Because the effect of vitrectomy on the PK properties of intravitreal drugs might not be determined only from these studies and the intravitreally injected materials are different between the previous studies and ours, it would be inappropriate to extrapolate and conclude that eyes undergoing vitrectomy have a faster clearance rate of ranibizumab. Moreover, our previous study[11] showed that vitrectomy had no significant effect on the PK properties of intravitreal bevacizumab in rabbit eyes, which is consistent with the results of the present study.

A direct comparison between the PK properties of bevacizumab and those of ranibizumab is now feasible by using our previous data.[11] Our results show that there are no remarkable differences between the half-lives of intravitreal bevacizumab (6.99 and 7.06 days in vitrectomized and nonvitrectomized eyes, respectively) and those of ranibizumab (2.51 and 2.75 days in vitrectomized and nonvitrectomized eyes, respectively). As ranibizumab is a smaller molecule (48 kDa) than bevacizumab (149 kDa), it may be cleared faster from the vitreous cavity than bevacizumab, which explains the differences in half-lives. These results suggest that vitrectomy, or the absence of the vitreous, will have little effect on the clearance of current ophthalmic anti-VEGF agents. We did observe an additional difference between the clearance rates of the two drugs: bevacizumab was cleared during the first fast-distribution phase and the subsequent slow-elimination phase, whereas ranibizumab was cleared only during the elimination phase. Our previous study of bevacizumab showed that vitrectomy affected mainly the first fast-distribution phase, which lasts for approximately 1 day, whereas the second slow-elimination phase, which determines the overall half-life of bevacizumab, showed minimal change following vitrectomy.[11] Ranibizumab, being smaller than bevacizumab, probably has a shorter distribution phase (1 hour or less based on our data), and thus, we can overlook the first distribution phase of ranibizumab, and the effect of vitrectomy on the PK properties of ranibizumab may be minimal.

Because the vitreous consists of gel-like, highly viscous materials, it has been thought to act as a barrier to diffusion of intravitreal drugs. However, using fluorescein, Knudsen et al.[17] revealed that the transport rate and extent of drug penetration of intravenous fluorescein into the vitreous increased, whereas drug elimination from the vitreous remained unchanged after vitrectomy. However, 1 recent study using radiolabeled agents showed that the average clearance half-life for ranibizumab was 2.81 ± 0.05 days in unoperated eyes and 2.13 ± 0.05 days after vitrectomy.[9] The strength of that previous study is in its use of serial imaging and measurement of radioactivity emission, which may avoid interindividual variation in the amount of ranibizumab initially injected. However, that study used a relatively novel method for the measurement of intravitreal drug concentrations, which has limitations such as the undetermined nature of the uncoupling of iodine-124 from the antibody in the vitreous cavity. Therefore, the 0.68-day difference in half-life between vitrectomized and nonvitrectomized eyes requires further validation with larger samples and different methods. Using an immunoassay, the most frequently used analytical method for assaying therapeutic proteins in biological samples, we showed that the differences in half-lives between vitrectomized and nonvitrectomized eyes were less than that reported by Christoforidis et al.[9] and may be considered pharmacokinetically and clinically insignificant.

To further examine the discrepancy between previous anti-VEGF PK studies and ours, we conducted a literature review regarding the methodologies used in PK studies of anti-VEGF and summarized the results in Supplementary Table S1. Compared to our study, previous studies showed significant differences between vitrectomized and nonvitrectomized eyes, and different PK properties which varied widely among different studies. The surgical procedures used varied in different studies and were often combined with lensectomy. The subsequent amount of vitreous humor removed may also differ, which may affect the PK results in vitrectomized eyes. Thus, for direct comparison of PK properties between our results and those reported in the literature, our results obtained from nonvitrectomized eyes should be compared with those obtained from nonvitrectomized eyes in the literature. Our methodology is most similar to that described by Bakri et al.,[8] and the vitreous half-lives measured are comparable between both studies. The initial dose used in our study (0.25 mg) was 50% of the

corresponding value used by Bakri et al.,[8] which may explain the discrepancy regarding the maximum concentration measured (91.6 μg/mL in our study, 57% of the corresponding concentration obtained by Bakri et al.[8]). In the other studies, lensectomy also was often performed during vitrectomy.[9,13] Lensectomy enables complete vitreous removal, but the significant differences observed between vitrectomized eyes and nonvitrectomized eyes may result not only because of vitrectomy but also because of lensectomy. Indeed, after removal of the lens, the vitreous cavity and the anterior chamber become one compartment, which may affect the PK properties of the intravitreally administered drug. Vitrectomy with lensectomy is not a commonly used surgical procedure, and we performed vitrectomy without lensectomy in order to generate an animal model of the more common form of vitrectomized human eyes.

It is important to know the difference between the tissue distribution and clearance of intravitreal ranibizumab as it allows us to make better clinical decisions with regard to optimal drug dosing. As the retina is the target tissue of anti-VEGF, we measured the ranibizumab concentration in this tissue. Considering the almost identical retinal concentrations of ranibizumab in vitrectomized eyes and those in nonvitrectomized eyes throughout the 1-month period following injection, our study supports an efficacy and dosing schedule for ranibizumab in vitrectomized eyes that is identical to that in nonvitrectomized eyes. To our knowledge, this is the first study to compare the retinal concentration of ranibizumab in vitrectomized eyes with that in nonvitrectomized eyes and, thus, has clinical implications for dosing regimens of intravitreal ranibizumab in vitrectomized eyes.

There are some limitations in our study that require careful consideration for interpretation. First, the PK properties of drugs delivered to human eyes are different from those in rabbit eyes. Compared to human eyes, the smaller vitreous volume in the rabbit eye (1.5 mL, approximately 40% of human vitreous volume)[18] results in higher intravitreal ranibizumab concentrations. This is the reason why we injected 0.25 mg/0.025 mL ranibizumab rather than 0.5 mg/0.05 mL, which is used for human eyes. In addition, there is the possibility of different drug clearance mechanisms between human and rabbit eyes. Second, although we removed as much vitreous as possible, remnant vitreous behind the lens and on the retinal surface was inevitable and might have affected intraocular distribution and clearance of ranibizumab. More specifically, the size of the crystal lens in relation to that of the whole eye is much larger in rabbits than in humans.[18] Therefore, in rabbits, it is difficult to remove the vitreous completely without removing the crystal lens, especially when attempting to remove peripheral vitreous just behind the crystal lens. The remnant vitreous behind or around the lens might have affected our results.

We measured ranibizumab concentrations in retina samples that were homogenized as described in the study by Nomoto et al.[14] However, it is possible that the retinal samples (supernatant) obtained by homogenization and centrifugation do not contain all the anti-VEGF molecules actually present in the retina, and thus, the issue of percent of recovery should be considered carefully. Few studies of the intraocular PK properties of anti-VEGF addressed the percent of recovery issue (Supplementary Table S1) in the aqueous humor, vitreous humor, and retina. However, despite this unresolved issue, our comparative study using protocols for vitrectomized eyes that were identical to those used for nonvitrectomized eyes is valid as the percent of recovery in each tissue would be identical in both groups. We also acknowledge that the comparison between anti-VEGF concentrations in the retina and those in the other ocular tissues might not be valid unless the recovery issue is resolved.

In conclusion, the PK properties of intravitreal ranibizumab in vitrectomized eyes are not substantially different from those in nonvitrectomized rabbit eyes. Vitrectomized and nonvitrectomized eyes also showed similar concentrations of ranibizumab in the retina. We suggest that the role of the vitreous in the distribution and clearance of ranibizumab is insignificant and that similar dosing regimens for intravitreal ranibizumab injection may be used for patients that have previously received vitrectomy.

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