

# Intraocular Pharmacokinetics of 10-fold Intravitreal Ranibizumab Injection Dose in Rabbits

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**Purpose:** To investigate intraocular pharmacokinetics of 10-fold dose of intravitreally injected ranibizumab compared with the conventional dose in an experimental model.

**Methods:** Ranibizumab 30  $\mu$ L at 10 mg/mL (conventional) and 100 mg/mL (10-fold) doses was injected separately into each eye of 28 rabbits. Ranibizumab concentrations in the aqueous humor, vitreous, and retina were estimated at each time period after injection, using enzyme-linked immunosorbent assay. The pharmacokinetic properties of ranibizumab were determined using a one-compartment model in all three ocular tissues. The time-concentration profile and predictive trends were plotted to determine drug efficacy in the retina.

**Results:** Maximum concentrations after conventional and 10-fold dosing were observed in the retina at 1 and 4 days after injection, respectively. The half-life of ranibizumab after conventional and 10-fold dosing did not differ in the anterior chamber and vitreous, whereas the half-life was prolonged approximately twice with the 10-fold dose in the retina (36.74 h vs. 76.85 h) and serum (91.93 h vs. 179.01 h). Similarly, the estimated time for ranibizumab concentration in the retina over 27 ng/mL (minimum effective concentration of ranibizumab) was prolonged approximately twice with the 10-fold dose (1315 h [55 days] vs. 2393 h [100 days]). No adverse effects were observed in either group.

**Conclusions:** The retinal half-life and concentration of ranibizumab in rabbit eyes were increased approximately twice after a 10-fold dose compared with the conventional dose. This finding indicates a possibility to lengthen the injection interval to improve the efficacy of ranibizumab in human eyes.

**Translational Relevance:** Our results highlight the potential for clinical application of a high-dose (10-fold) of anti-VEGF agents to prolong the intravitreal injection intervals, simultaneously improving the drug efficacy.

## Introduction

Ever since the development of anti-vascular endothelial growth factor (VEGF) agents, notably ranibizumab and bevacizumab, these drugs have been

used as first-line treatment for exudative age-related macular degeneration (AMD), retinal vascular occlusion, and diabetic macular edema.<sup>1-4</sup> Recent treatment guidelines recommend the administration of a monthly loading dose, as required, followed by fixed dose and extended regimens. These doses have been proven

effective by pivotal clinical trials.<sup>5,6</sup> Recently, real-world studies suggest that undertreatment owing to the administration of fewer injections than required is a key factor responsible for poor visual outcome.<sup>7–10</sup> Furthermore, the huge socioeconomic burden associated with frequent intravitreal injections has been considered a barrier to optimal treatment.<sup>9</sup>

Solutions have been proposed for decreasing the treatment burden associated with anti-VEGF therapy. The duration of action of anti-VEGF drugs has been increased by developing new drugs, escalating dose of the proven drugs, or administering sustained-release formulations. HARBOR<sup>11,12</sup> and SAVE<sup>13,14</sup> are the two notable clinical trials on dose escalation of ranibizumab in exudative AMD. According to these two hallmark studies, escalation of the dose of ranibizumab to 2.0 mg, that is, four times higher than the conventional dose (0.5 mg) resulted in a fewer number of intravitreal injections or improved outcomes in terms of visual acuity. Hutton-Smith et al.<sup>15</sup> supported the results by evaluating the difference in duration of VEGF suppression after varying doses of intravitreal injection of anti-VEGF agents. The authors concluded that escalating the dose could prolong the duration of action of anti-VEGF agents and augment VEGF suppression in the retinal layer.

With reference to these prior studies, we postulated that administering a higher dose of ranibizumab intravitreally would reduce the required number of injections and would be likely to relieve the socioeconomic burden on patients. Previous human studies have used a four-fold dose of ranibizumab and no definite adverse effects were observed as compared with conventional dosing. However, studies are needed to identify the pharmacokinetics of higher doses of these agents on the human eyes. Hence, we performed an animal experiment to investigate pharmacokinetic profiles of ranibizumab, an anti-VEGF agent, at a higher dose (10-fold) compared with the conventional dose.

## Materials and Methods

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

## Procedures of Animal Experiment and Immunoassay

A total of 56 eyes were obtained from 28 healthy New Zealand white rabbits. They were categorized as 14 rabbits in the reference group (ranibizumab, 10 mg/mL injection [conventional dose]) and 14 rabbits in the high-dose group (ranibizumab, 100 mg/mL injection [10-fold dose]). All procedures were performed using the same technique as described in our previous pharmacokinetic studies on rabbit eyes.<sup>16–19</sup> First, the rabbits were anesthetized with 15 mg/kg of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil, Virbac Laboratories, Carros, France) and 5 mg/kg of xylazine hydrochloride (Rompun, Bayer Healthcare, Seoul, Korea). Topical anesthesia (1% proparacaine hydrochloride ophthalmic eye drops; Alcaine; Alcon Laboratories Inc., Fort Worth, TX) was applied after pupil dilation with phenylephrine hydrochloride and tropicamide eye drops (Mydrin-P; Santen Pharmaceutical Co., Osaka, Japan). After disinfecting the periocular area and the conjunctiva with 5% povidone-iodine, 30  $\mu$ L of the conventional and high-dose ranibizumab solutions were intravitreally administered into each eye 1 mm behind the surgical limbus of the superotemporal quadrant using a 30-gauge needle. The procedures were performed in both the eyes following the same technique. Four rabbits (two in each group) were killed at 1 hour and four were killed at each of the following time points: 1, 4, 8, 14, 30, and 60 days after injection. The eyes were enucleated and frozen at  $-80^{\circ}\text{C}$ .

From the frozen eyes, the aqueous humor, the vitreous, and the retinal tissues were isolated. First, the globe of the eye was opened and the aqueous humor was obtained. Then, the frozen iris and lens were removed carefully using tissue forceps. The frozen vitreous humor was separated from the remaining tissues of the retina and the choroid. The tissues were then thawed, defrosted, and the volume and weight were measured. The vitreous humor samples were solubilized in 1.0 mL of phosphate-buffered saline (PBS) containing 1% bovine serum albumin on a rotator overnight at  $4^{\circ}\text{C}$ , and centrifuged. Retina and choroid samples were homogenized, lysed, and centrifuged. After all three compartment samples were prepared, an indirect enzyme-linked immunosorbent assay was performed.<sup>20,21</sup>

In this study, we created the standard curve of known ranibizumab concentrations for quantification, similar to those in our previous studies.<sup>16–19</sup> First, the human recombinant VEGF was diluted to a concentration of 2  $\mu\text{g/mL}$  in 50 mmol/L carbonate buffer (pH 9), and aliquoted on the plate at 100  $\mu\text{L}$  per well for coating. Then, it was incubate overnight at

4°C and the plate was washed with 200  $\mu\text{L}$  of  $1\times$  PBS three times. Second, for the blocking process, we added 200  $\mu\text{L}$  of 1% bovine serum albumin in  $1\times$  PBS and washed the plate with 200  $\mu\text{L}$  of  $1\times$  PBS three times. Third, tissue samples were diluted in 0.1% bovine serum albumin,  $1\times$  PBS, divided into aliquots on the plate, and incubated overnight at 4°C. For each individual plate, standard curves of the known ranibizumab concentrations, ranging from 2.00 to  $0.97 \times 10^{-3}$  ng/mL, were included. After washing the plate with 200  $\mu\text{L}$  of 0.05% Tween-20 in  $1\times$  PBS three times, the anti-Fab antibodies were used to detect the samples and the optical densities were analyzed at the 450 nm wavelength. The optical densities of known ranibizumab concentrations and the dilution ratios of each tissue samples in three compartments were summarized in Supplementary Table S1. Finally, a four-parameter logistic curve fit was obtained using SoftMax Pro 5.4.1. (Molecular Devices, Sunnyvale, CA), and the standard curve was drawn as in Supplementary Figure S1. The four-parameter fit equation is as follows:

$$y = \frac{A - D}{1 + \left(\frac{x}{C}\right)^B} + D$$

Where  $A$  is the minimum value that can be obtained,  $B$  is the maximum value that can be obtained,  $C$  is the point of inflection (the point on the S-shaped curve halfway between  $A$  and  $D$ ), and  $D$  is Hill's slope of the curve (the steepness of the curve at point  $C$ ).

## Pharmacokinetic Analyses

The concentrations of ranibizumab in each compartment, aqueous humor, vitreous, and retina were estimated by a one-compartment model. The previous model described in our study<sup>19</sup> was used for the pharmacokinetic data analyses using the following equation:

$$C(t) = \frac{\text{Dose}}{V/F} \times e^{-kt}$$

Where  $C(t)$  (in micrograms per milliliter) is the concentration at time,  $V/F$  (in milliliters) is the apparent volume of distribution, and  $-k$  (1/h) is the elimination rate constant.

Several pharmacokinetic parameters (half-life, in hours; mean residence time in hours, maximum concentration [in micrograms per milliliter], time at maximum concentration in hours, area under the time-concentration curve [AUC, as hours  $\times$  micrograms per milliliter], and apparent clearance [in milliliters per hour]) were estimated. In addition, the efficacy and toxicity of ranibizumab were assessed. The efficacy

of intravitreally injected ranibizumab was considered as the total time of the retinal concentration of the drug at greater than 27 ng/mL,<sup>22</sup> which was defined as ranibizumab concentration inhibiting 50% of VEGF-A in vitro. Moreover, the serum ranibizumab concentration was estimated for monitoring the possible systemic adverse effects.

The concentration and pharmacokinetic parameter data were presented as the mean  $\pm$  standard deviation. Student's  $t$ -tests were used for obtaining continuous parametric data. We performed all statistical analyses using SPSS software version 21.0 for Windows (SPSS, Inc, Chicago, IL), and a  $P$  value of less than 0.05 indicated a statistically significant difference.

## Results

The concentrations of ranibizumab after conventional and 10-fold dosing in the anterior chamber, vitreous, and retina of the rabbit eyes as well as the serum are provided in Table 1. The concentrations of the drug in the anterior chamber and vitreous were the highest at 1 hour after injection after both conventional and 10-fold dosing, whereas the serum concentration was the highest at 1 day after injection. However, the retinal concentrations were the highest at 1 and 4 day after injection after conventional and 10-fold dosing, respectively. After reaching the peak, concentrations of the drug decreased rapidly.

A one-compartment model was adopted for analyzing the pharmacokinetic parameters. Table 2 summarizes the detailed pharmacokinetic parameters after conventional and 10-fold dosing of ranibizumab in rabbit eyes. The half-life of the drug in the anterior chamber (55.58 h vs. 53.36 h) and vitreous (51.70 h vs. 52.60 h) after conventional and 10-fold dosing showed no significant difference. However, the half-life in the retina (36.74 h vs. 76.85 h) and serum (91.93 h vs. 179.01 h) was prolonged approximately twice after 10-fold dose. Similarly, the AUC of the retina after the 10-fold dose was doubled (34,656.53 h  $\times$   $\mu\text{g/mL}$  vs. 16,491.45 h  $\times$   $\mu\text{g/mL}$ ), whereas the serum AUC was quadrupled after 10-fold dose ( $6885.24 \times 10^{-3}$  h  $\times$   $\mu\text{g/mL}$  vs.  $1644.41 \times 10^{-3}$  h  $\times$   $\mu\text{g/mL}$ ).

With the observed experimental concentration data at each time point (1 h, and 1, 4, 8, 14, 30, and 60 days), we constructed predictive trend lines of the concentration at each compartment (Fig. 1). Ranibizumab concentration in the anterior chamber and vitreous decreased exponentially immediately after intravitreal injection, whereas the concentrations in the retina and

**Table 1.** The Concentrations of RBZ and 10×RBZ in the Anterior Chamber, Vitreous, Retina of Rabbit Eyes and Serum at 1 Hour and 1, 4, 8, 14, 30, and 60 Days after Injection

Time	Anterior chamber Concentration (µg/mL)		Vitreous Concentration (µg/mL)		Retina Concentration (µg/mL)		Serum Concentration (ng/mL)		P* value
	RBZ 0.3 mg/0.03 mL	10×RBZ 3.0 mg/0.03 mL	RBZ 0.3 mg/0.03 mL	10×RBZ 3.0 mg/0.03 mL	RBZ 0.3 mg/0.03 mL	10×RBZ 3.0 mg/0.03 mL	RBZ 0.3 mg/0.03 mL	10×RBZ 3.0 mg/0.03 mL	
1 hour	23.08 ± 6.11	181.48 ± 13.72	140.01 ± 10.85	1637.54 ± 132.74	76.48 ± 9.32	90.15 ± 6.40	3.81 ± 0.52	19.73 ± 1.32	<0.001
1 day	14.08 ± 4.47	106.13 ± 9.64	100.61 ± 8.19	1192.33 ± 105.32	102.70 ± 11.27	112.90 ± 9.73	7.35 ± 1.14	22.07 ± 1.93	<0.001
4 days	8.69 ± 2.23	77.24 ± 8.48	51.72 ± 6.47	604.53 ± 48.78	72.26 ± 5.85	224.68 ± 15.77	4.56 ± 0.63	11.84 ± 1.46	<0.001
8 days	7.80 ± 2.06	64.40 ± 7.31	17.67 ± 4.26	450.87 ± 29.47	55.04 ± 4.46	83.80 ± 6.43	2.08 ± 0.29	6.93 ± 0.82	<0.001
14 days	6.23 ± 1.83	17.08 ± 5.27	2.13 ± 0.44	171.32 ± 12.79	0.95 ± 0.17	9.36 ± 2.28	1.47 ± 0.17	4.58 ± 0.37	<0.001
30 days	0.02	0.08 ± 0.02	0	0.55 ± 0.11	0.16 ± 0.03	0.57 ± 0.11	1.33 ± 0.18	3.37 ± 0.48	<0.001
60 days	0	0.01	0	0	0	0.33 ± 0.09	0	2.26 ± 0.31	<0.001

10×RBZ, 10-fold ranibizumab; RBZ, ranibizumab.

P values in boldface indicate statistical significance.

\*Student's t-tests for P values

**Table 2.** Estimated Pharmacokinetic Parameters of RBZ and 10×RBZ after Intravitreal Injection into New Zealand White Rabbits

	$t_{1/2}$ (h)	MRT (h)	$C_{max}$ ( $\mu\text{g/mL}$ )	$T_{max}$ (h)	AUC ( $\text{h} \times \mu\text{g/mL}$ )	V/F (mL)	CL/F (mL/h)
<b>Anterior chamber</b>							
RBZ 0.3 mg/0.03 mL	55.58 ± 7.72	171.04 ± 19.82	23.08	1	3431.81	7.01	0.087
10×RBZ 3.0 mg/0.03 mL	53.36 ± 5.14	138.03 ± 15.19	181.48	1	23,020.1	10.03	0.130
<b>Vitreous</b>							
RBZ 0.3 mg/0.03 mL	51.70 ± 6.03	81.59 ± 7.48	140.01	1	12,202.52	1.81	0.024
10×RBZ 3.0 mg/0.03 mL	52.60 ± 4.86	131.28 ± 10.67	1637.54	1	198,715.6	1.15	0.015
<b>Retina</b>							
RBZ 0.3 mg/0.03 mL	36.74 ± 3.79	107.19 ± 9.32	102.70	24	16,491.45	0.96	0.018
10×RBZ 3.0 mg/0.03 mL	76.85 ± 6.63	140.51 ± 11.37	224.68	96	34,656.53	9.58	0.086
<b>Serum</b>							
RBZ 0.3 mg/0.03 mL	91.93 ± 7.75	252.73 ± 18.46	$7.35 \times 10^{-3}$	24	$1644.41 \times 10^{-3}$	$21.85 \times 10^3$	$0.164 \times 10^3$
10×RBZ 3.0 mg/0.03 mL	179.01 ± 15.09	479.98 ± 31.25	$22.07 \times 10^{-3}$	24	$6885.24 \times 10^{-3}$	$103.73 \times 10^3$	$0.401 \times 10^3$

AUC, area under concentration-time curve; CL/F, apparent clearance;  $C_{max}$ , observed maximum concentration; MRT, mean residence time; 10×RBZ, 10-fold ranibizumab; RBZ, ranibizumab;  $t_{1/2}$ , half-life;  $T_{max}$ , time to  $C_{max}$ ; V/F, apparent volume of distribution.

the serum ascended sharply for 1 to 2 days after injection and then diminished rapidly owing to intraocular distribution of the drug. After 14 days of intravitreal injection, most of the drug was cleared both after conventional and 10-fold dosing. However, concentrations of the drug after 10-fold dosing were still detected at 30 and 60 days after injection (Table 1). Moreover, we calculated the extraction recovery ratio at different time points in each tissue compartments—the anterior chamber, vitreous humor, and the retina (Supplementary Table S2).

As discussed, 27 ng/mL has been considered as the effective cut-off level of ranibizumab concentration inhibiting 50% of VEGF-A in the retina in vitro. Therefore, in Figure 2, we plotted linear trend lines at 30 and 60 days after injection to calculate the time period at which the drug is above the retinal concentration of greater than 27 ng/mL. According to the predicted trend lines, estimated time for ranibizumab concentration in the retina to be greater than 27 ng/mL was prolonged approximately twice after 10-fold dosing (1315 h [55 days] vs. 2393 h [100 days]). This result coincided with the doubled half-life and AUC in the retina after 10-fold dosing.

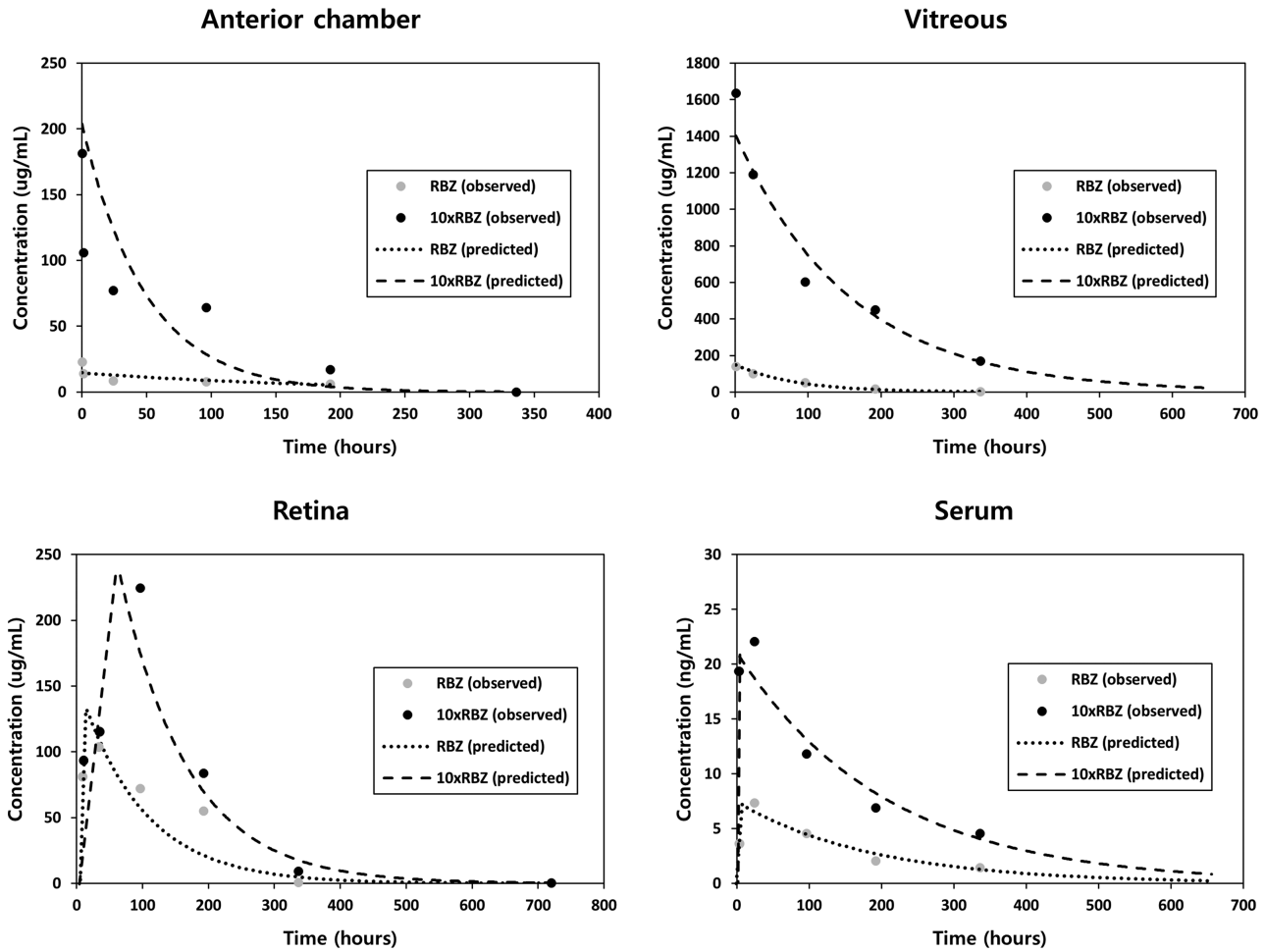
## Discussion

This study investigated the pharmacokinetic parameters of ranibizumab in the anterior chamber, vitreous, retina, and serum of rabbits after 10-fold dosing compared with those after conventional dosing. The results showed that the main pharmacokinetic parameters in the retina, such as the half-life, AUC, as well as efficacy duration, were doubled when a 10-fold dose of ranibizumab was administered intravitreally.

However, the half-life of ranibizumab in the anterior chamber and vitreous did not differ substantially after conventional and 10-fold dosing, which was considered to be due to simple elimination of the drug; the retinal half-life differed because of the multiple barriers to intraocular drug distribution when moving from the primary compartment to the secondary compartment.<sup>15</sup> Therefore, it can be inferred that the administration of a 10-fold dose of ranibizumab led to a prolonged release of the drug, thereby requiring fewer intravitreal injections.

In the serum, the half-life of ranibizumab was doubled and the AUC was quadrupled after administration of a 10-fold dose of the drug; however, there was no definite adverse effect until 60 days after injection. Moreover, the serum AUC of ranibizumab after 10-fold dosing ( $6885.24 \times 10^{-3} \text{ h} \times \mu\text{g/mL}$ ) was approximately 0.04% of that of the retinal AUC after conventional dosing ( $16491.45 \text{ h} \times \mu\text{g/mL}$ ). The serum maximum concentration of ranibizumab after 10-fold dosing was 22.07 ng/mL, which was still very low as compared with that after conventional dosing. The results are consistent with those of Gaudreault et al.,<sup>23,24</sup> who injected 0.5 mg and 2.0 mg of ranibizumab into rabbit eyes, implying that the concentration of the drug in the systemic circulation was significantly low as compared with vitreous and retinal tissue concentrations. Considering the different volume size of the serum compartments in humans relative to that in rabbits and monkeys, the systemic exposure to a 10-fold dose of ranibizumab would be much lower and, thus, the systemic adverse effects could be neglected.

Although there are debates on the interpretation of the study results of the two hallmark clinical trials (HARBOR and SAVE), using higher doses of

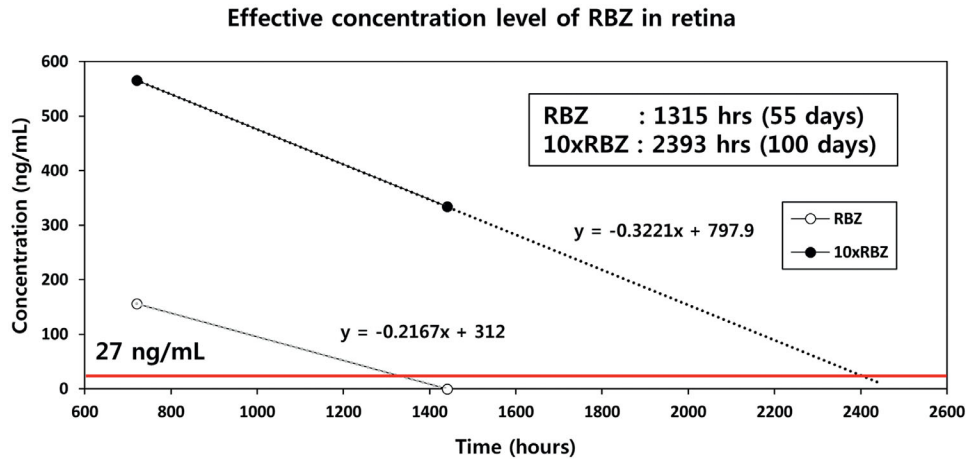


**Figure 1.** The observed concentration data at each time point and predicted trend lines in the anterior chamber, vitreous, retina, and serum. Concentration levels were measured in four rabbit eyes at each time point. *Gray dots* are data from the conventional dose of ranibizumab and *black dots* from the 10-fold dose of ranibizumab. *Small dot lines* are predicted trends of the conventional dose and *large linear dot lines* are predicted trends of the 10-fold dose.

ranibizumab have demonstrated potential benefits of 2 mg of ranibizumab. In the results of the 24-month HARBOR study,<sup>12</sup> the mean number of injections was 11.9 in the 2 mg as-needed (PRN) group, which was 2.3 fewer injections than the 14.2 in the 0.5 mg PRN group. The mean interval between the injections was 12.6 weeks in the 2.0 mg PRN group, which was 2.6 weeks longer than 9.9 weeks in the 0.5 mg PRN group. There were no clinically significant safety issues with 2.0 mg of ranibizumab compared with 0.5 mg of the drug (conventional dose). In the SAVE study,<sup>13,14</sup> patients with recalcitrant neovascular AMD were treated with 2 mg of ranibizumab injections monthly for 3 doses, and the mean visual acuity was increased by +3.7, +3.9, and +3.3 letters after 1, 2, and 3 months, respectively. However, there have been concerns that a higher dose of anti-VEGF in patients with wet AMD might induce macular atrophy. Nevertheless, in a post hoc analysis of patients of the HARBOR trial, the

hazard ratio for developing macular atrophy was 1.09 with 2.0 mg versus 0.5 mg regimen, which was not significantly high.<sup>25</sup>

Subsequent investigations have been performed since the completion of the HARBOR and SAVE studies. Chan et al.<sup>26</sup> used 2.0 mg of ranibizumab for the treatment of vascularized pigment epithelial detachments in patients with AMD. The authors concluded that both 0.5 mg and 2.0 mg doses of the drug resulted in similar anatomic and functional visual outcomes at 12 months, whereas the 2.0 mg dose showed more rapid improvements. The READ-3 study group<sup>27</sup> also compared the treatment outcomes in diabetic macular edema with 0.5 mg and 2.0 mg of ranibizumab for 24 months. The investigators suggested that both doses achieved anatomic and visual improvements without any significant additional benefits, such as better visual acuity or longer injection intervals. These studies left controversy on whether



**Figure 2.** The efficacy of the conventional dose and the 10-fold dose in the retina, with reference to the measured concentration of four rabbit eyes at 30 and 60 days after injection. The concentration level known to inhibit at least 50% of VEGF-A in vitro is 27 ng/mL, and we estimated the duration of retinal concentration predicted above 27 ng/mL (red line). White dots are the observed data from the conventional dose, and the following linear trend line is drawn. Black dots are the observed data from the 10-fold dose, and the following linear trend line is drawn. Linear trends are drawn rather than exponential curves because the exponential curves after 30 days after injection are similar to linear lines. The calculated duration of the effective concentration level of ranibizumab in the retina is 1315 hours (55 days) with the conventional dose and 2393 hours (100 days) with the 10-fold dose, approximately twice as long.

injecting a four-fold higher dose of ranibizumab is beneficial or not.

Recently, a new anti-VEGF agent named brolucizumab (RTH258)<sup>28,29</sup> is in a human trial. It is a small molecular-weight (26 kDa) compound and is more condensed than ranibizumab. It is estimated that 6 mg of brolucizumab equals about 12 times of the condensed concentration of 2.0 mg of aflibercept, and 22 times of 0.5 mg of ranibizumab. Recent clinical studies by Holz et al.<sup>28</sup> and Dugel et al.<sup>29</sup> pointed out that anatomic and visual improvements after administration of 6 mg of brolucizumab were comparable with those after administration of 2 mg of aflibercept, and a lesser number of injections could be administered in the 12-week treatment cycle. Overall, if ranibizumab is more concentrated as a 10-fold dose, like in our present experiment rather than just a four-fold dose as used in previous studies, we could argue that injecting a higher dose of ranibizumab might be beneficial for a longer duration. As shown in Figure 2, the duration of efficient concentration after administration of a 10-fold dose of ranibizumab was prolonged by approximately twice; therefore, a 8 to 12-week treatment cycle could be feasible rather than administering monthly or bimonthly intravitreal injections. This interpretation could also explain the brolucizumab trial result that about 50% of neovascular AMD eyes were maintained on q12 week dosing with 6 mg brolucizumab throughout the study period.<sup>29</sup>

There were several limitations to our study. First, it was an experiment on animals involving rabbit

eyes. Therefore, caution is required while extrapolating the results in comparison to human eyes. Second, we used a one-compartment model to analyze the pharmacokinetic parameters to maintain continuity with our previous experimental studies.<sup>18,19</sup> The one-compartment model is intuitive and easy to use for analysis; however, the real-world intraocular distribution and elimination processes are more complex than this model allows. Third, the concentrations measured after 30 and 60 days after the injection could be variable, leading to a change in the prediction of the trend lines because considerably lesser concentrations of ranibizumab were detected at those time points. Finally, a toxicologic assessment involving a high dose of ranibizumab should be carried out. Although there were no definite local or systemic adverse effects such as infection and inflammation, when we observed the rabbit eyes at 60 days after injection, histologic and functional hazard assessment must be considered. Furthermore, animal experiments should focus on investigating the safety of escalated drug dosing.

To conclude, administration of a 10-fold dose of ranibizumab in the rabbit eyes showed doubled half-life, AUC, and duration of effective concentration in the retinal tissues compared with those after administration of the conventional dose of the drug. No significant local or systematic adverse effects could be anticipated as low serum concentration was constantly maintained. These results highlight the potential for clinical application of a high-dose of ranibizumab to

prolong the intravitreal injection intervals, simultaneously improving the drug efficacy.

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